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Characterisation and clinical studies of an antimicrobial urinary catheter for long-term use

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Abstract

Background and Aims

Catheter-associated urinary tract infections (CAUTI) are a common and costly complication of indwelling urinary catheterisation. No commercial technology exists that protects against the major uropathogens for the lifetime of a long-term catheter. Silicone indwelling urinary catheters were impregnated with the antimicrobials (AUC) rifampicin, sparfloxacin, and triclosan to confer antimicrobial activity.

With the overall aim of delivering this technology to urinary catheters users, this thesis aims to firstly, understand the profile of micoorganisms attached to indwelling urinary catheters. Secondly, using a combination of studies investigating the impregnated catheter surface and in vitro models, determine the ability of the AUC to resist mineral encrustation. Thirdly, this thesis will assess the protective activity of the AUC against multi-drug resistant organisms and *Enterococcus spp*. Finally, the safety and patient acceptability of this AUC will be assessed by a single-centre clinical trial.

Methods

Urinary catheters were collected from patients at Nottingham University Hospitals NHS Trust (NUHT), and also over one year from one volunteer. General microbiological methods, including a new method of processing urinary catheters and MALDI-ToF were employed to identify and quantify attached microorganisms. Pulsed-field gel electrophoresis (PFGE) and similarity analysis determined the relationship between isolates of the same species isolated from catheters collected consecutively from the volunteer.

The catheter surfaces were investigated after antimicrobial impregnation and soaking using atomic force microscopy and bacterial attachment assays. Reduction of mineral encrustation on the AUC was investigated in the presence and absence of bacteria. Spectrophotocolourimetry quantified phosphate deposition in a static and in vitro flow model. The protective duration of the AUC against *Enterococcus*

spp. and multi-drug resistant bacteria was investigated in a clinically predictive in vitro model.

A single-centre, non-randomised safety and patient acceptability study was conducted. The primary outcome measure was rate of adverse events attributable to antimicrobial impregnation. Secondary outcome measures included patient acceptability, withdrawal before the end of the trial, and microorganism colonisation of trial catheters.

Results

Sixty-one urinary catheters were collected from patients at NUHT. *E. coli* and *Enterococcus faecalis* were the most commonly isolated organisms. A novel method was developed to isolate micoorganisms from the catheter lumens and balloons separately. Nine consecutive urinary catheters were collected from one volunteer over one year. Methicillin-susceptible *Staphylococcus aureus*, *E. faecalis*, *Citrobacter koseri*, and *Pseudomonas aeruginosa* were isolated from many of the nine catheters over the course of the year. PFGE revealed the isolates were indistinguishable, except for three *P. aeruginosa* isolates, which were closely related but differed by a 2-3 band difference by PFGE.

Surface characteristics of silicone urinary catheters were not adversely affected by antimicrobial impregnation. The AUC prevented blockage and reduced phosphate deposition when challenged with *Proteus mirabilis* in static and flow models. The AUC was not able to consistently eradicate *E. faecalis* and *E. faecium* isolates. The AUC resisted colonisation by methicillin-resistant *S. aureus*, methicillin-resistant *Staphylococcus epidermidis,* MSSA, *Staphylococcus saprophyticus*, extended-spectrum beta-lactamase producing *E. coli*, New Delhi metallo-beta-lactamase producing *E. coli* for approximately 12 weeks.

Thirty patients were recruited to the safety clinical trial, which demonstrated the AUC was safe and was acceptable to the majority of participants. Only one adverse event was reported that was ‘probably’ associated with antimicrobial impregnation of the catheters and it was mild and resolved within 48 hours. There were significantly less bacterial isolates

attached to the balloons of trial catheters compared to the matched original catheters.

Conclusions

The AUC is safe and effective technology for long-term catheter users. Next steps include a clinical trial of efficacy and commercialisation.

Declaration

This is to certify that work submitted in this thesis is the result of original research. It has been conducted substantially by me with assistance as outlined below:

- Preparation of samples and data analysis of the x-ray photoelectron samples was primarily carried out by myself. Dr. Emily Smith carried out the XPS analysis and provided CasaXPS software for a fee.
- Preparation of samples and data analysis of the atomic force microscopy samples was primarily carried out by myself. Professor Xinyong Chen carried out the AFM analysis and provided the NanoScope Analysis software for a fee.
- Clinical trial study design, ethical application, data collection, analysis, writing and general administration were conducted primarily by myself with support from Professor Roger Bayston and Mr. Richard Parkinson.
- Administrative governance, all laboratory work, upkeep of trial database was administered by myself. Helen Betts and Mr Richard Parkinson took informed consent from patients recruited to the clinical trial. Helen Betts followed-up patients in the trial with assistance from myself.
- All antimicrobial urinary catheters for use in the clinical trial were impregnated by myself. Sterilisation and sterilisation validation were carried out by Yushin Medical Co and this was arranged with assistance from Lesley Orme, Clinimed Ltd.

This work has not been submitted to any other degree or other professional qualification. Supervision of this thesis was undertaken by Professor Roger Bayston and Mr. Richard Parkinson.

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List of Abbreviations

AE	Adverse event
A&E	Accident and Emergency Department
AFM	Atomic force microscopy
ANOVA	Analysis of variance
AR	Acquired resistance
ASB	Asymptomatic bacteriuria
AU	Artificial urine
AUC	Antimicrobial-impregnated urinary catheter
CAPD	Continuous ambulatory peritoneal dialysis
CAUTI	Catheter-associated urinary tract infection
CDC	Centers for Disease Control and Prevention
CFU	Colony forming unit
Ch	Charrière unit
CLED	Cystine-lactose electrolyte deficient
CLSI	Clinical and Laboratory Standards Institute
CRN	Clinical Research Network
CSU	Catheter specimen of urine
CT	Cefotaxime
CTL	Cefotaxime + clavulanic acid
DI	Deionised
DPA	Dipicolinic acid
ECOFF	Epidemiological cut-off value
EDTA	Ethylenediaminetetraacetic acid
ESBL	Extended-spectrum beta lactamase
EUCAST	European Committee on Antimicrobial Susceptibility Testing
EVD	External ventricular drain
GC-MS	Gas chromatography-mass spectrometry
HPLC	High-performance liquid chromatography
HRA	Health Research Authority
IDSA	Infectious Diseases Society of America
IQR	Interquartile range

ISA	Iso-sensitest agar
Kbp	Kilobase pairs
MALDI-ToF	Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry
MBC	Minimum bactericidal concentration
MBL	Metallo-beta-lactamase
MDD	Medical Devices Directive
MDR	Multi-drug resistant
MDevR	Medical Devices Regulations
MHA	Mueller-Hinton agar
MHB	Mueller-Hinton broth
MH-F	Mueller-Hinton broth supplemented
MHRA	UK Medicines and Healthcare products Regulatory Agency
MIC	Minimum inhibitory concentration
MSSA	Methicillin-susceptible *Staphylococcus aureus*
MRSA	Methicillin-resistant *Staphylococcus aureus*
MRSE	Methicillin-resistant *Staphylococcus epidermidis*
MS-GC	Mass spectrometry- gas chromatography
MSU	Mid-stream urine
m/z	Mass to charge ratio
NDM-1	New Delhi metallo-beta-lactamase 1
NUHT	Nottingham University Hospitals Trust
PBP	Penicillin binding protein
PBS	Phosphate buffered saline
PFGE	Pulsed-field gel electrophoresis
PIC	Participant identification centre
PIS	Participant information sheet
PK/PD	Pharmacokinetic/ pharmacodynamics
PPI	Patient and public involvement
PPM	Parts per million
PTFE	Polytetrafluoroethylene
REC	Research Ethics Committee
RCT	Randomised control trial

RMC	Research management committee
SAE	Serious adverse event
SEM	Scanning electron microscopy
SCI	Spinal cord injury
TK100	Time kill 100%
TSB	Tryptone soya broth
TURP	Trans-urethral resection of the prostate
TURT	Trans-urethral resection of the tumour
TWOC	Trial without catheter
TZ	Ceftazidime
TZL	Ceftazidime + clavulanic acid
VRE	Vancomycin-resistant enterococci
WT	Wild-type
XPS	X-ray photoelectron spectroscopy

Chapter 1. Introduction and Aims

Much of modern medicine relies on implantable medical devices ranging from electrical devices, such as pacemakers to regulate heart rate, to simple tubular devices such as central venous catheters and urinary catheters. While these devices are essential for the care and dignity of many patients, all come with a risk of infection. Particularly, urinary catheters are the most common risk factor for a urinary tract infection[1], and catheter-associated urinary tract infections (CAUTI) cost the NHS approximately £99 million each year[2]. The clinical, personal, and financial burden of CAUTI is enormous and yet no technology is commercially available to prevent CAUTI in patients who require a long-term indwelling urinary catheter to manage their bladder.

For some patients, such as those undergoing surgery or with acute retention, they may require a urinary catheter only in the short-term (less than 28 days); a key strategy to preventing infection is removing the catheter as soon as possible. However, for some patients such as stroke survivors, those with neurodegenerative disease, those with chronic retention, and those with spinal cord injury, long-term catheterisation (over 28 days) is often the only solution.

Technology that would prevent repeated episodes of CAUTI and therefore reduce antibiotic use and its associated side effects would improve patient quality of life, help prevent increased antimicrobial resistance, and reduce healthcare-associated costs. An antimicrobial urinary catheter (AUC) with protective activity for the lifetime of a long-term urinary catheter is one such way to address the need for this preventative technology.

Please find in this chapter a general introduction to indwelling urinary catheters and their problems and how the AUC with long-term activity described herein aims to address them. This thesis consists of distinct chapters with distinct methods. The detailed introduction to the methods used in each chapter can be found at the start of each individual chapter.

1.1 Urinary system function and anatomy

The urinary system's major role in the human body is for the control and regulation of the body's fluids. The function of the urinary system can be broken down into three major activities:

1. Regulation of fluids to maintain correct fluid volume, the balance of ion concentrations and pH
2. Excretion of waste products and some drugs
3. Synthesis of endocrine-related molecules that regulate blood pressure[3]

The urinary system is composed of four major anatomical components with distinct functions to carry out the above processes. The kidneys are the filtration system which control the composition and volume of body fluids resulting in the production of urine. One ureter connects to each kidney and transports the urine from the kidneys using peristalsis to the bladder where the urine is stored. The urethra then transports urine from the bladder out of the body[4].

Voiding of urine when stored in the bladder is a voluntary process in which there is coordinated bladder detrusor muscle contraction with urethral sphincter relaxation[3]. However, this voluntary process can be affected by various conditions resulting in urinary incontinence or incomplete bladder emptying (urinary retention). Generally incontinence is caused by weakness of the pelvic floor and sphincter muscles (stress incontinence), overfilling of the bladder (overflow incontinence) or overactivity of the detrusor muscle (urge incontinence). Retention, which is the inability to void urine completely or partially, can be acute or chronic. It can be caused by bladder outflow obstruction such as prostatic enlargement in men, or by loss of detrusor contractility such as in neurological disease. Generally acute urinary retention is defined by a sudden and often painful onset, whereas chronic urinary retention is not painful with a large residual volume of urine in the bladder after voiding[5].

1.1.1 **Components of urine**

Urine is typically clear in appearance with a yellow colour. The mean pH of urine is 6.25 with a range of 5.52-6.97 in adults. The main components of urine include: nitrogen, amino acids, hormones, sugars, organic bases and inorganic bases. The complexity of urine means that there is an extensive list of constituents which will vary slightly depending on gender and age and other underlying health issues[6]. The major components and their concentrations can be found in Table 1-1. Generally, there is good similarity between the values provided by the two sources, especially considering that urine constituents vary by person, time of day, and diet[6, 7].

Table 1-1 Major constituents of urine and their average concentrations (mg/day unless otherwise stated) from two sources

Major Urine Constituents	**Mean mg/day (Free and Free, 1976)[7]**	**Mean mg/day (Diem, 1962)[6]**
Urea	24,500	20,000-35,000
Chloride	71 mg/L	7368
Creatinine	1,400	2,145
Phosphorous	849	1,100
Uric Acid	140	528
Potassium	2,500	2,740
Glucose	100	72
Protein	35	20-100

Typically bilirubin, haemoglobin, and ketones are completely absent from urine and can indicate disease if present. Microscopically a few epithelial cells, red blood cells, or white bloods cells may be present, but can indicate disease or infection if seen in large quantities[8, 9].

1.1.2 **Urinary crystals**

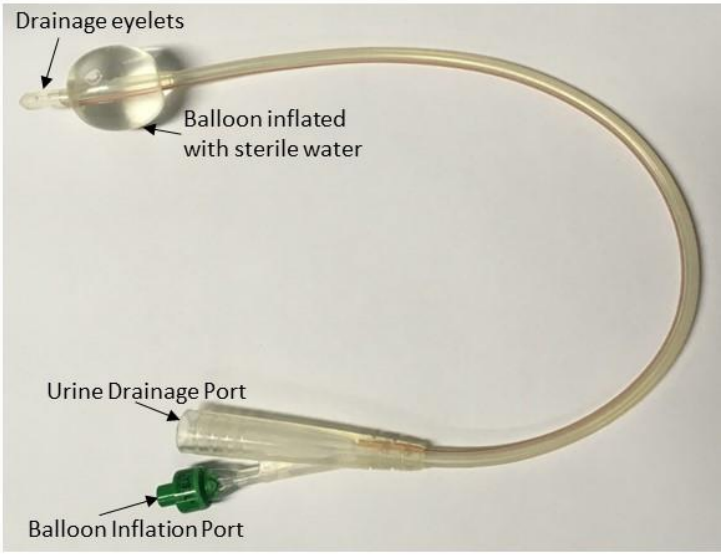
The presence and identification of urinary crystals is useful for identifying kidney disease and infection. Some urinary molecules that may promote crystallisation include calcium, oxalate, urate and phosphate ions[10]. Of importance; ammonium magnesium phosphate crystals (also known as struvite) are associated with an alkaline pH in response to infection by

urease-producing bacteria. Crystals can be produced by the bacterial enzyme urease, which hydrolyses the urea in the urine producing ammonia and carbon dioxide[11]. The ammonia raises the pH of the urine and as the pH increases, magnesium ammonium phosphate crystals precipitate out of the urine[12]. This is associated with blockage of urinary catheters.

1.2 Urinary catheterisation

Frederick Foley designed a rubber latex catheter in 1937 that was available for use as a urethral urinary catheter[13]. Since then, the design of the catheter has changed very little[14]. The current design of the Foley catheter consists of a long, hollow, flexible tube with ports at one end and an inflatable balloon at the other with drainage eyelets above the balloon (Figure 1-1). There can either be two ports (two-way catheter) or three ports (three-way catheter). All indwelling catheters have a port for urine drainage and one for inflation of the balloon once in the bladder. A three-way catheter has an additional port used to irrigate the bladder[15]. The catheter tip is inserted into the bladder where it is held in place by the balloon, which is inflated with sterile water. The balloon is deflated for removal.

Figure 1-1 Two-way Foley urinary catheter with components labelled



Urinary catheterisation can be classified as either indwelling or intermittent. Intermittent urinary catheters are inserted when needed and removed after urine has been passed, whereas indwelling catheters remain held in place by a balloon which is inflated once in the bladder as described

above. There is some evidence that the use of intermittent catheterisation instead of indwelling urethral catheters may lower the risk of CAUTI and bacteriuria[16]. Indwelling catheterisation can either be urethral or suprapubic depending on the site of catheterisation. In urethral urinary catheterisation the catheter is inserted into the bladder via the urethra. Suprapubic catheterisation bypasses the urethra by draining urine through a catheter placed through a tract in the abdominal wall.

Urinary catheters are available in female and standard lengths for adults which are either 200-260 mm or 400-450 mm in length, respectively. The shorter female length can be used only in female patients due to the risk of the balloon not reaching the bladder in men and being inflated in the urethra causing trauma[17]. Urinary catheters are also available in different lumen diameters, which are described in Charrière units (Ch). Adult sizes range from 12 Ch – 30 Ch and the lumen size of the urinary catheter is determined by patient comfort and what is being drained from the patient's bladder. The smallest size should be used for patient comfort, but if clots or encrustations need to be drained then a larger lumen diameter may be required[18].

Historically, urinary catheters were made of latex, but due to the risk of allergy, latex catheters are now coated with a silicone elastomer. Alternatively, many catheters, particularly for long-term use, are all-silicone and are advantageous in that they do not induce allergy and have a better flow[19]. Hydrophilic coatings have been applied to the catheter surfaces to reduce friction and trauma.

There are two types of drainage devices; a drainage bag and a valve. The drainage bag allows for urine to continuously flow into the bag for collection, and the bag is then emptied when full. Alternatively, when using a valve the urine is stored in the bladder, and the valve is opened to flush the urine and then reclosed[20].

Indwelling catheterisation can further be subdivided into short-term and long-term catheterisation. The duration of short-term catheterisation is 28 days or less and the duration of long-term-catheterisation may be from 28 days up to three months[2, 21]. The cut-off at 28 days between short-term and long-term catheterisation is determined by bacterial colonisation of the

urinary catheter; virtually all urinary catheters are colonised with bacteria by 28 days[2].

1.2.1 Complications of indwelling urinary catheterisation

As the catheter bridges between mucosal surfaces and the environment, the body's defence barrier is broken, and the patient is at risk of infection and other non-infective complications[12]. Therefore, it is of the utmost importance that the patient is thoroughly assessed for the need to have an indwelling catheter. Indications for short-term catheterisation include perioperatively, treatment of acute urine retention, monitoring of urine output in ill or unconscious patients, bladder irrigation to treat bleeding, and introduction of certain drugs into the bladder. Long-term catheterisation may be necessary for patients with chronic retention, patients with a small bladder capacity or to maintain skin integrity for patients with severe or untreatable incontinence[22].

Some minor non-infective complications include leakage of urine, accidental removal of the catheter, catheter expulsion and blockage of the catheter. More serious complications include urethral stricture (narrowing of the urethra due to trauma or infection), bladder stones and bladder cancer (1.0% frequency in spinal cord injury (SCI) patients)[23]. A very serious complication specific to those with SCI at or above the sixth vertebra (T6) is autonomic dysreflexia. In response to pain stimuli below the spinal lesion, such as CAUTI, the body produces heightened nerve signals mediated through the spinal cord, resulting in increased blood pressure, which can result in seizures, stroke, or death[24, 25] .

1.3 Catheter-associated urinary tract infections

Asymptomatic bacteriuria (ASB) is the presence of bacteria in the urine in the absence of symptoms and requires no antimicrobial treatment[16, 24]. In the long-term urinary catheter user population, all patients will be likely to have bacteria in their urine. It is only when symptoms are present that the diagnosis of CAUTI can be made. The risk of bacteriuria increases by 5%

each day the catheter remains in place and of those with bacteriuria 10-24% will develop symptoms indicative of infection[2, 16].

1.3.1 **Diagnosis**

The Centers for Disease Control and Prevention (CDC) defines CAUTI as having a catheter in place and having symptoms including fever greater than 38°C. Suprapubic tenderness or costovertebral angle pain or tenderness can also be useful symptoms if there are no other recognised causes for these symptoms. Urinary urgency, urinary frequency, or dysuria, while traditional symptoms in diagnosing urinary tract infection in non-catheterised patients, are not useful in those who are catheterised[26]. Further difficulty in diagnosis can be encountered in elderly patients and those with dementia as they may present with vague symptoms such as confusion, reduced mobility, and newly evident urinary incontinence[27].

Additionally, the CDC definition of CAUTI extends to include the microbiological results of which the urine culture of the patient can have no more than two microorganism species, one of which is bacterial with > 10^5 CFU/mL. There are conflicting recommendations in guidelines as to when to send a catheter specimen of urine (CSU) sample from a symptomatic patient. Local guidelines at Nottingham University Hospitals Trust (NUHT) state that CSU samples should be sent for analysis only if the patient is symptomatic and the catheter should be changed during the treatment course[28]. Therefore, the CSU comes from the current catheter suspected of infection. Alternatively, the Association for Professionals in Infection Control and Epidemiology guidelines recommend sending the urine specimen after removal of the catheter to avoid sampling catheter biofilm. If the patient requires a replacement catheter, the CSU can then be taken from the new catheter. Regardless, CSU samples should only be sent if the patient is displaying symptoms and not as routine.

1.3.2 **Microbiology of CAUTI**

The microorganisms commonly isolated from CAUTI patients include *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus* spp., *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Candida* spp., and *Staphylococcus*

spp.[29, 30]. *P. mirabilis* is a particularly menacing organism to long-term catheter users as it can cause blockage of catheters due to mineral encrustation, bladder and kidney stone formation, and ascending infections. It is able to access the catheter, bladder, and kidneys due to its peritrichous flagella that allow it to swim in fluid, and swarm over solid surfaces[31].

In long-term catheter users the infection is often polymicrobial. The rate of polymicrobial CAUTI in newly catheterised patients is approximately 6%[32] and 77% of those with CAUTI in long-term catheter users[33].

1.3.2.1 **Multi-drug resistant organisms in urological infections**

Multi-drug resistant organisms play an important role in the microbiology of CAUTI. *E. coli* and *K. pneumoniae* and other Enterobacteriaceae can produce beta-lactamase enzymes, particularly extended-spectrum beta-lactamases (ESBLs), which confer resistance to penicillins, first, second, and third generation cephalosporins and aztreonam[34]. ESBL-producing organisms may also be resistant to trimethoprim, tetracycline, and fluoroquinolones[35]. Throughout Europe, there is an increase in ESBL *E. coli* isolates detected from 2013 to 2016[36].

Methicillin-resistant *Staphylococcus aureus* (MRSA) infections have been declining in England and Wales as a result of government-led screening programmes and MRSA bacteraemia reduction targets[37]. MRSA bacteraemia rates have fallen 72.0% in 2016-2017 compared to 2008-2009[38]. Currently, the Department of Health recommends screening patients with a history of MRSA colonisation or infection and patients admitted to high risk units/wards such intensive care units and neurosurgery wards. Patients who are colonised with MRSA should be isolated until decolonised[39]. Despite the reduction in MRSA prevalence, urinary catheters and urinary tract infections can often be the original source for the MRSA bacteraemia[37]. The genes encoding for methicillin - resistance can be transferred between *Staphylococcus* species[40]. More *S. epidermidis* isolates are now methicillin-resistant (MRSE) than *S. aureus* isolates[41].

1.3.2.1.1 Major mechanisms of resistance by gram-positive organisms

Penicillinase enzymes, which confer resistance to penicillin and other beta-lactam antibiotics, were discovered in 1940, when the first clinical trials of penicillin had just begun and by 1948 58% of *S. aureus* isolates were resistant to penicillin[42, 43]. The activity of methicillin, the first semisynthetic beta-lactam antibiotic, was stable against penicillinase enzymes and could be used to treat penicillin-resistant staphylococci[44]. In 1960 methicillin was introduced for clinical use, and in 1961 methicillin-resistant *S. aureus* was identified[45]. Resistance to methicillin was not regulated by enzymatic degradation of the antibiotic, but rather by the structural change to the antibiotic target: penicillin binding protein (PBP). The alternative PBPs produced by MRSA (PBP2a) had less affinity to methicillin than those produced by MSSA[46]. PBP2a also has a lower affinity for most penicillins as well as cephalosporins providing protection against many additional beta-lactam antibiotics[47]. The gene encoding for PBP2a was identified as *mecA* and is located on the staphylococcal cassette chromosome *mec* (SCC*mec*), which is a genomic antibiotic resistance island, which can be integrated into the staphylococcal genome by recombinase enzymes[48], meaning it is mobile and transferable between resistant and sensitive strains[47], including transfer between *Staphylococcus* species[40]. The mecA gene is also widespread in coagulase-negative staphylococci[49]. More *S. epidermidis* isolates are now methicillin-resistant (MRSE) than *S. aureus* isolates. MRSA in urology is most often isolated from indwelling devices, mostly urinary catheters[50].

Enterococcus spp. are intrinsically resistant to many beta-lactams and cephalosporins due to the production of another low-affinity PBP (PBP5)[51]. Enterococci are also naturally resistant to clindamycin, fluoroquinolones, trimethoprim-sulfamethoxazole, and aminoglycosides and rapidly acquire resistance to chloramphenicol, erythromycin, rifampicin, nitrofurantoin and tetracyclines[52, 53]. Glycopeptides, which include vancomycin and teicoplanin, are important antibiotics for treatment of enterococcal infections. Glycopeptides inhibit cell wall synthesis and in particular, vancomycin prevents cross-linking of peptidoglycan chains which form the outer layer of the bacterial cell wall of gram-positive bacteria[53]. *E. faecium* isolates have a

higher prevalence of vancomycin resistance than *E. faecalis* isolates. The prevalence of vancomycin-resistant *E. faecalis* in Europe ranges from 0.0% - 4.4% with 3.6% of *E. faecalis* isolates in the UK being vancomycin resistant. 0.0% - 45.8% of *E. faecium* isolates in Europe are vancomycin resistant with 17.0% of *E. faecium* isolates in the UK being vancomycin resistant[36]. There are five phenotypes associated with vancomycin resistance in enterococci but VanA and VanB are the most commonly found in *E. faecium* and *E. faecalis*. The *vanA* and *vanB* clusters of genes coding for the respective phenotypes are both transferable between isolates and species as transposons. However, VanA is the more common phenotype as *vanB* genes must be transferred on larger mobile elements[53].

1.3.2.1.2 Major mechanisms of resistance by gram-negative organisms

Resistance to beta-lactams is an increasing trend also with gram-negative organisms and is most commonly mediated by the production of beta-lactamase enzymes[34]. Beta lactamases can be grouped according to their structure and function (Table 1-2).

Table 1-2 Classification of β-lactamase enzymes, the common enzyme types, and general antibiotics hydrolysed by each class. OXA: Oxacillin beta-lactamase, IMP: Imipenemase metallo-beta-lactamase, NDM: New Delhi metallo-beta-lactamase, VIM: Verona integron-encoded metallo-beta-lactamase

Groups according to Bush-Jacoby-Medeiros[34, 54, 55]	Ambler β-lactamase classes [56, 57]	Representative β -lactamases	Resistance to:
Group 1 Cephalosporinase	C	AmpC, CMY	Penicillins; 1st, 2nd, 3rd generations of cephalosporins, aztreonam, β-lactam inhibitors
Group 2 Serine- β-lactamase	A	TEM, SHV, CTX	Penicillins, and at least early generation cephalosporins
	D	OXA	Penicillins and oxacillins, some resistant to cephalosporins and carbapenems [58]
Group 3 Metallo-β-lactamase	B	IMP, NDM, VIM	penicillins, cephalosporins, and carbapenems, but not aztreonam [59]

The Bush-Jacoby-Medeiros classification scheme groups beta-lactamases functionally according to antimicrobial hydrolysis and susceptibility to beta-lactamase inhibitors. For example, Group 2 β-lactamases differ from Group 1

cepahlosporinases in that AmpC is not inhibited by clavulanic acid, whereas the Group 2 β-lactamase are, and will not hydrolyse oxacillins like the Class D β-lactamases[60]. The Ambler classification system organises β-lactamases according to molecular structure; for example, Ambler originally divided the β-lactamases into Class A (serine β-lactamases) and Class B (metallo β-lactamases (MBL)) based on the enzymes' amino acid sequences and that the Class B MBLs require zinc for its hydrolysis activity[56].

The Group 2 β-lactamases (Subgroup 2be) includes the extended-spectrum beta-lactamases (ESBLs), which specifically include TEM, SHV, and CTX-M from Enterobacteriaceae. Some OXA enzymes, such as OXA-11 largely isolated from *P. aeruginosa* also are considered ESBLs[58]. ESBLs are broadly defined as enzymes that hydrolyse penicillins, cephalosporins and are inhibited by β-lactamase inhibitors such as clavulanic acid and tazobactam[34].

Carbapenemase enzymes confer resistance to carbapenems in addition to penicillins and cephalosporins and include Group 2/Class D OXA enzymes such as OXA-23 and OXA-48, Group 2/Class A KPC enzymes and Group 3/Class B MBLs[34, 55]. The OXA – type carbapenemase hydrolysing enzymes have been mostly found in *Acinetobacter baumanii* isolates[61].

The Group 3/Class B MBLs are different from Group 1/Class A, D and Group 2 β-lactamase in that they are not inhibited by β-lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam, but are inactivated by EDTA and other metal chelators. One such MBL enzyme, the New Delhi metallo-beta-lactamase (NDM -1) was originally isolated in *K. pneumoniae* and *E. coli* isolates in the UK from patients returning from travel in the Indian subcontinent[59]. In November 2012 Health Protection Scotland confirmed their first NDM-1 *E. coli* isolated from a urinary tract infection from a patient that had no relevant travel history or medical care[62] highlighting the development of a native UK population. Furthermore, a study of 12,304 urine samples found two ESBL genes in 56% of isolates highlighting UTI as a major source of resistance genes[63].

1.3.2.2 **Microbial access**

Bacteria can access the catheter extraluminally or intraluminally. Extraluminal colonisation can result from two different routes. The first route occurs when the bacteria are pushed into the urinary tract by the catheter tip during insertion and the second is by which bacteria colonising the urethral opening can move along the catheter-mucosa interface by motility and growth[64]. Intraluminal contamination of the urinary catheter usually results from reflux of urine from a contaminated drainage bag [2]. Bacteria are able to move along the intraluminal catheter surfaces under flow conditions in *in vitro* models. Extraluminal colonisation is more common as colonisation by the intraluminal route is dependent on how often the catheter drainage system is compromised[65].

1.3.2.3 **Biofilm formation**

The majority of bacteria responsible for CAUTI are capable of forming a biofilm[66], which is a distinct mode of growth important in CAUTI pathogenesis. A biofilm is defined by its structure and function. Structurally, it is a community of microorganisms attached to an interface and surrounded by an exopolysaccharide matrix[67, 68]. The matrix contributes to biofilm persistence as it protects the bacteria from phagocytosis[69] and may facilitate horizontal gene transfer[70]. Other persistence strategies relate to the functional characteristics of the biofilm, such as recalcitrance to antimicrobials[71], and 'persister cells' that can repopulate the biofilm[72]. Recalcitrance and resistance to antimicrobials are the defining functional characteristic of a biofilm. Those bacteria buried within the matrix have less oxygen and nutrients available so an altered phenotypic state is switched on by stress mechanisms[70]. In this state, only essential processes remain active, and therefore many target sites for antibiotics are down-regulated [73]. Some populations in the biofilms may develop resistance, which differs from recalcitrance in that it results from genetic mutations caused by an increased state of hypermutability[74]. Therefore, to eradicate biofilm, 10-1,000 times the minimum inhibitory concentration (MIC) of antibiotics normally needed to inhibit the planktonic form is required[75]. These levels of antibiotics would be difficult to safely achieve systemically.

The formation of a biofilm is characterised by four major stages. The first stage is adhesion to a surface and the initial adhesion is reversible occurring as the result of nonspecific physiochemical attraction. Irreversible attachment is then mediated by specific interactions by flagella, fimbriae, and adhesins on the cell surface. Secondly, once irreversibly attached the bacterial cells multiply, aggregate, and secrete the exopolysaccharide to encase and form microcolonies. Thirdly, the microcolonies proliferate and mature. Finally, individual planktonic bacteria are dispersed from the biofilm to establish biofilm at other sites[76].

In regard to urinary catheters and preventing biofilm colonisation, attachment increases as surface roughness increases, therefore any modification to the catheter should not increase surface roughness[68]. Furthermore, urine can produce a conditioning film on the catheter surface[77], which can increase the ability of bacteria to attach to the material[78].

1.3.3 **Mineral encrustation**

All urinary catheter types are susceptible to mineral encrustation, which is a complication often resulting from crystal deposits on the catheter surface which can cause blockages[78]. Although build-up of cellular debris, such as cellular material from increased urothelial shedding, may also contribute to blockages. As previously mentioned, some bacteria produce a urease enzyme which hydrolyses the urea in the urine into ammonia and carbon dioxide. The production of ammonia raises the pH of the urine and a change to a more alkaline urinary pH can cause minerals in the urine to precipitate out of solution and deposit on the catheter surface[12]. The crystal deposits can occlude the catheter lumen or drainage eyelets causing reflux of urine to the renal calyces and/or bypassing or urine around the outside of the catheter[12, 79]. For some catheter users, their catheter may block as frequently as every 7 – 10 days and those patients termed as ‘blockers’ typically have more alkaline urine that catheter users that are ‘non-blockers’[80].

Urease-producing bacteria which commonly colonise urinary catheters include *Proteus* spp., *Providencia rettgeri*, *Morganella morganii*, *S. aureus*, *S. saprophyticus*, and *K. pneumoniae* [12]. Of these species, *P. mirabilis* is the one most commonly associated with encrustation and catheter blockage due

to the ability of its urease to hydrolyse urea 6-25 times faster than other ureases from other species[11]. Furthermore, the polysaccharide matrix formed by the *P. mirabilis* biofilm attracts crystals by binding calcium and magnesium ions[81]. The *P. mirabilis* biofilm in a urinary catheter is known as a crystalline biofilm in which the biofilm bacteria grow amongst crystals such as ammonium magnesium phosphate (struvite) and calcium phosphate. The structure is able to sustain crystallisation and encrustation of the urinary catheter.

1.3.4 **Treatment and management**

The first step in management and treatment of CAUTI is to confirm that it is CAUTI as opposed to catheter-associated asymptomatic bacteriuria, which is determined by the patient's clinical condition. NICE and SIGN guidelines currently do not recommend routinely prescribing antibiotics when changing long-term indwelling urinary catheters unless the patient frequently experiences CAUTI after catheter changes, or if there has been trauma during catheterisation[24, 82, 83]. The Infectious Diseases Society of America (IDSA) agrees that antibiotic prophylaxis is not recommended for routine short-term or long-term catheterisation[65]. If antibiotics are prescribed for symptomatic CAUTI, then the catheter should be changed before starting antibiotic therapy[24, 65].

IDSA and local Trust guidelines further recommend performing a urine culture and susceptibility testing before starting antimicrobial therapy, and replacing the catheter if it has been *in situ* for greater than two weeks and CAUTI is suspected[28, 65]. Furthermore, the duration of antibiotic treatment, if antibiotics are recommended, is seven days if the symptoms resolve quickly and 10-14 days if there is delayed resolution of symptoms[65]. Locally, it is recommended that women who respond rapidly and have had the catheter removed may require only three days of antibiotics. Choice of antibiotic should be guided by symptoms and reflect the antibiotic policy in place locally empirically, and then refined by the sensitivities if available.

Public Health England recommends nitrofurantoin, trimethoprim and pivmecillinam as first-line antibiotics for UTI depending on local sensitivities and kidney function. If first-line antibiotics are unsuitable amoxicillin can be

used if the causative organism is susceptible or fosfomycin if the patient is at risk of causative organisms with increased resistance[83]. Locally at NUHT first line treatment includes fosfomycin, nitrofurantoin and pivmecillinam on the basis that they can be given orally, are concentrated in the urinary system, and are likely to maintain activity against gram-negative organisms with increased resistance such as ESBL-producers[28].

1.3.5 **Burden of CAUTI and health economics studies**

According to the NHS Digital Safety Thermometer, between February 2017-2018 approximately 3.18% – 3.40% of patients seen by the NHS that year had a long-term urinary catheter and as of February, 2018 the national median was 13.54% per month of patients catheterised with a short term or long-term catheter. Of those patients with a catheter 4.80% - 5.72% had a urinary tract infection (CAUTI)[84]. Although there are no robust economic assessments of CAUTI available, the best figure available estimates the cost of CAUTI to the NHS to be £99 million each year with each CAUTI episode costing approximately £1968[2]. A more recent estimation from 2015 suggests that costs to the UK are approximately £1.0-2.5 billion[19]. Costs may also be influenced by the infecting organism where an episode of CAUTI caused by *E. coli*, staphylococci, or enterococci is significantly cheaper than infections caused by other Enterobacteriaceae, *Pseudomonas spp*. and yeasts[85]. CAUTI is the second most common cause of secondary bacteraemia and catheter-associated bacteraemic episodes have a seven day mortality rate of 30.1%[86]. In this study by Melzer and Welch, 16.7% of *E. coli* causing bacteraemic CAUTI resulting in death were ESBL producers[86].

Encrustation is an important part of the economic burden as one study found that frequent blockage due to encrustation affects 50% of long-term catheter users[87]. A study following 457 long-term catheter users showed that within a six month period 90 patients received emergency treatment in hospital, 111 patients received nursing night-referrals and 91 nursing day-time referrals for catheter blockage, all of which are associated with significant costs[88].

1.3.6 **Prevention of CAUTI**

Attention to aseptic technique, handwashing, personal protective equipment use, lubricant use to minimise trauma and infection, and cleaning the urethral meatus prior to catheterisation are recommended techniques that can help reduce CAUTI[2, 21]. Once the catheter is in place steps should be taken to ensure that the connection between the urinary drainage system and catheter valve remains sterile, urinary drainage bags should remain below the level of the bladder and not rest on the floor, the drainage bag should be emptied often to prevent reflux of urine, and the urethral meatus should be cleaned daily as part of personal hygiene[2, 21].

NICE Infection Prevention and Control quality standard (QS61) advises that the urinary catheter should be removed as soon as no longer needed as the risk of infection increases with the duration of catheterisation[21]. A systematic review and meta-analysis demonstrated that stop orders are an effective way of ensuring that the catheter is removed as soon as possible and subsequently reduced the rate of CAUTI[89]. This is mostly relevant for short-term catheter users as for many long-term catheter users urinary catheters are part of the long-term healthcare management plan and are essential to care and dignity of the patient. For those who require an indwelling urinary catheter, different commercial products including several antimicrobial urinary catheters have been developed in an effort to decrease CAUTI.

One such commercial product is Farco-fill Protect (Clinimed Ltd.), which is a 10.0 mL solution containing 0.3% triclosan packaged in a sterile syringe for inflation of the urinary catheter balloon[90]. The product is based on laboratory studies by Stickler in which catheter balloons inflated with 3g/L of triclosan were placed in a bladder model and inflated in a bladder chamber perfused with artificial urine (AU) and inoculated with communities of uropathogens. Their model showed that inflating the balloon of the catheter with triclosan prevented blockage by *P. mirabilis* for seven days[91] and was able to eliminate *E. coli*, *P. mirabilis*, and *K. pneumoniae* from the bladder model[92]. Its activity is governed by the diffusion of triclosan from the balloon and into the bladder[91, 92]. Case studies of use of Farco-fill Protect in

indwelling urinary catheter users who could be characterised as ‘blockers’ saw the life of each catheter increase and reduced the need for catheter maintenance solutions to wash out the catheter and bladder[93]. However, the maximum lifetime of the Farco-fill Protect is four weeks[90], which would not be suitable for a long-term catheter user.

Another commercial technology is UroShield (NanoVibronix), which exploits ultrasonic vibrations to prevent bacterial attachment to the urinary catheter[94]. The actuator is clipped onto the catheter itself above the balloon and drainage ports so it remains out of the body and is attached by wire to an external battery. The actuator generates surface acoustic waves which are transmitted directly to the urinary catheter to prevent biofilm formation. Animal studies show that this technology maintained urine sterility for 5-9 days compared to the controls which showed bacteria in the urine between 1-2 days. While it may delay biofilm formation for several days this is unlikely to be significant to long-term catheter users. Furthermore, the authors/inventors state that disruption of the acoustic waves renews bacterial attachment to the catheter so the device would need to be continuously active[95]. It also remains to be seen if the battery attached by wire would be acceptable to patients.

1.4 **Antimicrobial urinary catheters**

An ideal biomaterial should marry both efficacy and patient acceptability. For long-term urinary catheters this means that the antimicrobial or antiseptic urinary catheter should have activity against the major CAUTI causative organisms for the length of time the catheter remains in place (maximum 12 weeks). Importantly, if antimicrobials are integrated into the biomaterial they should be carefully considered to prevent the emergence of resistant bacteria. The protective activity should extend to both the outer and inner surfaces of the urinary catheter as well as the balloon. Any modifications to the urinary catheter should not adversely affect the mechanical properties, such as the inflation and deflation of the balloon. Any drugs or chemicals added to the urinary catheter should not be toxic and not cause irritation to the patient. Long-term catheter users generally have many additional co-

morbidities, and for ease of use for the patient it should mimic their standard care regime.

1.4.1 Approaches to making biomaterials antimicrobial

There are a variety of methods for giving biomaterials an antimicrobial advantage and the major mechanisms are detailed below.

1.4.1.1 Biomaterial nanostructures

Not all antimicrobial biomaterials technologies rely on incorporating drugs and chemicals into the biomaterial, and some anti-adhesion biomaterials have been designed by changing the structure of the material. For example, nanostructures on titanium surfaces, such as those used in hip and knee replacements have been investigated for their ability to resist bacterial adhesion. Puckett et al. demonstrated that nanorough titanium, which is titanium specifically manufactured by an electron beam, decreased adhesion by *S. aureus* and *P. aeruginosa*[96]. Hizal et al. demonstrated that nanostructured pillars on titanium worked synergistically with an antibiotic coating to reduce bacterial adherence by *S. aureus*[97]. Although titanium may not be a relevant material for urinary catheters, the same theory has been applied to black silicon, which is a synthetic silicon, in which nanoprotusions exert a bactericidal effect due to the mechanical stresses they exert on the cell wall and inner membrane[98]. This strategy is advantageous in that nanostructures will not generate antimicrobial resistance. However, if the structures aren't able to completely prevent bacterial adherence or eradicate attached bacteria it may be possible that the subsequent biofilm development will then cover the nanostructures rendering them useless. Furthermore, the black silicon surfaces are metallic and not polymeric like silicone and are unlikely to be useful for catheters.

1.4.1.2 Coatings

There is an extensive list of coatings which have been applied to biomaterials to prevent bacterial adhesion and biofilm formation. Of particular relevance is silver, which has been coated onto commercial catheters and wound dressings. Silver nanoparticles, ionic silver (Ag^{+}) and metallic silver (Ag^{0}), which can react with water in body fluids and become ionised, have

bactericidal activity[99], such as altering cell membrane permeability, inhibiting DNA replication, and causing irreversible damage to cells, although the exact mechanisms are still under investigation[100]. The variety of bacterial target sites means it is unlikely that bacteria are able to develop resistance to silver. However, the toxicity of silver nanoparticles and silver ions is poorly understood, it is universally agreed that there is some toxicity depending on the concentration and chemical composition[100]. Silver-coated catheters have not performed well in randomised controlled trials (RCT)[101, 102], with the authors speculating that the reason they do not perform well is that that silver is not released sufficiently, or the coating design is not optimal[103].

Cationic polymers such as quaternary ammonium have broad spectrum antimicrobial activity by interfering with ion exchange across the cell membrane resulting in compromised membrane integrity[104]. Another well-characterised polymer is poly(ethylene glycol), which is a well-hydrated polymer that prevents absorption of proteins and bacteria[105]. This is in addition to antibiotic coatings. One such coating is a hydrogel coating that incorporates gentamicin or polymyxin B into the hydrogel and is released in a pH-dependent manner. When bacteria attach and produce acid as a metabolic by-product the change in pH triggers the release of the antibiotic. This is advantageous as the antibiotics are released only in the presence of bacteria so they are conserved until needed. However, experiments under flow showed a significant reduction of live bacteria attached to the coated-antibiotic loaded samples, but it was not able to eradicate attached bacteria[106]. Generally coatings are often heterogeneous and mechanically weak[107].

1.4.1.3 Incorporation of antimicrobials into biomaterials

An alternative strategy for making biomaterials antimicrobial is to add the antimicrobial agent(s) directly into the material. One method is to mix the antimicrobial agent with the raw silicone before being milled and moulded into the device shape[108]. Post-manufacture impregnation of the biomaterial with the antimicrobial agent(s) is an additional approach in which the antimicrobials are incorporated into the biomaterial matrix. One such example of this are Bactiseal® hydrocephalus shunts and external

ventricular drains (EVD)(Codman Neuro, Depuy Synthes), which are impregnated with rifampicin and clindamycin. The impregnation process is beneficial as the antimicrobial molecules are evenly distributed throughout the silicone shunt and drain matrix[109].

1.4.2 **Experimental antiseptic urinary catheters**

Urinary catheters impregnated with gaseous nitric oxide, which has general antimicrobial properties due to its role in the inflammatory response, release nitric oxide over a 14-day period[110]. In laboratory studies the nitric oxide impregnated catheter eradicated *P. aeruginosa, E. coli* and *C. albicans* planktonic microorganisms in a suspension surrounding the catheter in 24-48 hours. Initial studies showed that it was safe in patients catheterised for 7-28 days[111]. The clinical efficacy of this catheter has yet to be evaluated. Regardless of its efficacy it does still not offer adequate protection for long-term catheter users.

Alternatively, urinary catheters coated in non-pathogenic *E. coli* have shown reduced colonisation of the catheter surface by *Providencia stuartii*, *E. coli*, *E. faecalis*, and *C. albicans*, but did not entirely prevent colonisation[112, 113]. This technology is based on the concept of bacterial interference in which the competition for nutrients, production of antimicrobial molecules, competition for attachment sites, disruption of biofilm, and disruption of virulence factor genes may be possible mechanisms of action for the prevention of colonisation by other organisms[114]. A RCT of inoculating this same non-pathogenic *E. coli* strain directly in the bladders of catheter users via their catheter reduced the likelihood of developing CAUTI. The bladder instillations were required twice daily for three consecutive days and the length of bladder colonisation after this regime varied between patients[115]. Patient acceptability and compliance will need to be investigated as well as cost-effectiveness due to the cost of nursing time for administering the bladder instillation.

Hydrogel-coated urinary catheters coated with a mixture of bacteriophage targeting *P. aeruginosa* and *P. mirabilis* reduced attached bacteria over 48 hours, but did not eradicate either organism[116]. This phage cocktail would not display any efficacy against other uropathogens due to their specificity.

Similarly, *S. epidermidis* bacteriophage pre-treatment of hydrogel-coated catheters reduced *S. epidermidis* attached at 24 hours[117]. However, the authors speculate that biofilm bacteria may be non-susceptible or have reduced susceptibility to phage infection due to the altered metabolic biofilm state[116].

Urinary catheters impregnated with rifampicin and minocycline demonstrated reduced bacteriuria for up to two weeks in post-prostatectomy patients, but did not demonstrate reduced symptomatic UTI (CAUTI)[118].

1.4.3 **Commercially available antiseptic urinary catheters**

Currently, the only commercially available antiseptic indwelling urinary catheter is BARDEX® I.C. Infection Control Foley catheters (Bard Medical). The BARDEX® I.C. catheter is composed of a silver-alloy coating with a hydrogel coating on a latex urinary catheter. The manufacturer states that this catheter can prevent infection by preventing bacteria from adhering to the catheter and thus preventing biofilm formation and subsequent CAUTI and bladder infection. The silver is chemically bound to the catheter surface and its release is mediated by a gold and palladium layer[119].

A second antimicrobial urinary catheter previously commercially available was ReleaseNF® Foley Catheters sold by Rochester Medical, but it was discontinued by the company in 2012. The ReleaseNF® catheter incorporated nitrofurazone into the catheter which was released in a controlled dose[120]. However, the same technology has been applied to intermittent catheters, which are commercially available as MAGIC$^{3®}$ Antibacterial Catheter and is produced by BARD CARE (Bard Medical).

Our research group has tested these commercial catheters and showed that the reported antibacterial activity assayed by multiple tests was short-lived. The silver-alloy catheter did not prevent bacterial migration, and did not prevent colonisation by *E. coli* after a single bacterial challenge. The nitrofurazone catheter delayed bacterial migration for up to 48 hours, did not prevent colonisation after a single challenge, and appeared to release nitrofurazone for only two days[121]. A Cochrane Review of urethral urinary

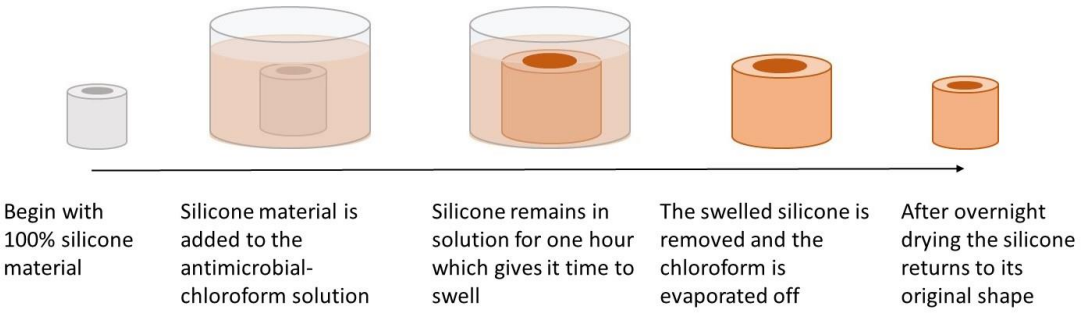
catheters demonstrated that the silver alloy catheter did decrease bacteriuria but not necessarily the number of cases of CAUTI. The same review concluded that the nitrofurazone–impregnated catheter may reduce CAUTI, but this was unlikely to make an impact on the clinic or patients[122]. This was further supported by the multi-centre RCT of the silver-alloy and nitrofurazone catheter compared to a standard polytetrafluoroethylene (PTFE) catheter, in which the silver-alloy catheters did not reduce CAUTI and the reduction by the nitrofurazone-impregnated catheter was small and the catheter was not recommended for clinical use [101]. Furthermore, the nitrofurazone catheters are vulnerable to blockage by *P. mirabilis*[123] as nitrofurantoin, a closely related antibiotic, has weak activity against this bacterium[124].

Therefore, the available antiseptic catheters are licensed and marketed are for short-term use only, and high-quality reviews and trials state that neither silver nor nitrofurazone catheters clinically reduced CAUTI rates. Furthermore, no existing commercially available technology addresses the problem of mineral encrustation and catheter blockage. There are no antiseptic urinary catheters available for long-term catheter users.

1.4.4 **Novel antimicrobial urinary catheter with long-term activity**

A process was previously developed by our research group in which antimicrobials could be impregnated into the silicone biomaterial itself[108, 109]. The antimicrobials are dissolved in chloroform, therefore the antimicrobials chosen must be chloroform soluble, and then the silicone catheter is added. The chloroform acts as a swelling agent allowing the antimicrobials to enter the silicone matrix as the silicone matrix swells. Then the chloroform is evaporated and the antimicrobials remain evenly distributed throughout the matrix[77](Figure 1-2).

Figure 1-2 Diagram of antimicrobial impregnation process



This process has been applied to hydrocephalus shunts and EVD (Bactiseal®) which are impregnated with rifampicin and clindamycin [109] and are commercially available. The antimicrobial-impregnated shunt and EVD are in use worldwide and have demonstrated an average reduction from 11.5% to 3.0% in infection rates at several institutions[125, 126]. Despite the increase in price of the antimicrobial impregnated shunt compared to a non-antimicrobial impregnated shunt, the shunt is cost-beneficial due to reduction of infection-related treatment costs[125, 127]. The antimicrobial-impregnated shunt is currently being evaluated nationally in the BASICS trial to examine its nationwide infection prevention rate and cost effectiveness compared to silver-coated shunts and all-silicone shunts[128]. Preliminary economic analysis demonstrates that the antimicrobial impregnated hydrocephalus shunt prevents 370 brain infections and 38 deaths each year in England saving the NHS £18.4 million annually[129].

The impregnation process has also been applied to continuous ambulatory peritoneal dialysis (CAPD) catheters, which are impregnated with rifampicin, trimethoprim, and triclosan[130] and are currently undergoing commercialisation. The antimicrobials are chosen based on the susceptibility of microorganisms responsible for infections of each device.

Recently this same process of antimicrobial impregnation was applied to silicone urinary catheters. The catheter is impregnated with a solution of 1.0% w/v triclosan, 0.2% w/v rifampicin and 1.0% w/v sparfloxacin achieving a total drug content of 0.17% w/w triclosan, 0.006% w/w rifampicin, and 0.16% w/w sparfloxacin in the catheter. The antimicrobial-impregnated urinary catheter (AUC) has demonstrated protection against colonisation by *P. mirabilis*, *S. aureus*, and *E. coli* for seven to twelve weeks. This long-term protective activity of the catheter is important and unique as there are no

antimicrobial catheters on the market offering long-term protection for long-term catheter users. Also of importance, the impregnation process did not negatively affect the mechanical properties of the catheter or of the balloon[77, 131].

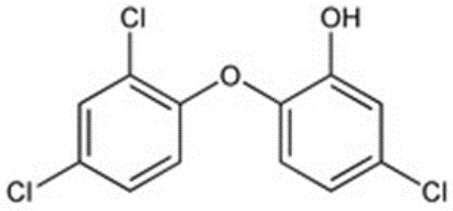
1.4.4.1 Selection of antimicrobials for impregnation of urinary catheters

Rifampicin, triclosan, and sparfloxacin were chosen for impregnation of urinary catheters as they are all soluble in chloroform and able to migrate through the silicone polymer. They were also chosen for their spectrum of activity against gram-positive and gram-negative uropathogens[77]. Three antimicrobials were selected so that at least two antimicrobials would be active against the majority of uropathogens to prevent the development of resistance in accordance with the principle of dual drug therapy. Dual drug therapy uses two antibiotics of two different classes to prevent emergence of resistance as the likelihood of bacteria developing two simultaneous mutations is greatly reduced[132].

1.4.4.2 Triclosan

Triclosan (5-Chloro-2-(2,4-dichlorophenoxy)phenol), is a broad-spectrum antimicrobial with activity against gram-positive bacteria, gram-negative bacteria, yeasts, and moulds[133, 134]. The structural formula can be seen in Figure 1-3 and its empirical formula is $C_{12}H_7Cl_3O_2$.

Figure 1-3 Structural formula of triclosan [134]



Its mechanism of action is that it interferes with fatty acid synthesis, specifically binding to enoyl-acyl carrier protein reductase (FabI), which is encoded for by the *fabI* gene[135]. Triclosan increases the affinity of FabI to NAD^+ (oxidized form of nicotinamide adenine dinucleotide) and this ternary structure of FabI, NAD^+, and triclosan is stable, preventing fatty acid synthesis[136]. Fatty acids are essential to cell membranes and as sources of

energy[137]. Furthermore, the majority of bacteria are regulated by the fatty acid synthetic (FAS) type II pathway, whereas mammalian cells are regulated by FAS type I pathway[138] therefore there is low risk of toxicity to human cells.

Due to its widely accepted safety profile, triclosan is commonly used in cosmetic and household products such as hand soaps, dishwashing soaps, toothpaste, face washes, body sprays, deodorants, shampoos for domestic animals, and makeup[133].

It also has many applications in the healthcare setting as well as a surgical scrub and as a handwashing agent for health care workers due to its gentleness on the skin and ability to exhibit continued antimicrobial activity on the skin[139]. One such product is Bacti-Stat (EcoLab) which is a 0.3% triclosan solution for handwashing by healthcare professionals, which has also been used to bathe infants before being admitted to a hospital nursing for MRSA decolonisation[140]. A 2% triclosan bath/shower in combination with nasal mupirocin has also been shown to reduce surgical site infection, and also the combination of the two drugs prevents mupirocin resistance by MRSA[141, 142].

Triclosan has also been applied to many experimental devices such as ureteral stents[143, 144], the balloons of urinary catheters[90], CAPD catheters, and sutures (Coated VICRYL Plus Antibacterial (polyglactin 910) sutures, Ethicon US, LLC)[145, 146].

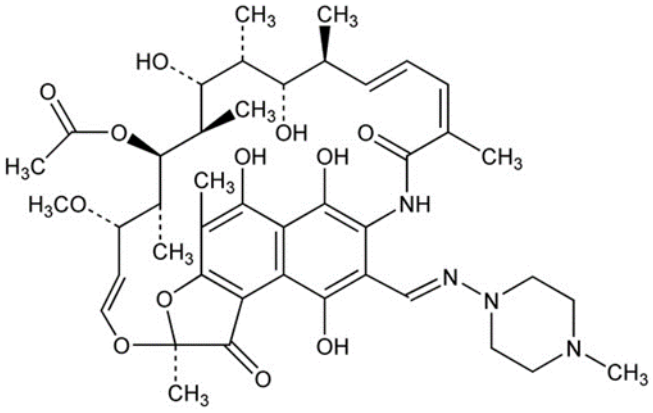
The safety of triclosan has been widely studied due to its widespread commercial use, and to reinforce its safety in surgery and medicine. The European Community Cosmetic Directive has approved a maximum concentration of 0.3% triclosan in cosmetic products and the European Commission Scientific Committee on Consumer Products determined that triclosan is not acutely toxic by oral administration or by dermal administration[147, 148]. However, the Federal Drug Administration in the USA ruled that consumer antiseptic wash products containing triclosan and 18 other antiseptics did not offer an advantage over nonbacterial active ingredients and were determined that their benefits did not outweigh the risks. However this ruling does not apply to what they refer to as ‘first aid antiseptics’, which include skin wound cleansers, skin antiseptics, and skin

wound protectants. Nor does it affect healthcare personnel hand washes, preoperative skin preparations, surgical hand scrubs, or medical devices[149].

1.4.4.3 **Rifampicin**

Rifampicin (3-[[(4-Methyl-1-piperazinyl)imino]methyl]rifamycin) is an antibiotic of the rifamycin class, particularly known for its activity against *Mycobacterium tuberculosis* but it also has activity against staphylococci and streptococci, *Neisseria* spp., *Legionella pneumophila*, *Listeria* spp., and *Haemophilus influenza*[150]. Its structural formula can be seen in Figure 1-4 and its chemical formula is $C_{43}H_{58}N_4O_{12}$[151].

Figure 1-4 Structural formula of rifampicin [151]



Its mechanism of action is that it forms a complex with the bacterial DNA-dependent-RNA polymerase (DNA-dep-RNA-pol) which interferes with RNA synthesis [152]. Specifically, rifampicin binds to the β-subunit of the DNA-dep-RNA-pol, which contains the active site for DNA to bind for production of mRNA. However, rifampicin does not bind to the active site, but binds adjacent to it thus physically blocking the synthesis of mRNA beyond 2-3 nucleotides, therefore preventing synthesis of amino acids and thus proteins [153]. Resistance to rifampicin occurs due to mutations in the *rpoB* gene affecting the DNA-dep-RNA-pol β-subunit preventing rifampicin from binding [154]. Rifampicin monotherapy is discouraged due to this single-point mutation which drives resistance.

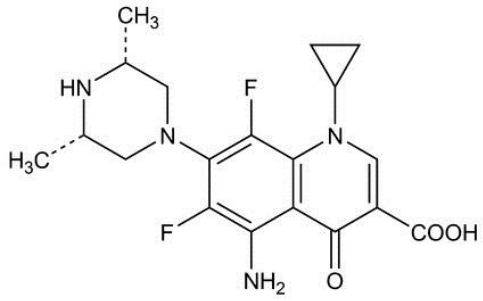
Rifampicin is an inducer of the liver enzyme cytochrome P450, which can accelerate the metabolism of other drugs, therefore there may be serious interactions with other medications[155]. Other adverse effects of rifampicin

include hepatotoxicity and renal dysfunction with extended exposure, and general flu-like symptoms. It is not a carcinogen, but may be a teratogen [156].

1.4.4.4 **Sparfloxacin**

Sparfloxacin (*el*-5-amino-1-cyclopropyl-7-[(3*R*,5*S*)-3,5-dimethyl-1-piperazinyl]-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid) is a fluoroquinolone antibiotic[157] with activity against staphylococci (including MRSA), streptococci, enterococci, Enterobacteriaceae, anaerobes, *Mycoplasma* spp., and intracellular pathogens such as *Chlamydia* spp. Its structural formula can be seen in Figure 1-5 and its chemical formula is $C_{19}H_{22}F_2N_4O_3$.

Figure 1-5 Structural formula of sparfloxacin [157]



Relative stereochemistry

Sparfloxacin has an advantageous pharmacokinetic profile with good tissue penetration and a long half-life in plasma and tissue; therefore less dosing is required[158]. It targets bacterial DNA synthesis, specifically DNA gyrase and topoisomerase IV, which are two enzymes involved with the supercoiling of DNA. Sparfloxacin, and other quinolones, form a complex with the gyrase/topoisomerase IV enzymes and DNA preventing DNA replication[159]. Resistance can develop by mutations in GyrA subunit of the DNA gyrase, mutations in parC subunit of topoisomerase IV, or as a result of decreased accumulation in the cell[159, 160]. Adverse events associated with sparfloxacin include photosensitivity (the most common event), gastrointestinal upset, prolongation of electrocardiographic QTc interval, and insomnia[161]. Due to the seriousness of some of these adverse events, the European Agency for the Evaluation of Medicinal Products withdrew approval for 100 mg strength sparfloxacin, but maintained approval for the 200 mg strength formulation. It was concluded that the benefits of 100 mg

sparfloxacin were outweighed by the risks at that concentration[162]. Sparfloxacin has been discontinued for sale in the USA[163]. However, studies of a latex urinary catheter coated with sparfloxacin found it to be biocompatible with green monkey kidney cells suggesting its safety in the urinary system[164].

1.4.4.5 **Safety of drug release from the AUC**

Despite the adverse effects of the three agents listed above, it is unlikely that the antimicrobials in the catheter will give rise to any local or systemic effects as the concentration of the antimicrobials in the catheter and released from the catheter are less than achievable plasma levels from therapeutic doses. 0.006% w/w (0.49 mg) rifampicin, 0.17% w/w (14.00 mg) triclosan, and 0.16% (13.25 mg) w/w sparfloxacin is impregnated into the urinary catheter[77, 131]. Over 28 days drug release studies showed that the amount of rifampicin released was undetectable, 2.6 mg of sparfloxacin was released, and 4.2 mg of triclosan was released[77]. Triclosan is approved for use in the European Union at a maximum concentration of 0.3%[165] and the total drug content of triclosan in the entire catheter is 0.17% w/w. Sparfloxacin is released from the AUC at approximately 1.25 μg/mL/day[77] and a peak serum concentration of sparfloxacin when given intravenously at 40 mg/kg is 12.42 μg/mL. The amount released per day from the catheter equates to the serum concentration after one oral dose and it is envisaged that approximately half of the antimicrobials that are eluted will be released into the urine and rinsed away. The low levels of drug released are advantageous for patient safety, and also do not affect the antimicrobial activity as the levels at the catheter surface are high and likely to be above the majority of bacterial MICs. The antimicrobials do not need to be released, but rather be retained at the catheter surface because they are protecting the catheter not the patient.

1.5 **Aims**

Initial characterisation of the antimicrobial activity of the catheter has previously been assessed. Therefore, to expand on the initial work with the goal of commercialising this antimicrobial catheter for use in the clinic by long-term catheter users, the overall aims of this project were to:

1. Characterise the microorganisms colonising the surfaces of conventional urinary catheter lumens and relate this to patient clinical details. This will inform/validate the design of the catheter as the bacteria responsible for biofilm formation and symptom presentation could be different from those cultured from urine samples.
2. Investigate the ability of the AUC to prevent mineral encrustation. Currently, no catheter is commercially available that can prevent, reduce or delay mineral encrustation, a problem that is common to longterm catheter users.
3. Assess the antimicrobial spectrum against multi-drug resistant (MDR) CAUTI pathogens. Unfortunately, as the prevalence of MDR pathogens is increasing worldwide, it is therefore increasingly important to assess the antimicrobial activity of this catheter against MDR strains.
4. Plan and execute a safety clinical study and patient acceptability study. The clinical study recruited 60 long-term catheter users who received the AUC to determine if any adverse events were associated with the catheter. To plan for a future RCT, there was a component of assessing the feasibility of a larger trial.

This thesis consists of varied methods and approaches with the overall aim of producing and validating an antimicrobial urinary catheter that is ready to be CE-marked for use by long-term catheter users.

Chapter 2. Investigation of microorganisms attached to the lumens and balloons of indwelling urinary catheters

2.1 Introduction

The majority of data on causative bacteria of both CAUTI and ASB are derived from urine samples (though these are discouraged in catheterised patients) and little is known about the bacteria that attach on to the catheter surface[166]. As the mechanism of action of the AUC is that it kills attached bacteria it is important to verify whether microorganisms attached to the catheter correlate with urine culture studies. Furthermore, it is well established that 100% of urinary catheters in place for 28 days[2] or greater will be colonised with bacteria, and a longitudinal study of consecutive catheters over a year may offer insight on the evolving microbial environment of the urinary catheter and how this may influence symptom development.

2.1.1 Previous studies of urinary catheter biofilms

Three studies[66, 167, 168] have examined the contents of the urinary catheter surfaces using different methods for culturing the attached organisms. Matsukawa et al. analysed the intraluminal contents of urinary catheters by cutting open the luminal space and swabbing a 5cm long segment[168]. Djeribi et al. analysed the inner contents of catheters by sterilising the outer surface of the catheter, cutting the catheter into discs and placing these on blood agar. Biofilm forming ability of the organisms was visualised by scanning electron microcopy (SEM) by immersing silicone discs into a solution of the isolated bacteria, and semi-quantitatively by crystal violet biofilm assay[167]. A study by Holá et al. used sonication to remove attached bacteria into surrounding broth, but could not differentiate between organisms on both the outer and inner lumen surfaces as the whole catheter was sonicated in broth[66]. Likewise, Sherertz et al. sonicated 1,681 central venous catheters in broth and concluded that sonication provided a high resolution in terms of identifying the number of organisms and different species, but could not differentiate between inner and outer colonisation[169].

The study described here avoids the above limitations and aims to provide more detailed and accurate data on the colonisation of the catheter lumen and balloon, and biofilm formation in situ. This study will also add to the existing literature by relating the catheter culture and catheter specimen urine results to patient-specific information such as symptoms, antibiotic usage, and mineral encrustation. A longitudinal catheter collection study has not been performed to date and this will examine the catheter microbiota and assess the relationship of the colonising organisms over time.

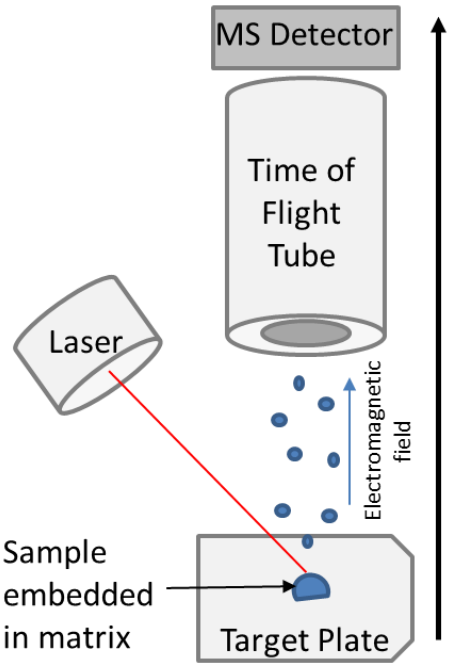
Identification of the attached microorganisms can be performed by conventional and molecular microbiological techniques. Conventional culturing, although often time-consuming, is a very sensitive, low-cost method of identification. For identification of difficult to identify organisms, matrix-assisted laser desorption/ionisation time of flight (MALDI-ToF) mass spectrometry is an additional fast and accurate method of identification[170]. Pulsed-field gel electrophoresis (PFGE) is a sensitive method of determining the relatedness of isolates.

2.1.2 MALDI-ToF

MALDI-ToF analysis is a technically simple, rapid, accurate and inexpensive method of bacterial and fungal identification relying on differences in cellular proteins of microorganisms[171]. Specifically, the proteins analysed by MALDI-ToF are mainly ribosomal proteins[170]. However, for some bacteria such as mycobacteria, corynebacteria, and *Acinetobacter spp*. they are highly genetically similar and species identification with MALDI-ToF is difficult[172]. All identification is dependent on the database of reference spectra[173].
For use in microbiological identification the subcultured organism is placed onto the target plate surface with the matrix solution, which is energy absorbent. The matrix co-crystallises with the sample so that the microorganism is embedded within the matrix. The target plate is placed in the mass spectrometer where a vacuum is established. The sample, including the matrix, is vaporised by a laser which ionises the structural proteins of the sample so that one proton is added to the protein forming protonated ions[170]. The matrix assists in absorbing some of the photo-energy as heat and releases this heat to the sample so it sublimes forming a gas phase of ions. The ions are

accelerated into a flight tube by an electromagnetic field. The time of flight of the ions to reach the detector at the end of the tube is measured. The time of flight of analytes is determined by the mass to charge ratio and degree of ionisation[172, 173] (Figure 2-1).

Figure 2-1 Diagram of the essential components of MALDI-ToF mass spectrometry



Each species has a unique mass spectrum and the generated spectrum can be compared to a database of known recorded spectra. As there may be variations due to noise within the equipment the identification of the isolate comes with a score. A score above 2.0 indicates that the identification is valid at a species level. A score between 1.7-2.0 indicates that the identification is valid at the genus level[173]. MALDI-ToF has more recently been used to investigate outbreaks and screening of emerging clones, including *E. coli* and staphylococci[174, 175].

2.1.3 **Pulsed-field gel electrophoresis**

Electrophoresis relies on electricity to induce DNA molecules to migrate through a gel matrix, where smaller DNA molecules are able to move through the gel more easily and separation based on DNA size can be achieved. However, DNA segments of approximately 20 kilobase pairs (kbp) and larger are able to migrate only so far and then the DNA molecules remain indistinguishable. Separation by PFGE is achieved by the reorientation of the

DNA molecules with a a reorientated electrical field. Larger DNA molecules take longer to realign with the new field and therefore spend more time reorientating than migrating and separation is achieved in this manner[176]. Restriction enzymes that cleave the DNA infrequently digest the chromosomal DNA so the whole genome can be analysed. Due to the ability of PFGE to separate whole bacterial genomes, it has been used in strain typing and epidemiological studies and has the ability to determine the relatedness of isolates[177].

2.1.4 Chapter aims

This study aimed to examine removed indwelling urinary catheters for the microorganisms attached to the lumens and balloons of the catheters, with the balloon contents being representative of the bladder environment. A method was developed for this purpose. The microbiological findings were related to clinical details such as antibiotic use, CAUTI symptom presentation, and CSU results. The same method was used to examine the attached organisms to the balloons and lumens of consecutive indwelling urinary catheters collected from a single patient over one year to determine changes to the catheter microenvironment over time.

2.2 Materials and Methods

2.2.1 Urinary catheter collection

2.2.1.1 Catheter collection at NUHT

Between 5 June 2015 and 28 July 2016 indwelling urethral urinary catheters were collected from patients at NUHT, Nottingham, UK. Indwelling urethral urinary catheters that remained in situ for greater than ten days from male and female patients over 16 years of age were collected by the clinical staff according to clinical need. Clinical staff were provided with instructions on the inclusion criteria for catheters and an audit form to accompany the removed catheter. Clinical staff were instructed to remove the catheter according to standard local protocols and if possible, drain, but not flush, the lumen of any residual urine. The catheters were then placed in a resealable freezer bag and stored in the specimen fridge with an anonymous patient identifier and the

audit form containing information about antibiotics received and current symptoms of CAUTI. The catheters were collected within 24 hours of being placed in the specimen fridge and transported for laboratory analysis.

An audit to retrospectively review CSU results during the period of catheterisation of the 61 patients of whom had catheters included in this study was approved by Nottingham University Hospitals NHS Trust (Audit ID: 17-309Q) in December 2017. CSU results from this period were obtained from microbiology reports available on a hospital-wide information system, NotIS (Nottingham Information System).

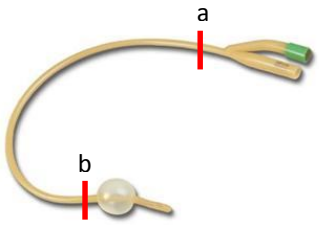
2.2.1.2 Consecutive collection of catheter in the community from one volunteer

The volunteer who agreed to have his catheters collected over the course of the year, had the indwelling catheters changed at home every six weeks as per his standard care. The catheter was removed by the district nurse and placed in a resealable freezer bag. Transportation was arranged to deliver the catheter to the laboratory for analysis within two hours of the catheter removal. Details of antibiotic and antifungal use and episodes of CAUTI over the course of the year was provided by the volunteer.

2.2.2 Sampling of microorganisms attached to the lumens and balloons

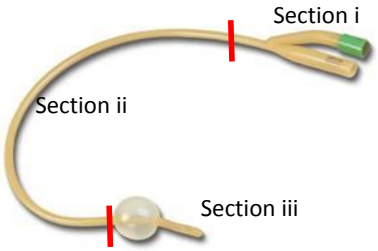
Upon arrival in the laboratory, the catheter was removed from the specimen bag using sterile forceps and placed onto a sheet of aluminium foil sterilised by autoclaving at 121°C for 15 minutes. Stainless steel, straight-jawed surgical clamps clamped the catheter on the lumen before the drainage and balloon inflation ports (point ‘a’ in Figure 2-2) and on the lumen after the balloon (point ‘b’ in Figure 2-2).

Figure 2-2 Part 1 method demonstration of processing of collected urinary catheters. Collected indwelling urinary catheters were clamped using sterile surgical straight-jaw clamps at points 'a' and 'b' to prepare the catheter for analysis of lumen and balloon contents.



The balloon (section iii in Figure 2-3) was separated from the lumen, the interior section of tubing, of the catheter using a sterile scalpel (Swann-Morton, Sheffield, UK) and placed into a sterile universal container (Sterilin, UK). A 1.0cm section was cut away from the base of the balloon section and placed in 2.0 mL of acetone and refrigerated for future scanning electron microscopy (SEM). The drainage and balloon inflation ports (section i in Figure 2-3) were separated from the lumen and discarded. This left the lumen section (section ii in Figure 2-3) with its ends clamped.

Figure 2-3 Part 2 method demonstration of processing of collected urinary catheters. The red lines indicate the section of the catheter previously clamped. Using a sterile scalpel the catheter was cut into three sections after the clamps to separate the balloon and ports from the catheter tubing. Section I, the ports, were discarded, Section ii, the catheter tubing, remained clamped, and Section iii, the balloon tip, was placed separetely in a sterile universal container.



Opening both ends of the clamps briefly, 1-2 mL phosphate buffered saline (PBS, Oxoid, UK) filled the lumen depending on the size of the catheter. The ends were quickly reclamped and placed into a resealable freezer bag. PBS was added to the universal container so that it covered the entire surface of the balloon section. The balloon section and catheter lumen were sonicated for five minutes at 30 kHz to detach the bacteria into the surrounding PBS (the sonicate). After sonication, the catheter lumen was removed from the resealable freezer bag and both ends were cleaned with an alcoholic pre-injection swab (Steret, Molnylycke Healthcare, Göteburg, Sweden). The lumen sonicate was then drained into a sterile Bijou bottle (Sterilin). 200µL of the

balloon and lumen sonicates and appropriate dilutions were pipetted onto cystine-lactose electrolyte deficient (CLED) agar (Oxoid) and incubated overnight in air at 37°C. If culture positive, the colonies were quantified and general microbiological identification performed. If culture negative, the plates were incubated for a further 24 hours.

After plating the lumen sonicate, its pH was determined using pH indicator strips (spectral 5.0-9.0, Hydrion, Micro Essential Laboratory, New York, USA). A catheter lumen was considered blocked if it was not possible to insert 1-2 mL of PBS required for sonication without breaking up the existing occlusions.

2.2.3 Identification of microorganisms isolated from the balloons and lumens

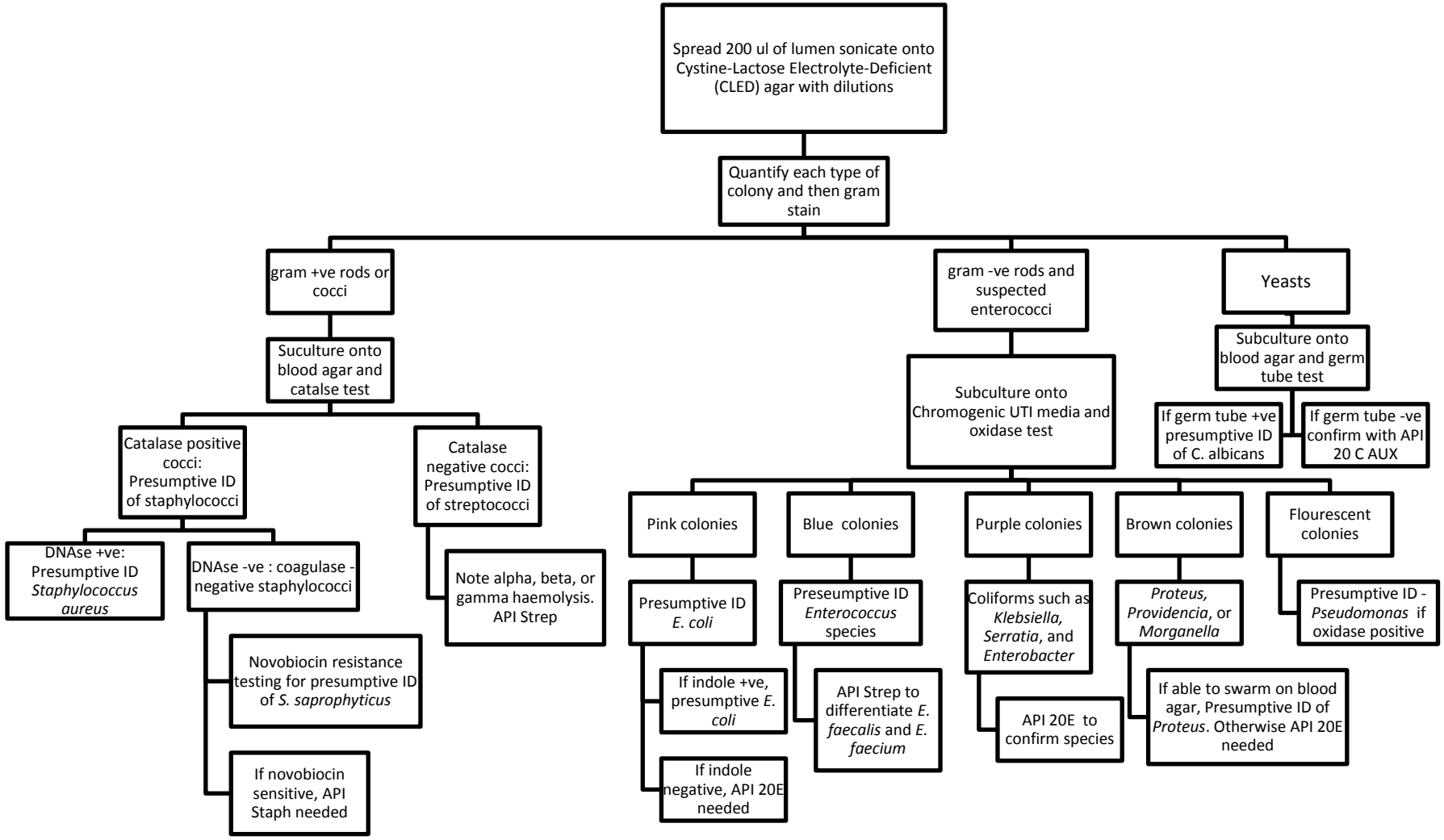
When culture positive on CLED, different colony types (if more than one) were quantified by counting and subcultured onto the appropriate agar according to the algorithm in Figure 2-4. Gram-stain, catalase and/or oxidase test, and subculture were performed for all colony types isolated from both the lumens and balloons. If there were organisms on both the lumen and balloon with the same gram-stain, catalase and/or oxidase test, and colony morphology and API was needed to confirm the species, only the isolate from the lumen was used to identify the isolate. Chromogenic UTI agar (Oxoid) is a differential medium that can presumptively identify the main uropathogens. A single colony of the gram-negative organisms was streaked onto Chromogenic UTI medium and incubated overnight at 37°C.

2.2.3.1 Indole test

Pink colonies on Chromogenic UTI medium are presumptive for the identification of *E. coli*. To confirm identification, an indole test was performed according to Cowan and Steel's Manual[178]. Briefly, two colonies were picked from a well-isolated plate and emulsified in TSB overnight at 37°C. After incubation, approximately 1.0 mL of xylene was added to the bacterial suspension and gently agitated to separate the indole into the xylene layer. Approximately 0.5 mL of Ehrlich's reagent was gently pipetted down the side of the bottle. The production of a pink colour in the xylene layer was a positive

reaction. For isolates that were indole-negative they were identified with API 20E and if identified as *E. coli*, the indole test was repeated with the isolates incubated at 30°C instead.

Figure 2-4 Algorithm to identify microorganisms isolated on CLED agar from lumens and balloons



2.2.3.2 Germ tube assay for *Candida* spp.

A germ tube test was performed for presumptive *Candida* spp. A single colony was emulsified in 0.5 mL of human serum and incubated for two hours at 37°C. 10 µL of the suspension was placed on a microscope slide, covered with a coverslip, and examined under the microscope at 400x magnification for the presence of germ tubes. The presence of germ tubes presumptively identified the isolate as *Candida albicans.* To confirm identification and for germ tube negative isolates API 20 C AUX was used.

2.2.3.3 Adonitol broth fermentation

The API 20E database is unable to differentiate between *Citrobacter koseri* and *Citrobacter amalonaticus* and thus provides an identification of *Citrobacter koseri/amalonaticus*. To differentiate between the two an adonitol broth was prepared (Table 2-1) and adjusted to a pH of 7.4 ± 0.2 using 1M sodium hydroxide.

Table 2-1 Adonitol broth recipe

Chemical	Amount (g/L)
Tryptone (Oxoid)	10.0
Sodium chloride (Fisher Scientific, Loughborough UK)	5.0
Phenol Red (Sigma-Aldrich St. Louis, Missouri, USA)	0.018
Adonitol (Sigma)	10.0

The formulation for the broth is based on the commercial preparation by Thermo Fisher Scientific. The adjusted broth was filter-sterilised through a 0.2 µm cellulose nitrate membrane (Sartorius Stedim Biotech) into an autoclaved container.

5.0 mL of the adonitol broth was aseptically transferred to a sterile universal container. To inoculate the broth several well-isolated colonies 18-24 hours old were transferred to the broth. This included an adonitol fermentation positive control of a known *Citrobacter koseri* (F3983) isolate verified by MALDI-ToF and an adonitol-negative control of *E. coli* (F4247). The inoculated tubes and an uninoculated control were incubated overnight at 37°C aerobically with the lids of the universal containers slightly loosened. A positive

result was a change of the colour of the broth to yellow. A negative result was no change of the colour or reddening of the broth colour.

2.2.3.4 MALDI-ToF MS

Isolates that were difficult to identify using conventional methods were identified by MALDI-ToF (Microflex LT Mass Spectrometer, Bruker Daltronics) at Sheffield Teaching Hospital NHS Foundation Trust. As the facilities were not available locally at this time for uniform MALDI-ToF identification of all isolates, only those that were difficult to identify by biochemical testing were transported to Sheffield. The isolate of interest was swabbed from a fresh (18-24 hours old) pure culture of the isolate on blood agar plate and placed in a sterile transport swab (Sterilin Ltd) for transport. Isolates were identified using MALDI Biotyper RealTime Classification (Bruker Daltronics) software.

2.2.3.5 Susceptibility testing of microorganisms isolated from the balloons and lumens

All isolates were tested for resistance according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines[179, 180]. All Enterobacteriaceae were screened for ESBL production by disc diffusion using cefpodoxime (10 µg), ceftazidime (10 µg), ceftriaxone (5 µg) discs (Oxoid) on Mueller-Hinton agar. If the isolate was resistant or intermediate to ceftazidime or ceftriaxone, or resistant to cefpodoxime the isolate was confirmed for ESBL production. The method for confirmation testing is determined by species[179]. Confirmation testing of Group 1 Enterobacteriaceae, which includes *E. coli, Klebsiella spp.*, and *P. mirabilis,* was carried out using the gradient test method using both cefotaxime/cefotaxime + clavulanic acid (CT/CTL) and ceftazidime/ceftazidime + clavulanic acid (TZ/TZL). For the Group 2 Enterobacteriaceae, which includes *Enterobacter spp., Morganella morganii*, and *Serratia spp.*, and *Pseudomonas spp*. ESBL production was confirmed by a combination disc diffusion assay. A cefepime (30 µg) disc and a cefepime + clavulanic acid (30 + 10 µg) disc were placed apart on a bacterial lawn on Mueller-Hinton agar and incubated overnight at 37°C. If the zone of inhibition is ≥ 5mm for the disc with clavulanic acid compared to cefepime alone, the test is positive.

Isolates that were resistant to one of the screening antibiotics were also then screened for carbapenemase resistance using a meropenem (10 µg) disc (Oxoid) as it has the best balance of sensitivity and specificity for detecting carbapenemase resistance.

All staphylococci isolated were tested for methicillin resistance by disc diffusion assay using a cefoxitin (30 µg) disc according to the EUCAST disc diffusion method[180].

All enterococci were screened for vancomycin resistance by disc diffusion using a 5 µg vancomycin disc on Mueller-Hinton agar according to the EUCAST disc diffusion method. If resistant according to disc diffusion, resistance was confirmed using MIC gradient method (Etest, Oxoid).

2.2.3.6 **Light microscopy**

A wet mount of the lumen sonicate was prepared and examined under light microscopy for the presence of crystals and other structures. After processing the catheters for microbiological isolation, 10µL of the lumen sonicate was placed on a microscope slide, covered with a coverslip, and examined for crystals under the light microscope at 200x, 400x, and 1000x magnification if necessary.

2.2.4 **Pulsed-field gel electrophoresis**

The make-up of buffers used for PFGE can be found in Table 2-2. A stock solution of 0.5M EDTA (dibasic ethylenediaminetetraacetic acid, Fisher Scientific) adjusted to pH 8.0 using sodium hydroxide pellets (Fisher Scientific) in molecular biology grade water (MBGW) (VWR International Ltd) and 1.0M Tris (Ultrapure Tris, MP Biomedicals LLC, Illkirch France) adjusted to pH 8.0 using 1M hydrochloric acid (Fisher Scientific) were prepared for use in making certain buffers described in Table 2-2 below. They were autoclaved after pH adjustment.

Table 2-2 Composition of buffers and manufacturer details for use in PFGE experiments . MBGW: molecular biology grade water, EDTA ethylenediaminetetraacetic acid

Buffer	Components	Manufacturer Details
Cell suspension buffer	100mM Tris: 100mM EDTA, pH 8.0 in MBGW	1.0M Tris, pH 8.0 and 0.5M EDTA, pH 8.0 stock solutions diluted in MBGW (VWR)
TE buffer	10mM Tris, 1mM EDTA, pH 8.0	BioUltra Tris-EDTA buffer solution for molecular biology, pH 8.0, Sigma Aldrich
10X TBE buffer	1M Tris, 0.9M boric acid, and 0.01M EDTA	Ultrapure TBE Buffer 10X (Invitrogen)
0.5X TBE running buffer	10X TBE diluted in MBGW	Ultrapure TBE Buffer 10X diluted in MBGW
Cell lysis buffer	50 mM Tris, 50 mM EDTA, pH 8.0 + 1% N-laurylsarcosine, sodium salt (Sarcosyl)	1.0 M Tris, pH 8.0 and 0.5M EDTA, pH 8.0, and 10% Sarcosyl (Sigma-Aldrich) stock solutions diluted in MBGW
Proteinase K solution	20 mg/mL Proteinase K in MBGW	Proteinase K from *Tritirachium album* (Sigma-Aldrich)
Proteinase K/cell lysis buffer	50 mL cell lysis buffer, 250 uL 20 mg/mL proteinase K	
Lysozyme solution	20 mg/mL lysozyme in TE buffer	Lysozyme from chicken egg white (Sigma-Aldrich)
Lysostaphin solution	1.0 mg in 2.0 mL MBGW	Lysostaphin from *Staphylococcus staphylolyticus* (Sigma-Aldrich)

The PFGE protocol was modified for analysis of gram-negative bacteria and gram-positive bacteria. An additional isolate of each species isolated from catheters, not from the volunteer, was also analysed as a control to show if PFGE is a sensitive enough technique to detect differences.

2.2.4.1 **Gram-negative cell lysis and restriction for PFGE**

Gram-negative bacteria were subjected to PFGE according to the PulseNet protocol for *E. coli*, *Salmonella*, and *Shigella*[181] with some modification. Bacteria were grown overnight at 37°C on blood agar. Using a swab moistened with MBGW, several colonies were removed from the plate

and dispersed in 2.0 mL cell suspension buffer. The bacterial suspension was adjusted to an optical density between 0.8 -1.0 at 610nm. 20.0 µL of 20 mg/mL proteinase K stock solution was added to 400 µL adjusted bacterial suspension and then gently mixed with 400 µL of cooled 1% Seakem Gold agarose (Lonza, Basel, Switzerland). The agarose/proteinase K/cell suspension mixture was pipetted into disposable plug moulds (Bio-Rad, Hercules, California, USA) in duplicate and allowed to solidify in the refrigerator.

Plugs were lysed in 5.0 mL in proteinase K/cell lysis buffer at 55°C for two hours with shaking at 150 rpm. After lysis, the buffer was removed and replaced with warm (55°C) MBGW and incubated for 10 minutes at 55°C with shaking at 150 rpm. This was repeated twice more with MBGW and then three additional times with TE buffer.

Plugs were cut into 2.5 mm wide slices using a sterile scalpel and placed into 200 µL 1X pre-restriction buffer solution (SuRE-Cut™ buffer H, Roche) and incubated at room temperature for 10 minutes. After incubation, the buffer was removed and replaced with 200 µL of the *XbaI* restriction enzyme master mix (Table 2-3) and incubated for two hours in a 37°C water bath.

Table 2-3 Composition of *XbaI* restriction enzyme master mix for use in PFGE of gram-negative bacteria

XbaI Master Mix	
Reagent	**µL/plug slice**
MBGW	173
10X Buffer H	20
Bovine serum albumin	2
XbaI (10U/µl)	5

2.2.4.2 ***Staphylococcus aureus*** **cell lysis for PFGE**

Bacteria were grown overnight at 37°C on blood agar. Using a swab moistened with MBGW, several colonies were removed from the plate and dispersed in 3.0mL cell suspension buffer. The bacterial suspension was adjusted to an optical density between 0.8 -1.0 at 610nm. 1.0mL of the cell suspension was transferred to a 1.5mL microcentrifuge tube and centrifuged at 13,000 rpm for one minute. The supernatant was discarded and the pellet was re-suspended in 500 µL cell suspension buffer. The cell suspension was again

centrifuged at 13,000 rpm for one minute, the supernatant discarded, and the pellet was suspended in 200 µL cell suspension buffer. 10 µL of the lysostaphin solution was added per tube and gently mixed with 200 µL of 1% Seakem Gold agarose. The agarose/enzyme/cell suspension mixture was pipetted into disposable plug moulds and allowed to solidify. The plugs were transferred to 5.0mL cell lysis buffer with 25 µL lysostaphin for the staphylococci and incubated for one hour at 37°C. After incubation, 100µL of 20 mg/mL proteinase K solution was then added to the cell lysis buffer and incubated for two hours at 55°C with shaking at 150 rpm. The plugs were washed in MBGW for three washes and TE buffer for three washes at 55°C for 10 minutes each.

2.2.4.3 *Enterococcus faecalis* cell lysis for PFGE

Enterococci were subjected to PFGE according to the PulseNet protocol for PFGE of *Listeria monocytogenes*[182] with some modification. Bacteria were grown overnight at 37°C on blood agar. Using a swab moistened with TE buffer, several colonies were removed from the plate and dispersed in 2.0mL TE buffer. The bacterial suspension was adjusted to an optical density between 0.8 -1.0 at 610nm. 20 µL of lysozyme stock solution was added to 400 µL adjusted bacterial suspension and incubated at 37°C for 20 minutes and then at 55°C for 10 minutes. 20 µL of proteinase K stock solution was added to 400µl cooled 1% Seakem Gold agarose. The agarose/cell suspension/lysozyme/proteinase K was cast into disposable plug moulds and allowed to solidify. The solidified plugs were transferred to 5.0mL cell lysis/proteinase K buffer and incubated at 55°C for two hours with shaking at 150 rpm. The plugs were washed in MBGW for two washes and TE buffer for four washes at 55°C for 10 minutes each.

2.2.4.4 *Staphylococcus* and *Enterococcus* restriction for PFGE

Plugs were cut into 2.5mm wide slices using a sterile scalpel and placed into 200 µL 1X pre-restriction buffer solution (SuRE-Cut™ buffer A, Roche) and incubated at room temperature for 10 minutes. After incubation, the buffer was removed and replaced with 200 µL of the *SmaI* restriction enzyme master mix (Table 2-4) and incubated for two hours in a 30°C water bath.

Table 2-4 Composition of *SmaI* restriction enzyme master mix for use in PFGE of gram-positive bacteria

SmaI Master Mix	
Reagent	**µL/plug slice**
MBGW	170
10X Buffer A	21
Bovine serum albumin	2
SmaI (10U/µl)	8

2.2.4.5 **Running the PFGE Gel**

After restriction, the restriction enzyme mix was replaced with 200 µL 0.5X TBE buffer and incubated at room temperature for five minutes. All samples were run in duplicate. The plug slices were cut to fit the wells using a sterile scalpel and placed into the wells of a 15 well 1% Seakem Gold agarose gel prepared in 0.5X TBE buffer. Slices of one small graduation from the Lambda PFG Ladder (GelSyringe, New England Biolabs, Ipswich, Massachusetts, USA) were cut using a sterile scalpel and placed every four-five lanes into the gel. The tops of the wells were then sealed with 1% Seakem Gold agarose cooled to 48°C.

Electrophoresis was performed in 0.5X TBE buffer cooled to 14°C in a CHEF-DRII apparatus (Bio-Rad). The conditions of the electrophoresis run can be found in Table 2-5

Table 2-5 PFGE running conditions for CHEF-DRII apparatus

	Set 1	**Set 2**
Run Time	10 hours	13 hours
Initial Switch	5.0 seconds	15.0 seconds
Final Switch	15.0 seconds	60.0 seconds
Voltage	200V	200V

PFGE gels were stained for 30 mins in a 1 µg/mL ethidium bromide solution and then rinsed twice for 30 mins in distilled water. Gels were photographed using a UV transilluminator (BioDoc-It Imaging System, Ultra-Violet Products Ltd, Cambridge, UK). Banding patterns were compared with GelCompar II software (v6.6 Applied Maths, Saint-Martens-Latem, Belgium).

Variability was determined by the Dice's coefficient and isolates were clustered according to the unweighted pair group average method (UPGMA).

2.2.5 Statistical analysis

Data were analysed in GraphPad Prism 7.01. Normality was assessed by histogram, and some data not distributed according to Gaussian were transformed to a logarithmic scale. Correlation was assessed using the Pearson correlation. Significance was defined as $p<0.05$ and was calculated by unpaired t-test.

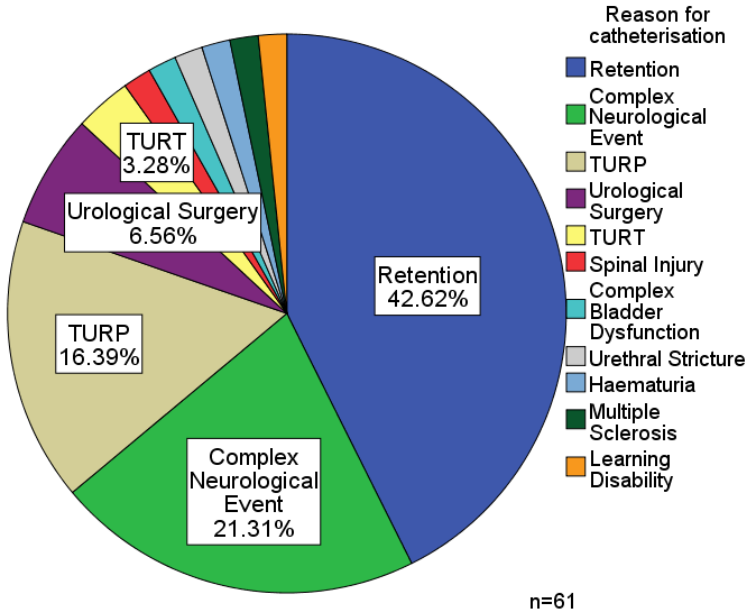
2.3 Results - catheter collection at NUHT

2.3.1 Setting and catheter collection demographics

Sixty-three urinary catheters were collected from four wards and urology theatres at NUHT, specifically, female neurosurgery ward (D10), male neurosurgery ward (D11), male urology ward (Harvey 2) and the neurorehabilitation centre (Linden Lodge). Two catheters were excluded from the final analysis as they did not meet the inclusion criteria for the study as one was a suprapubic catheter instead of urethral and one catheter was in situ for three days only. The majority (54.1%) of catheters were collected from Harvey 2 ward, followed by 24.59% from urology theatres, 14.75% from Linden Lodge, 4.92% from D10, and 1.64% from D11.

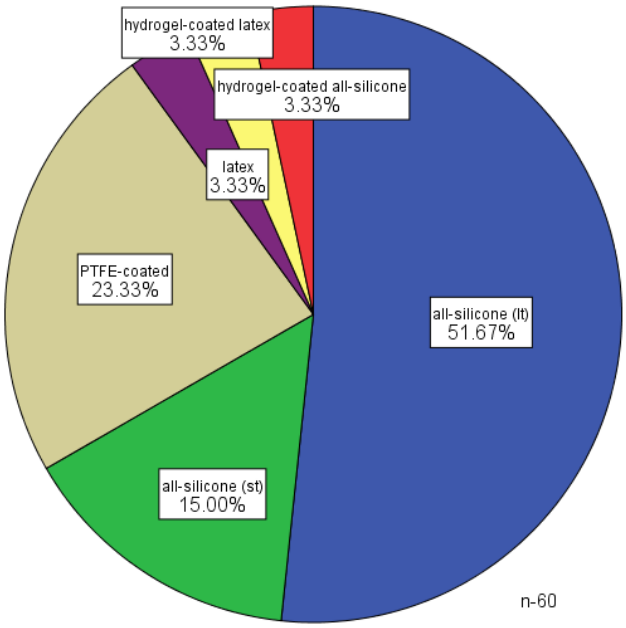
The reason for catheterisation was recorded for each catheter collected and the reasons reflect the patient populations sampled. The predominant reasons for catheterisation included management of retention (42.62%), management of a complex neurological event (21.31%), and transurethral resection of the prostate (TURP) (16.39%). Other reasons for catheterisation included management of bladder drainage as the result of long-term conditions such as spinal injury, multiple sclerosis, and learning disability (Figure 2-5).

Figure 2-5 Reasons for catheterisation in this study. TURP: transurethral resection of the prostate, TURT: transurethral resection of a tumour



The types of catheters collected also varied with the most common catheter type being all-silicone catheters for long-term use (51.7%), PTFE-coated (23.33%), and all-silicone for short-term use (15.0%) (Figure 2-6). The catheter type was not available or able to be determined for one catheter. The lumen sizes ranged from 12Ch to 20Ch.

Figure 2-6 Percentage of the catheter types collected from 60 of the 61 catheters included for analysis



2.3.2 Identification of microorganisms attached to the lumens and balloons of collected urinary catheters

107 and 103 organisms were isolated from the lumens and balloons, respectively, of 58 urinary catheters (Table 2-6). Three lumens (4.9%) and three balloons (4.9%) were culture negative. 34.4% of lumens were colonised by a single microorganism and 60.7% were polymicrobial. For the balloons, 37.7% were singly colonised and 57.4% were polymicrobial. The same microorganisms were isolated from the lumen and the balloon of the same catheter in 65.6% of collected catheters. The most commonly isolated organisms in both the balloons and lumens were *E. coli* and *Enterococcus faecalis*. There were more staphylococci than *Pseudomonas* spp. in the balloon and vice versa for the lumen.

Table 2-6: Number of each microorganism isolated from the balloons and lumens of 58 indwelling urethral urinary catheters in situ for 10 days or greater

Organisms isolated from catheter lumens			Organisms isolated from catheter balloons	
Enterobacteriaceae	**47**		**Enterobacteriaceae**	**40**
E. coli	24		*E. coli*	20
Klebsiella pneumoniae	5		*Klebsiella pneumoniae*	5
Enterobacter cloacae	4		*Enterobacter cloacae*	4
Klebsiella oxytoca	3		*Klebsiella oxytoca*	3
Proteus mirabilis	3		*Morganella morganii*	2
Morganella morganii	2		*Citrobacter koseri*	2
Serratia liquefaciens	2		*Hafnia alvei*	1
Citrobacter koseri	2		*Pantoea* spp.	1
Hafnia alvei	1		*Proteus mirabilis*	1
Pantoea spp.	1		*Serratia liquefaciens*	1
Enterococci	**18**		**Enterococci**	**20**
Enterococcus faecalis	17		*Enterococcus faecalis*	19
Enterococcus faecium	1		*Enterococcus faecium*	1
Pseudomonas spp.	**16**		**Pseudomonas spp.**	**12**
Staphylococci	**14**		**Staphylococci**	**19**
Staphylococcus epidermidis	4		*Staphylococcus epidermidis*	9
Staphylococcus aureus	3		*Staphylococcus aureus*	3
Staphylococcus capitis	2		*Staphylococcus caprae*	2
Staphylococcus caprae	1		*Staphylococcus haemolyticus*	2
Staphylococcus haemolyticus	1		*Staphylococcus xylosus*	1
Staphylococcus lugdunensis	1		*Staphylococcus hominis*	1
Staphylococcus saprophyticus	1		*Staphylococcus saprophyticus*	1
Staphylococcus xylosus	1			
			Yeasts	**7**
Yeasts	**6**		*Candida albicans*	4
Candida albicans	4		*Candida glabrata*	1
Candida guilliermondii	1		*Candida guilliermondii*	1
Candida parapsilosis	1		*Candida parapsilosis*	1
Others	**6**		**Others**	**5**
Micrococcus spp.	2		*Streptococcus agalactiae*	1
Corynebacterium propinquum	1		*Streptococcus bovis*	1
Streptococcus agalactiae	1		*Streptococcus gordonii*	1
Streptococcus bovis	1		*Streptococcus intermedius*	1
Streptococcus intermedius	1		*Corynebacterium propinquum*	1
Total:	**107**		**Total:**	**103**

2.3.2.1 **Unusual organisms**

Two *E. coli* isolates were indole negative, which is unusual as indole production is a defining characteristic of this species. Isolates F4123 and F4080 did not produce indole at 30°C or 37°C. They were isolated from two separate catheters that did not originate from the same patient (Table 2-7).

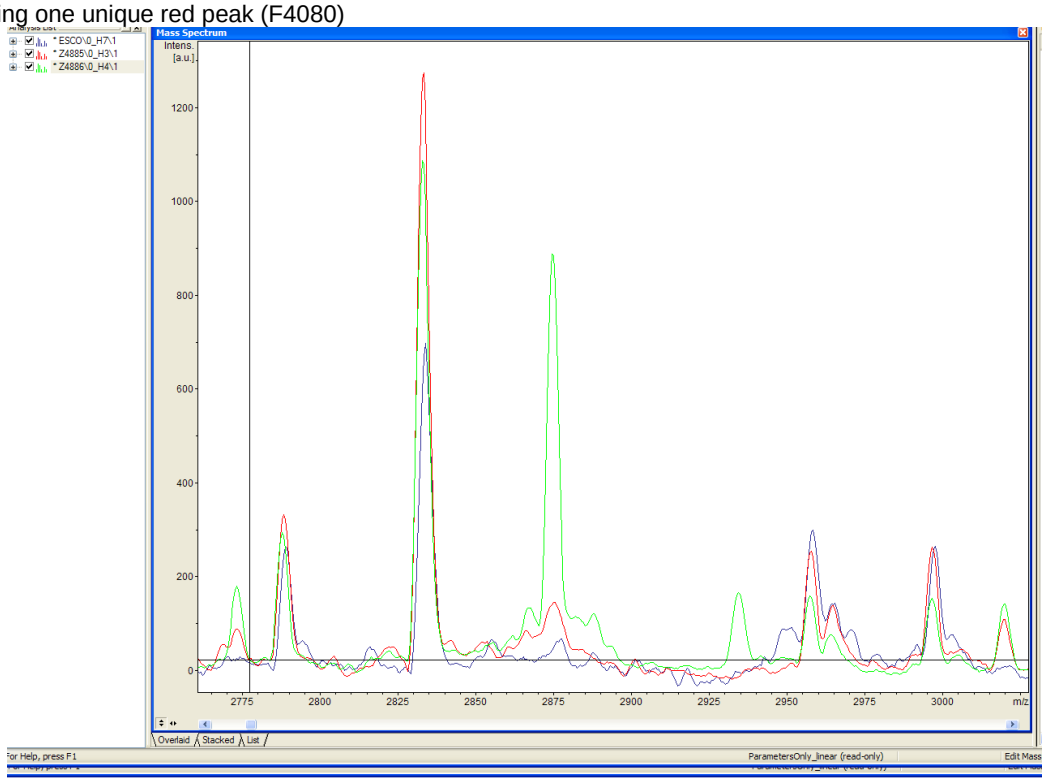
Table 2-7 Indole negative *E. coli* isolates

Catheter Number	**Hospital site of patient**	**Organisms isolated from lumen**	**Organisms isolated from balloon**
NB4202	Ward D10 - Neurosurgery	*S. epidermidis, Candida albicans, E. faecium,* ***E. coli*** **(indole –ve)**	*S. epidermidis, Candida albicans, E. faecium*
NB4223	Linden Lodge - Neurorehabilitation	*E. coli* (indole +ve), ***E. coli*** **(indole –ve)**	*E. coli* (indole +ve), ***E. coli*** **(indole –ve)**

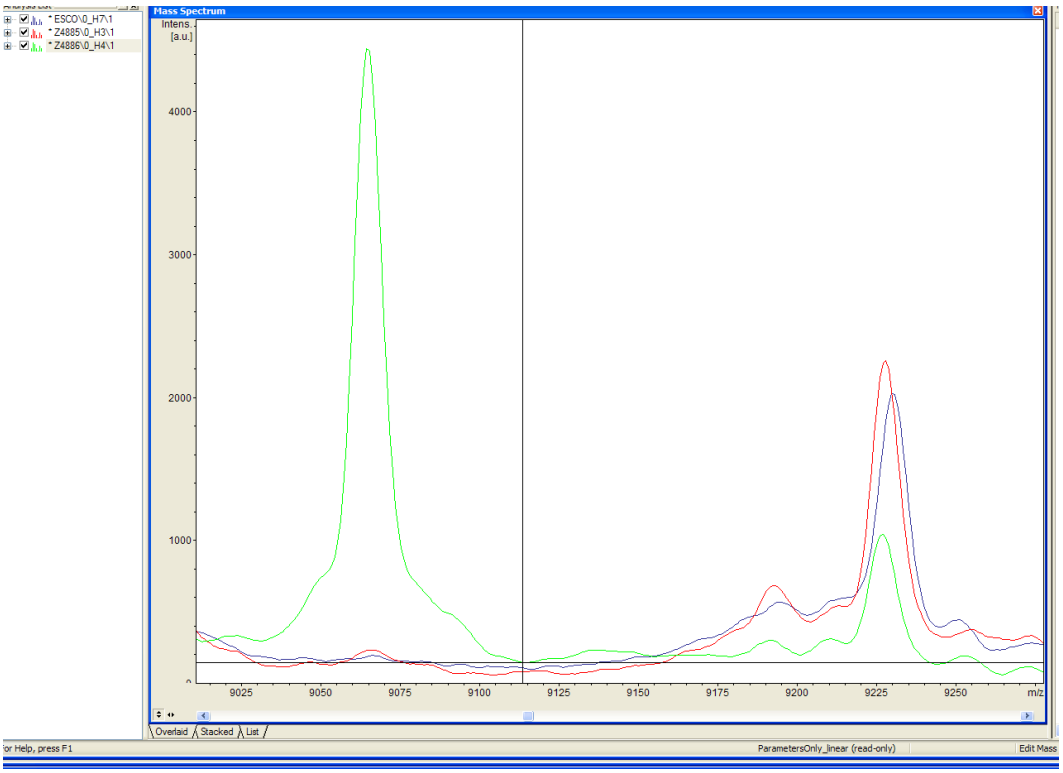
Considering 2/24 *E. coli* isolate from catheter lumens (8.3%) were indole negative, the isolates were compared by MALDI-ToF to understand if they were hospital isolates that were shared amongst patients and/or staff. Generation of at least six unique peaks are needed in order to show that strains of the same organism are not similar. According to MALDI-ToF analyses, the two indole –ve *E.coli* were different isolates as they had greater than six unique peaks. Some examples of unique peaks on the MALDI-ToF mass spectrum can be found in Figure 2-7.

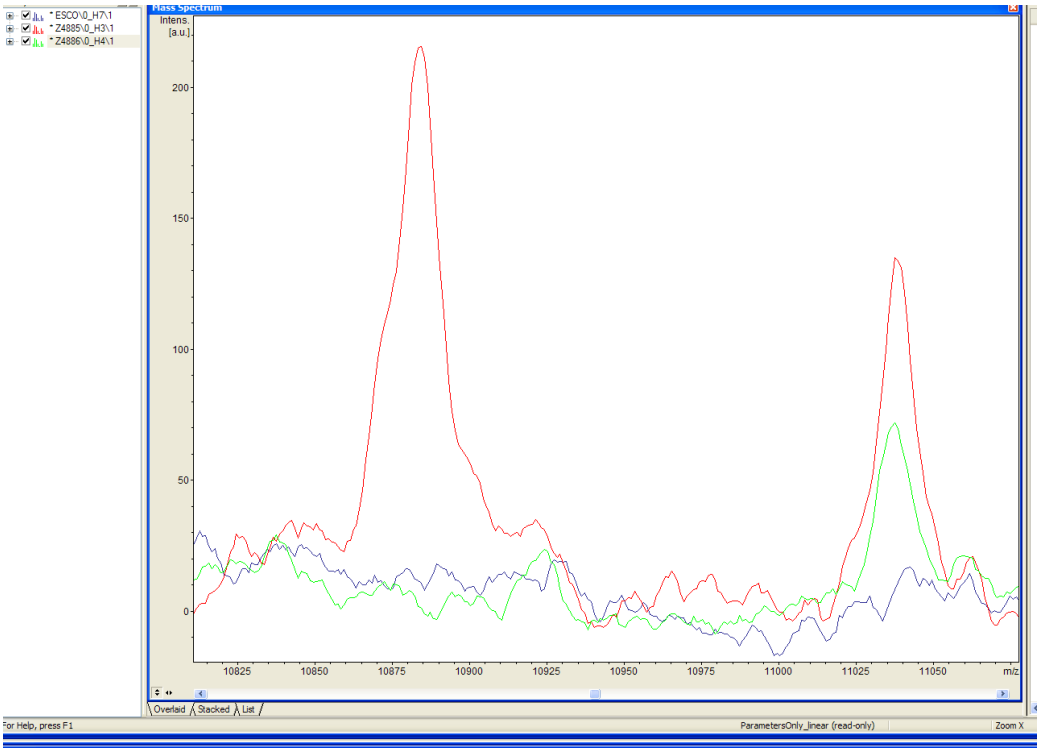
Figure 2-7 MALDI-ToF mass spectrum of *E. coli* reference strain (blue) line, F4080 indole negative E. coli (red line) and F4123 indole negative *E. coli* (green line) A) mass spectrum showing two unique green peaks (F4123), B) mass spectrum showing one unique green peak (F4123), C) mass spectrum showing one unique red peak (F4080)

A



B

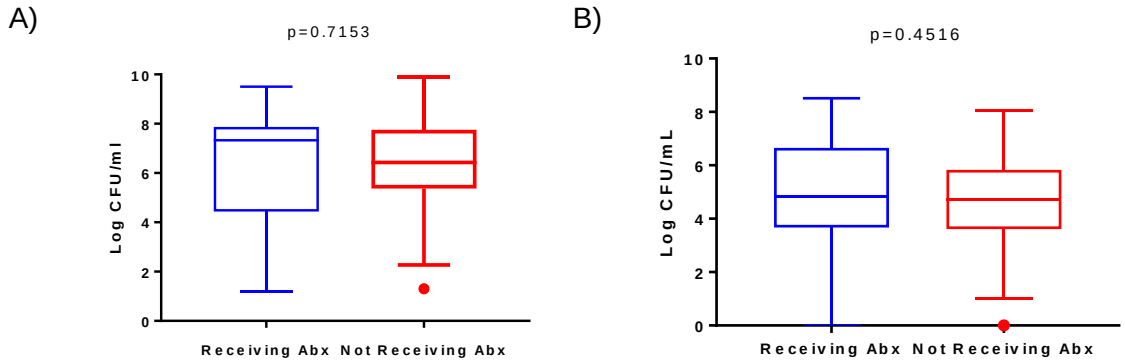


C 

2.3.2.2 **Antimicrobial resistance and antibiotic exposure**

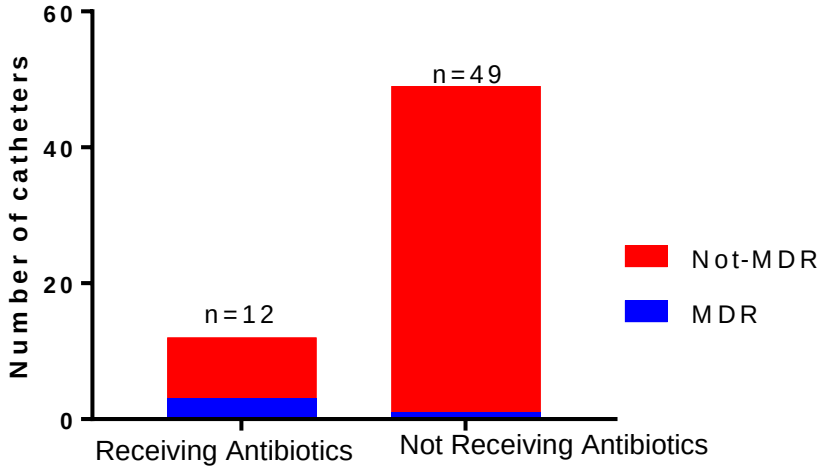
Sixteen urinary catheters (26.2%) were from patients known to be receiving antibiotics and of those, 75% were known to be receiving antibiotics for treatment of CAUTI. Others were receiving antibiotics prophylactically for other conditions and for treatment of other infections, such as a chest infection. Of the 16 catheters from patients known to be receiving antibiotics, all lumens were colonised by at least one microorganism and 15/16 of the balloons were colonised. Antibiotics did not significantly reduce colonisation of catheter lumens ($p=0.7153$) or balloons ($p=0.4516$) (Figure 2-8).

Figure 2-8 Tukey's Box Plot of the LogCFU/mL of each microorganism isolated from the A.) lumens and B.) balloons of catheters from patients receiving antibiotics and those not known to be receiving antibiotics. abx: antibiotics



Four multi-drug resistant organisms were isolated, including two ESBL-producing *E. coli* and two methicillin-resistant *S. epidermidis.* There was a significantly higher proportion (p=0.0216, Fisher's exact test) of MDR organisms isolated from catheters from patients known to be receiving antibiotics (25.0%) than from catheters from patients not known to be receiving antibiotics (2.04%) (Figure 2-9). MDR was defined according to the internationally agreed definition in which the bacterium was non-suceptible to one or more antimicrobial agent in three or more antimicrobial classess[183].

Figure 2-9 Number of catheters from patients either known to be receiving antibiotics or not receiving antibiotics and the number of catheters with a multi-drug-resistant (MDR) organism isolated



2.3.2.3 Duration of catheterisation and colonisation of catheter lumens

Information regarding the exact duration of catheterisation was available for 48/61 catheters. There was no correlation between the duration of catheterisation and the number microorganisms (Figure 2-10) that were colonising the lumens and the quantity of each microorganism (Figure 2-11) in the lumen from catheters from patients symptomatic for CAUTI and non-symptomatic. Eight of 61 patients were symptomatic for CAUTI (despite 12 being treated for CAUTI, only 8 had symptoms recorded) and duration of catheterisation was available for 7/8.

Figure 2-10 The number of isolates in each catheter of patients symptomatic and asymptomatic of CAUTI plotted against duration catheter was in situ.

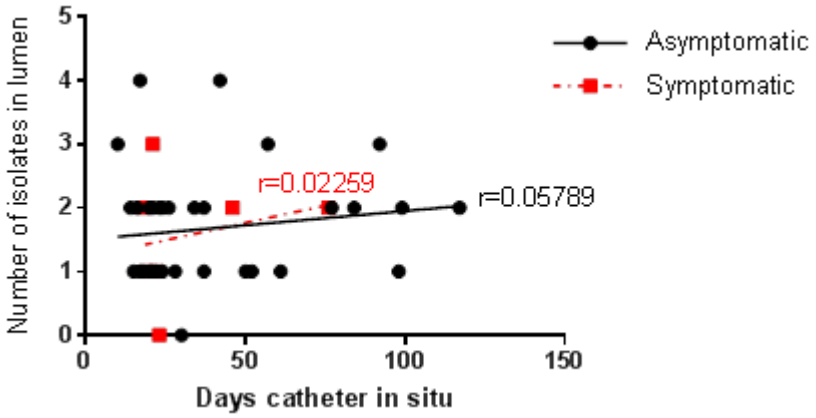
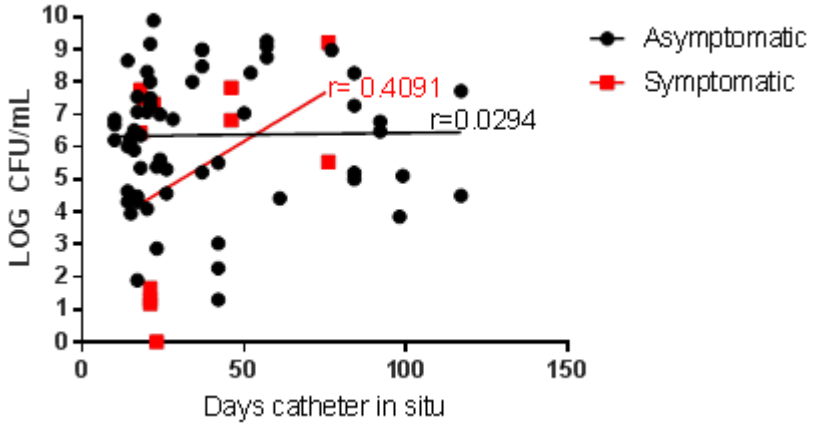


Figure 2-11 The quantity of isolates in each catheters from patients symptomatic and asymptomatic of CAUTI plotted against duration catheter was in situ



There was no significant difference between the number of species (p=0.7741) or the quantity (CFU/mL) (p=0.0976) of microorganisms isolated in the lumens of catheters from symptomatic and non-symptomatic patients over time. These results indicate that a heavy burden of microorganisms may be found in the absence of CAUTI symptoms and that the number and burden of microorganisms does not increase with the period of catheterisation after ten days in situ. Furthermore, there was no difference in the quantity and number of microorganisms in symptomatic and non-symptomatic patients which reinforces the importance of symptoms rather than microbiological culture in differentiating between CAUTI, which requires treatment and ASB, which does not.

2.3.2.4 Encrustation and blockage of catheter lumens

Five of the 61 collected catheters were completely occluded and *P. mirabilis* was isolated from 2/5 blocked catheters. As seen in Table 2-8 below, catheters 4211 and 4214 were not colonised by any organisms with urease activity. *E. faecalis* and *E. coli* were the most commonly isolated organisms from blocked catheters.

Table 2-8: Microorganisms isolated from the lumens of blocked urinary catheters

Catheter Number	pH of lumen contents	Organisms isolated from lumen	Magnesium phosphate crystals present?
4136	8.5	*P. mirabilis, Pseudomonas spp.*	No
4147	8.5	*S. aureus, Micrococcus spp.*	No
4201	6.5	*E. faecalis, E. coli, P. mirabilis, S. capitis*	Yes
4211	6.0	*E. coli, E. faecalis, C. albicans*	No
4214	7.5	*E. coli, E. faecalis*	No

The majority of crystals found in the catheters were of an undefined shape and therefore not clinically significant although granular casts were seen in one wet mount. Magnesium ammonium phosphate (also known as triple phosphate or struvite) crystals were seen in only one blocked catheter and are typically associated with alkaline urine and the presence of urease producing bacteria[184]. An additional four catheters contained magneisum ammonium

phosphate crystals, but were not blocked. Two of the five blocked catheters unusually had acidic luminal contents which is surprising since catheter 4201 contained *P. mirabilis*, which is known for alkalinising the urine. Of particular interest is catheter 4235 which had a significantly alkaline pH of the lumen sonicate of 8.5, but the only organism isolated was *E. faecalis*, which has no urease activity. *E. faecalis* was isolated from three catheters in which these crystals were found. In catheter 4217 *E. coli* was present alongside *E. faecalis* in the lumen, but *E. coli* also does not produce urease either.

2.3.3 CSU and catheter colonisation correlation

Three of the 61 patient notes were unavailable on NOTIS and were excluded in the following analyses. 28 CSU samples were taken from 22/58 patients during the period of catheterisation. Some patients had multiple CSU results during the period of interest.

Generally, the consensus between CSU results and catheter colonisation data is poor (Table 2-9). The majority of CSU samples were either 'mixed growth' or *E. coli* in significant quantity. Only one CSU result was '*Enterococcus* spp.' despite *E. faecalis* being the second most common organism found on the lumens or balloons, although it is possible *E. faecalis* may have been captured by the 'mixed growth' result.

Table 2-9 Catheter specimen of urine (CSU) results and the proportion that match the organisms colonised onto the balloon or lumen of the catheter from the corresponding patient

CSU Result	Number with this result (N=28)	Proportion that matches the organisms on the catheter lumen	Proportion that matches the organisms on the catheter balloon
Mixed growth	8 (28.6%)	6/8	6/8
E. coli	8 (28.6%)	1/8	1/8
No growth	7 (25.0%)	0/7	0/7
Proteus spp.	2 (7.1%)	0/2	0/2
Coliform	2 (7.1%)	0/2	0/2
Enterococcus spp.	1 (3.6%)	0/1	0/1
S. aureus	1 (3.6%)	1/1	1/1

Antibiotic choice does not appear to have been guided by the CSU results and available susceptibilities. Two of the 12 patients prescribed antibiotics for CAUTI did not have a CSU sent and an additional 2/12 had a CSU result of 'no growth' but all received antibiotics for treatment of CAUTI. An additional patient was prescribed cephalexin for an ESBL *E.* coli, meaning the isolate was resistant to cephalexin, which was identified in the catheter collection data and in the CSU result. Another patient was prescribed iv piperacillin-tazobactam for an *E. coli* that was identified as susceptible to oral antibiotic options such as nitrofurantoin and pivmecillinam in the CSU report. The catheter collection results identified *E. coli* and *E. faecalis* in both the lumen and balloon in significant quantities (>10^5 CFU/mL); nitrofurantoin could still remain a suitable antibiotic choice. An additional patient was prescribed pivmecillinam despite the CSU report of a pivmecillinam-resistant *Proteus* spp. It is not entirely surprising that more MDR organisms were isolated from catheters from patients receiving antibiotics when the antibiotic choice did not reflect the organism susceptibilities. This assumes that the CSU result encompassed all the organisms in the bladder environment and it is clear there were major discrepancies between the CSU and catheter colonisation data.

2.4 Results - longitudinal catheter collection

Nine catheters were collected from a single volunteer in the community setting from 10 October 2016 – 4 October 2017 . The volunteer was a 59-year-old male with a spinal cord injury (C6 tetraplegic) and thus requires an indwelling suprapubic urinary catheter to manage his bladder. During the daytime, he used a catheter valve to tone the bladder and flush debris, and at night urine freely drained into a night bag. The catheter valve, leg bag, and night bags were changed every seven days and flushed with tap water and then flushed again with a Dettol solution. At every catheterisation he was catheterised with an all-silicone 16 Ch standard length catheter.

During the year of catheter collection, the volunteer did not experience any episodes of CAUTI and was, therefore, not prescribed any antibiotics to treat

CAUTI. He was prescribed antibiotics and antifungals on several occasions throughout the year for treatment of other conditions and prophylactically (Table 2-10).

Table 2-10 Details of catheters collected from a single volunteer over one year including self-reported antibiotic/antifungal prescribing.SPC: suprapubic catheter, t.i.d.: three times a day, q.d.: once daily, b.d.: twice daily, i.m.: intramuscular

Date catheter inserted	**Date catheter removed and collected**	**Date antibiotics or antifungals prescribed**	**Antibiotics or antifungals prescribed**	**Reason for antibiotic/ antifungal administration**
26/8/16	10/10/16			
10/10/16	22/11/16			
22/11/16	02/1/17	30/12/2016	250 mg t.i.d. amoxicillin for 7 days	Chest infection
2/1/17	9/2/17	17/1/2017	150 mg fluconazole	skin infection
		31/1/2017	250 mg terbinafine q.d	Persistence of skin infection
9/2/17	24/3/17			
24/3/17	4/5/17			
Suprapubic tract lost on 4/5/17 and a urethral catheter inserted. Urethral catheter lost at creation of new SPC tract		4/5/2017	625 mg co-amoxiclav t.i.d. for 3.5 days	Prophylaxis over concerns of failed SPC tract widening leading to haematuria
		4/5/2017	i.m. gentamicin	Prophylaxis for cystoscopy
31/5/17	12/7/17	31/5/2017	i.m. gentamicin	Prophylaxis for creation of new SPC tract
		13/6/2017	500 mg clarithromycin b.d. for 7 days	*S. aureus* infection at SPC wound site
12/7/17	22/8/17			
22/8/17	4/10/17			

2.4.1 Characterisation of collected catheters

Only four different organisms, *Citrobacter koseri*, *E. faecalis*, MSSA, and *P. aeruginosa* were isolated from the balloons and lumens of the nine catheters. The four were not present all together in the catheter at each catheter removal

but were variably present throughout the year (Table 2-11). *C. koseri* and MSSA were not found in the same catheter lumen or balloon.

Table 2-11 Organisms isolated from the lumens and balloons, and the pH of the lumen sonicate of nine catheters collected from a single volunteer over one year*E. faecalis: Enterococcus faecalis*; P. aeru: *Pseudomonas aeruginosa*; MSSA: methicillin-susceptible *Staphylococcus aureus*; *C. koseri*: *Citrobacter koseri*

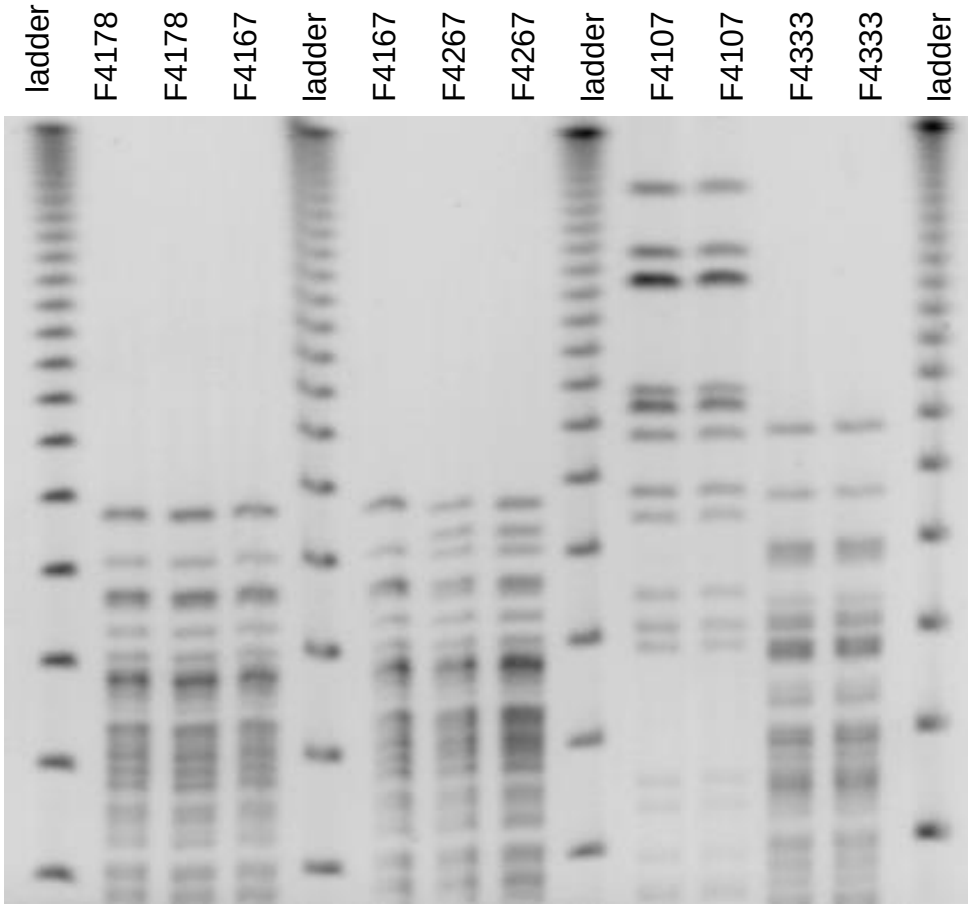
			Organism isolated from the lumen				**Organism isolated from the balloon**			
Date catheter inserted	**Date catheter removed**	**pH pf lumen sonicate**	***E. faecalis***	***P. aeru***	**MSSA**	***C. koseri***	***E. faecalis***	***P. aeru***	**MSSA**	***C. koseri***
26/8/16	10/10/16	8.0	√			√	√			√
10/10/16	22/11/16	8.0		√		√		√		√
22/11/16	02/1/17	8.5	√	√		√	√	√		√
2/1/17	9/2/17	7.5		√	√			√	√	
9/2/17	24/3/17	8.0		√		√				√
24/3/17	4/5/17	7.0		√		√		√		√
Suprapubic tract lost on 4/5/17 and a urethral catheter inserted. Urethral catheter lost										
31/5/17	12/7/17	7.0	√	√			√	√	√	
12/7/17	22/8/17	7.0	√	√	√		√	√	√	
22/8/17	4/10/17	7.0	√	√	√		√	√	√	

As the volunteer was prescribed amoxicillin, co-amoxiclav, clarithromycin, and gentamicin while the catheters were in situ the susceptibilities to these antibiotics were determined. None of the organisms developed resistance over the year.

2.4.2 PFGE of isolates from collected catheters

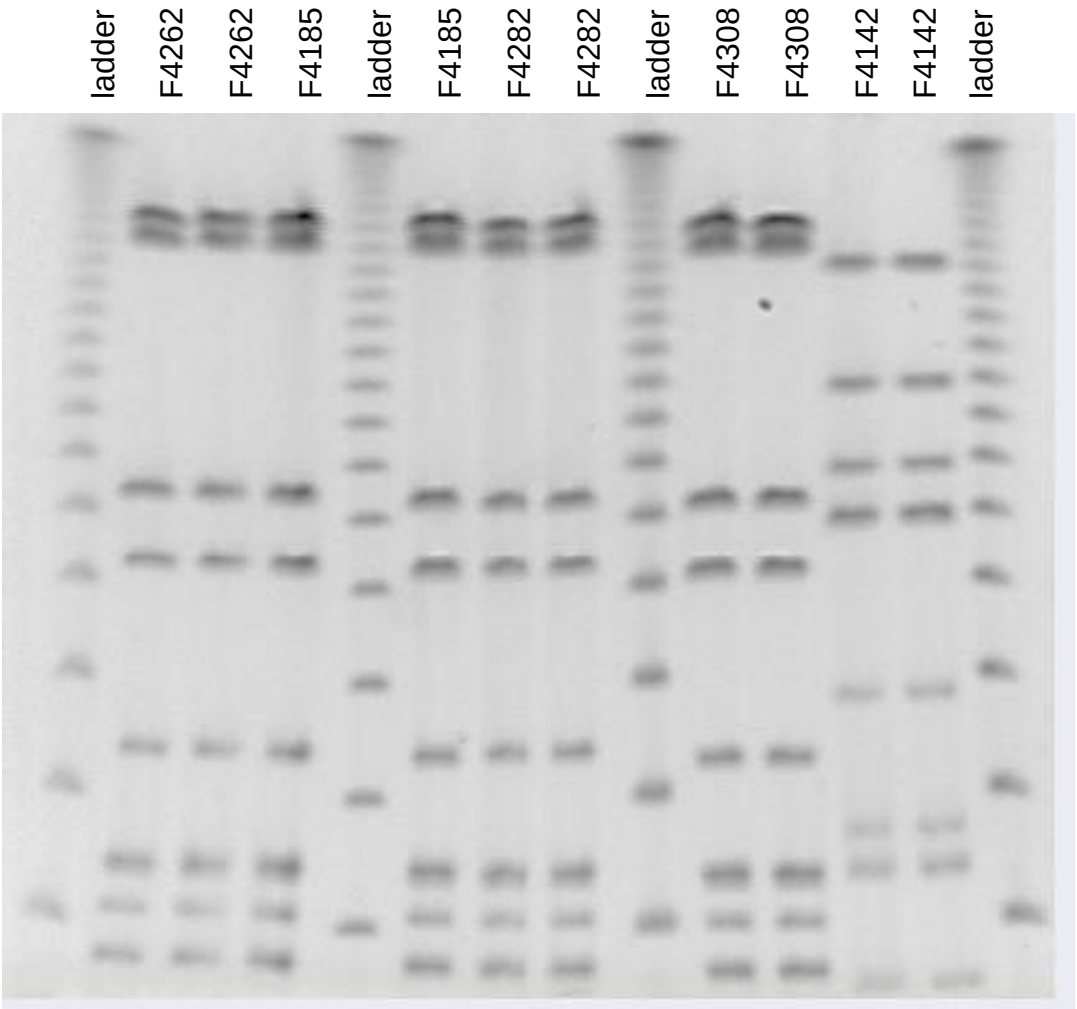
*Xba*I restriction profiles of *C. koseri* and *P. aeruginosa* isolates from the volunteer were successfully obtained (example, Figure 2-12). Digestion of *C. koseri* isolates from the volunteer's catheter yielded 12 bands measuring 61.4 – 661.9 kbp, and digestion of the *C. koseri* control (F4107) yielded 14 bands from 39.12-741.86 kbp. *Xba*I digestion of the eight *P. aeruginosa* catheter isolates yielded 13-14 bands ranging from 50.4-233.68 kbp. Digestion of the *P. aeruginosa* control (F4333) resulted in 12 bands ranging from 46.2-276.55 kbp.

Figure 2-12 PFGE gel of gram-negative isolates restricted by *Xba*I. Isolates F4178, F4167, and F4267 were *P. aeruginosa* isolates from the volunteer's catheter and F4333 was the *P. aeruginosa* control. F4107 was the *Citrobacter koseri* control. Lanes 1,5,9, and 14 contain the Lambda PFG ladder



*Sma*I restriction profiles of MSSA and *E. faecalis* were also successfully obtained (example, Figure 2-13). Digestion of the four MSSA catheter isolates yielded 7 bands from 47.5–686.8 kbp, and digestion of the unrelated control (F4142) resulted in 7 bands ranging from 63.4-587.3 kbp. Digestion of five *E. faecalis* catheter isolates resulted in 9 bands ranging from 62.19-469.05 kbp, and digestion of the unrelated control produced 9 bands from 62.97-597.42 kbp.

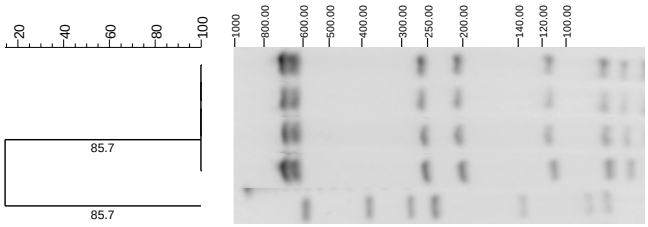
Figure 2-13 PFGE gel of MSSA restricted by *Sma*I.Isolates F4262, F4185, F4282, and F4308 are patient isolates and F4142 was the unrelated control. Lanes 1,5,9, and 14 contain the Lambda PFG ladder



Due to the consistency of the same four isolates over the course of the year, the isolates were assessed for strain relationship by PFGE. The four MSSA isolates were indistinguishable according to the interpretive criteria by Tenover et al., and also according to the analysis by GelCompar II in which

there is a 0% difference between the strains (Figure 2-14). This in contrast to F4142, the control *S. aureus*, which is clearly unrelated according to both methods of assessing relationship. This helps to demonstrate the sensitivity of this method.

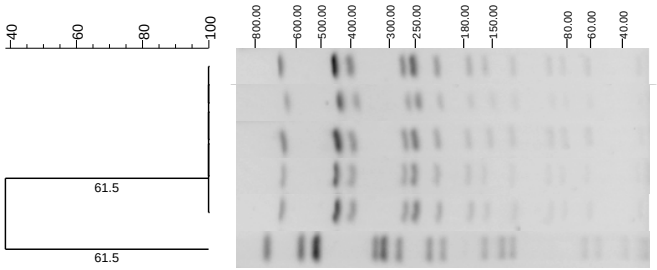
Figure 2-14 Dendogram of four *Staphylococcus aureus* restriction patterns. F4185, F4262, F4282, and F4308 were from the longitudinal catheter collection study and one *S. aureus* isolate (F4142) from an unrelated urine specimen. * = control



Key	Genus	species
F4185	Staphylococcus	aureus
F4262	Staphylococcus	aureus
F4282	Staphylococcus	aureus
F4308	Staphylococcus	aureus
F4142	Staphylococcus	aureus

Likewise, the five *C. koseri* isolates from this longitudinal catheter collection study had indistinguishable banding patterns in contrast to F4107 *C. koseri* which was isolated from a catheter collected from NUHT (Figure 2-15).

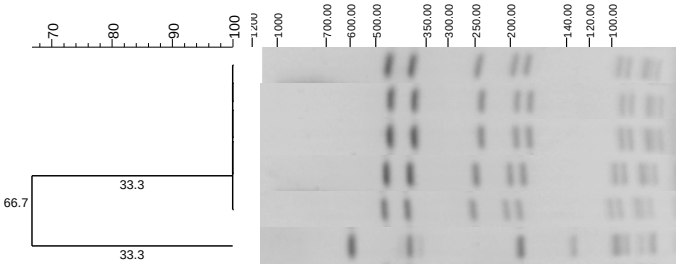
Figure 2-15 Dendogram of five *Citrobacter* restriction patterns. F4209, F4150, F4166, F4176, F4231 were from the longitudinal catheter collection study and one *C. koseri* isolate (F4107) from the **lumen of an unrelated urinary catheter. * = control**



Key	Genus	species
F4209	Citrobacter	koseri
F4150	Citrobacter	koseri
F4166	Citrobacter	koseri
F4176	Citrobacter	koseri
F4231	Citrobacter	koseri
F4107	Citrobacter	koseri

The five *E. faecalis* isolates from the longitudinal catheter collection were indistinguishable according to their restriction patterns. F3946, the non-epidemiologically related control, was unrelated (Figure 2-16)

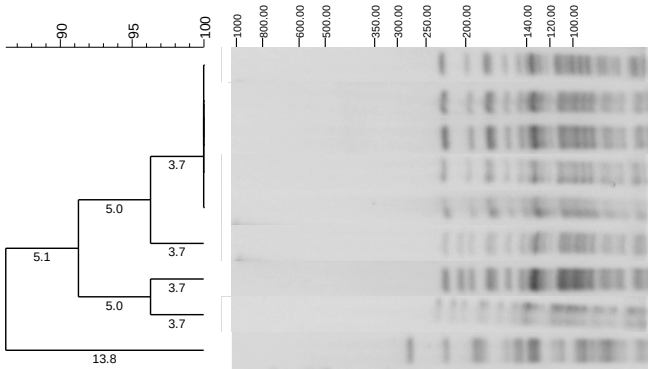
Figure 2-16 Dendogram of five *E. faecalis* restriction patterns. F4177, F4155, F4281, F4307, F4261 were from the longitudal catheter collections study and one *E. faecalis* isolate (F3946) from the lumen of an unrelated catheter. * = control



Key	Genus	species
F4177	Enterococcus	faecalis
F4155	Enterococcus	faecalis
F4281	Enterococcus	faecalis
F4307	Enterococcus	faecalis
F4261	Enterococcus	faecalis
F3946	Enterococcus	faecalis

The relationship of the *P. aeruginosa* isolates was slightly more complex. The five *P. aeruginosa* isolates cultured from the lumens of catheters 2-6 from the volunteer were indistinguishable. Isolates F4267, F4283 and F4311 were cultured from catheters 7-9 after the intermission in suprapubic catheterisation due to loss of the suprapubic tract. These isolates have a band at approximately 205.60 kbp which is absent from the three previous *P. aeruginosa* isolates (Figure 2-17).

Figure 2-17 Dendogram of eight *P. aeruginosa* restriction patterns. F4167, F4178, F4184, F4213, F4232, F4267, F4283, F4311 were from the longitudinal catheter collection study and one *P. aeruginosa* **isolate (F4333) from the lumen of an unrelated urinary catheter. * = control**



Key	Genus	species
F4213	Pseudomonas	aeruginosa
F4167	Pseudomonas	aeruginosa
F4178	Pseudomonas	aeruginosa
F4184	Pseudomonas	aeruginosa
F4232	Pseudomonas	aeruginosa
F4283	Pseudomonas	aeruginosa
F4267	Pseudomonas	aeruginosa
F4311	Pseudomonas	aeruginosa
F4333	Pseudomonas	aeruginosa

Isolate F4267, which is the next isolate chronologically from the five indistinguishable isolates, is missing the band at approximately 91.0 kbp and instead has a band at 205.69 kbp that is not present from the previous five isolates. This two band difference classifies F4267 as ‘closely related’ to the previous five isolates according to Tenover et al. and would be classified as part of the outbreak in an outbreak setting. It is likely that the two band difference is the result of a single genetic event and Tenover et al. further highlighted that these variations by two or three bands have been observed when isolated repeatedly from the same patient, as in this study. F4283 which was cultured chronologically after F4267 has 14 bands compared to 13 bands of the previous isolates. The band at 205.69 kbp remains, but the band at approximately 91.0 kbp has reappeared. This is mostly likely why the comparative analysis by the GelComparII software has grouped F4283 more closely to the first five isolates than F4267. F4311, which was isolated from the ninth, and final catheter also has 14 bands including the band at approximately 205.69 kbp and 91.0 kbp but is missing the band at

approximately 85.0 KB and instead has an additional band at 111.16 kbp, which is not present on the restriction patterns from any other isolates. It is apparent the genetic changes leading to different restriction patterns follow a sequential order. Despite F4311 being the least closely related to the original five isolates it still has only three fragment differences (addition of 205.69 kbp, removal of 85.0 kbp, addition of 111.16 kbp) making F4267, F4283, and F4311 classed as 'closely related' to the initial five isolates. The change from suprapubic catheterisation to urethral catheterisation back to suprapubic catheterisation is the point at which the mutations appear and may be as a result of a change in environmental pressures, such as antibiotic use.

2.5 Discussion

2.5.1 Lumen and balloon sonication

One method of quantifying attached biofilm bacteria can be to remove the organisms from the surface by sonication or enzymatically and then plating the sonicate onto agar to quantify the colony counts[185]. The method of sonication to isolate and quantify the attached micoorganisms is advantageous compared to the other methods as it increases the sensitivity of detecting microorganisms[186, 187]. For example, Djeribi et al. identified bacteria in urinary catheter lumens by sterilising the outer catheter surface, cutting the catheter into disks, and placing onto blood agar plates for 48 hours. They were unable to quantify bacteria according to this method and noted there was dense growth around the catheter segments on blood agar suggesting that the ability to differentiate different colony types could be compromised[167]. Matsukawa swabbed the luminal surface of the 5cm tip and spun the swab in saline. The saline suspension was plated and colonies could be enumerated from the plates. However, swabbing traditionally has low sensitivity and is dependent on the technique and skill of the swabber[188]. Studies comparing sonication and swabbing favours sonication for its increased colony recovery[189, 190]. Furthermore, neither study examined the balloon and catheter tip in isolation, which is likely to be the source or seed of bacteria in the bladder.

Previous experimentation in our laboratory showed that five minutes of sonication extracted the majority of bacteria that were attached to silicone discs and also did not kill bacteria[191]. Sonication has been used successfully to improve identification and quantification of bacteria attached to vascular catheters[169]. For vascular catheters a cut-off of 10^2 CFU/mL was used to determine colonisation of the catheters as the primary source of infection or if the bacteria had seeded there from an infection at a distant site. This is in contrast to the sonication of a urinary catheter in which quantification of organisms does not correlate with symptomatic infection and this method of analysis could not be used for such purposes most likely due to the fact that the limit of colonisation of the catheters is reached by 10 days. However, as Sherertz et al. found, sonication was able to separate and identify the mixed cultures attached to the catheters which is important given that the majority of urinary catheters are colonised by at least two organisms as seen in this study and by Warren et al.[33]. Separation of the balloon and the luminal contents for sonication may give an indication as to which bacteria in a mixed culture are more likely to affect the bladder epithelium as organisms limited to the lumen are merely colonising the catheter. This level of detail is missed with CSU culture. For example, one catheter grew 10^7 CFU/mL and 10^6 CFU/mL *E. coli* and *E. faecalis,* respectively, in the balloon whereas only *E. coli* was recorded in the CSU result despite both being present in significant quantities. As a consequence, only the antibiotic ssusceptibilities of the *E. coli* were presented in the report.

Discrepancies between organisms isolated from urinary catheters and CSU were also reported by Matsuwaka et al. in which 21 catheters that were culture positive by swabbing on the luminal surface, were culture negative according to CSU samples. They reported that a further 11 patients had at least one difference between urine and catheter isolates[168]. Similarly, all seven catheters identified as culture negative according to the CSU results all were culture positive after sonication of both the catheter and the lumen. The study described herein also highlights discrepancies between microorganisms on the balloon and in the catheter lumen, of which 34.4% had at least one difference. This could account for CSU differences in which

luminal organisms are picked up in the CSU as they are flushed out of the catheter.

2.5.2 **Identification of organisms**

UK Standards for Microbiology Investigations provide recommended identification algorithms, and of particular relevance, for urine specimens [192]. In these standards, CLED agar or chromogenic agar is recommended for targeted organisms such as Enterobacteriaceae, enterococci, staphylococci, *Pseudomonas* spp., which are the main organisms expected to be found from the catheters according to the previous studies[167, 168]. Sabouraud agar can be added if yeasts are suspected, but it was found in this study that yeasts are capable of growing on CLED. CLED and chromogenic media are particularly useful for these studies as they stop *P. mirabilis* from swarming due to the lack of electrolytes in the media, but they do not inhibit its growth[193] to allow separation of different colony types. However, due to differences in cost, dilutions of the lumen and balloon sonicate were initially plated onto CLED agar. Chromogenic UTI agar is particularly helpful for the presumptive identification Enterobacteriaceae and enterococci[194] so only suspected Enterobacteriaceae and enterococci were subcultured from CLED onto the chromogenic medium. Further confirmatory identification tests can be carried out according to the colour of the colonies.

MALDI-ToF is a useful tool for microbiological identification requiring little preparation other than subculturing the organism of interest and can provide species level identification within minutes. Databases are constantly being added to commercial systems so that identification is becoming more reliable and valid. For example, a study by Bizzini et al. obtained a score greater than 2.0 for 1278 (93.2%) isolates and among these 95.1% matched identification to the species level. Extracting the protein, particularly for yeasts may help in producing higher quality spectra[195, 196]. Bizzini et al. also noted in the same study that on three occasions *Shigella* isolates were misidentified as *E. coli* due to the limit of resolution of MALDI-ToF, but extraction of proteins and expansion of the databases were able to improve identification[195].

Enterobacteriaceae and enterococci were the most commonly isolated microorganisms from the lumens and balloons of indwelling urinary catheters collected at NUHT. Although CSU results from the same catheters explicitly identified enterococci in only one catheter. *E. coli* and *E. faecalis* were found co-colonising 10 catheter lumens and seven catheter balloons including some catheters which were blocked. Co-culture of the two organisms found that they can co-exist in biofilms without one outcompeting the other[197, 198]. Indole production by *E. coli* is useful in inhibiting virulence genes of other bacteria, although its inhibitory effect is not applicable to *E. faecalis* biofilm bacteria[198]. Due to indole's well-recognised importance in identification, it was surprising that two of the 24 *E. coli* isolates from these experiments did not produce indole, which was evident by indole tests at two different temperatures and also that these isolates lacked the typical aromatic indole smell on culture.

Identification of peaks unique to each *E. coli* pathotype may allow sub-typing of bacteria by MALDI-ToF[199]. MALDI-ToF was exploited herein for such purposes of trying to identify the emergence of clones. As two indole-negative *E. coli* were isolated from the urinary catheters, they were investigated for clonal similarity. Logistically, many patients from Ward D10, female neurosurgery, are transferred to Linden Lodge for long-term neurorehabilitation after resolution of the acute problem, and it is possible the indole negative *E. coli* from the D10 catheter could have been transferred to another patient when transferred to Linden Lodge. However, according to Bruker Biotyper methodology, there were sufficiently unique peaks to determine they were not indistinguishable. It then begs the question if there is something in the urinary catheter microenvironment that alters indole production. pH has a role with indole production increasing as the pH rises from 4.0 -9.0[200]. Catheter NB4223 had a luminal pH of 5.0 and catheter NB4202 had a luminal pH of 7.0. A pH of 5.0 may reduce indole production. NB4202 had a 100X greater pH and also contained an indole-negative *E. coli*. The luminal pH as measured here is a total pH of the luminal contents and does not take into account micro-variations within the biofilm environment in which the pH can vary within the biofilm[201].

2.5.3 Longitudinal catheter collection

This is the first study, to the author's knowledge, that examines the colonisation and relationship of isolates of consecutive urinary catheters over a year in a long-term catheter user with no modification to the normal catheter maintenance regimen. It was suspected that the same strains of each organism re-colonised the urinary catheter with each catheter change based on clinical suspicions such as identical antibiotic sensitivity profiles within each species. Furthermore, *C. koseri* is not a common CAUTI causative organism so its reappearance suggested a native source. The volunteer also experienced an infection by MSSA at the site of the suprapubic tract, possibly by the same MSSA as the one colonising the catheter lumens and balloons. The wound or catheter could seed colonisation of the other site due to proximity.

A recent study by Bossa et al. examined the urinary catheter microflora using Terminal Restriction Fragment Length Polymorphism of three males with neurogenic bladders before and after probiotic therapy. They concluded that the catheter microflora was unique to each participant and it was a stable population even when antibiotics and probiotics were prescribed and quickly reverted to the original population after stopping therapy[202]. This is consistent with the findings of this study in that the same four isolates; *C. koseri*, MSSA, *E. faecalis*, and *P. aeruginosa*, were isolated from nine catheters over the year and did not alter despite multiple courses of antibiotics and antifungals. The volunteer experienced no episodes of CAUTI over the year of catheter collection. It supports the idea that catheters develop their own microenvironment, as demonstrated by universal colonisation by 10 days in this study and 28 days as reported elsewhere[2]. It is possible that the organisms form a protective microenvironment that prevents colonisation by other, possibly more invasive, organisms. This is the basis for the experimental catheters coated with a non-pathogenic strain of *E. coli*[112, 113].

The study by Bossa et al. identified the microbial population using culture independent methods, which excluded the phenotypic characteristics of the microbial population such as antibiotic susceptibilities. Furthermore,

they identified the bacteria present in the urinary catheter environment but did not show the relationship between one isolate of one species in one catheter to the next[202]. The relationship of the isolates was analysed by PFGE. This is especially important in regard to *P. aeruginosa*, a ubiquitous environmental organism that could easily re-colonise the catheter from environmental sources.

The source of re-colonisation may be the bladder epithelium itself in which the bacteria are able to become intracellular[203].

2.5.4 **PFGE**

While PFGE is an older molecular technique for analysing whole genome relationships, compared to techniques such as whole genome sequencing, it is still often the gold standard. For example, PulseNet, a network that's part of the CDC routinely uses PFGE as a fingerprinting tool to identify outbreaks[181]. PFGE has good discriminatory power, better than other molecular fingerprinting techniques such multi-locus sequence typing and *spa*-typing, and has been used for many *S. aureus* hospital outbreaks[204]. However, whole genome sequencing is becoming more popular due to increased affordability, greater discriminatory power it offers, and shortened time to results with PFGE often taking two-three days to achieve results[205]. Despite, increased affordability, WGS remained too expensive for these experiments and therefore PFGE was chosen as the method with the greatest sensitivity for cost alongside clear criteria for interpreting the results.

Tenover et al. provided criteria for interpreting PFGE results from small sets of isolates based on the assumption that they are epidemiologically related. The interpretative criteria rely on the further assumptions that the isolates are recent progeny of a single original organism (in this case the first catheter colonised by the organism), that strains will have the same genotype and that epidemiologically unrelated isolates will not have the same genotype[177]. This was the basis for including a control organism of the same species that was isolated from a catheter or urine specimen of an unrelated patient. The results showed that the control organisms were unrelated to the isolates from the volunteer's catheters, which was important for validating that epidemiologically unrelated isolates

generate unique restriction patterns. Tenover et al. also recommend that these criteria be used for analysis of isolates within an approximate three month period, possibly because in an outbreak setting theoretically the outbreak has been controlled and the isolates are less likely to be related epidemiologically. The isolates for this study by PFGE were collected over one year, during which time isolates may naturally accumulate mutations. The clear epidemiological link between the isolates will need to be taken into consideration during interpretation of the PFGE results.

The choice of restriction enzyme was based on recommendations in the literature, particularly provided by Tenover et al. The decision to use *Xba*I for digestion of *Citrobacter koseri* was challenging as there are few PFGE studies of *C. koseri*. Nawaz et al. used *Spe*I to digest *Citrobacter freundii, Citrobacter amalonaticus,* and *Citrobacter bakii* from catfish[206], Shao et al. used *Xba*I to digest *C. freundii*[207], and Kanamari used *Sfi*I to digest ESBL-producing *C. freundii* and *C. koseri*[208]. Due to the variability of restriction enzymes used and the lack of guidance in the Tenover et al. criteria, *Xba*I was chosen on the recommendations by Tenover et al. and the PulseNet protocol[177, 181] and use by Shao et al. for digestion of other *Citrobacter* DNA[207]. The digestion with *Xba*I yielded 12-14 bands, which had sufficient discriminatory ability to differentiate the non-epidemiologically related control and those from that were epidemiologically related. Tenover et al. required at least 10 bands for their interpretative criteria to apply and therefore, *Xba*I was deemed suitable for this study.

Alternatively, Tenover et al. and many other protocols recommend *Sma*I for digestion of *S. aureus*[177, 209] but it yielded seven DNA fragments of the isolates from the urinary catheter and also from the control. The seven fragments of the control DNA were all of a different length to the seven fragments of the isolates from the volunteer's urinary catheter so there was sufficient discriminatory power for this small study. In a study with more samples or populations that are highly clonal, it would be prudent to consider using other molecular techniques to enhance the fingerprinting discrimination[209].

2.5.5 Statistical analysis

Statistical analysis and graphing were carried out using GraphPad Prism 7 software. Normality was assessed by preparing a histogram of the data. The number of organisms in the catheters followed a normal distribution, but the quantity of organisms in the collected catheters did not and the data was therefore transformed to a logarithmic scale to normalise the data. This was carried out to ensure data were comparable.

2.5.6 Summary

A novel method of quantifying, separately, colonising organisms attached to the catheter lumen and balloons was developed. Sixty-one urinary catheters were collected from patients at NUHT. Enterobacteriaceae, *Pseudomonas* spp., and enterococci were the most commonly isolated organisms. The findings from the lumen and balloon did not correlate with the available CSU results from these patients during catheterisation with the collected catheter. Furthermore, the CSU results did not appear to influence antibiotic treatment. A significantly higher proportion of catheters from patients known to be receiving antibiotics were colonised by multi-drug resistant organisms compared to catheters from patients not known to be receiving antibiotics during this catheterisation. Antibiotic administration for CAUTI or otherwise did not reduce the burden of microorganisms or the number of species of microorganisms colonising the lumen or balloons of urinary catheters. In total, 97% of catheters in this study were colonised within 10 days of catheter insertion. There was no correlation between the burden of microorganisms nor the number of species per catheter and the duration of catheterisation.

The longitudinal collection of urinary catheters from a single volunteer over one year provided nine suprapubic catheters for analysis. The same four organisms were isolated from the nine catheters and their relationship was investigated by PFGE. This study supports the idea of a catheter microenvironment and colonising organisms that are part of the catheter environment that recolonise the catheter with each subsequent catheterisation. DNA restriction patterns determined by PFGE demonstrated

that indistinguishable MSSA, *E. faecalis*, and *C. koseri* isolates colonised one volunteer's urinary catheter over the year. Five indistinguishable *P. aeruginosa* isolates colonised catheters 2-6 and then subsequently colonising *P. aeruginosa* were closely related but accumulated mutations. The population is stable despite probable exposure to antibiotics. The patient did not develop symptoms of CAUTI during the year. This reinforces CSU results, which inevitably are positive for bacteruria, but should not be used for diagnosis of CAUTI. It also suggests instead that despite catheter changes, appropriate hygiene measures, and antibiotics, a stable colonising, non-pathogenic bacterial community may form on urinary catheters of long-term catheter users.

Chapter 3. Studies of the antimicrobial urinary catheter and its effect on mineral encrustation and MDR organisms

3.1 Introduction

A successful antimicrobial urinary catheter would not only prevent MDR organisms but in preventing colonisation by organisms that cause mineral encrustation and blockage it could also prevent the need for early catheter changes as a result of blockage. The ability of the AUC to prevent colonisation by MDR organisms and blockage-causing organisms will be investigated in this chapter. Furthermore, the surface characteristics of the AUC which could predispose to bacterial attachment and mineral attachment will also be investigated.

Previous studies of the antimicrobial activity of the AUC have shown that it has protective activity against *E. coli* (including an ESBL-producing strain) and MRSA for 12 weeks, *K. pneumoniae* for approximately eight weeks, *P. mirabilis* for approximately 11 weeks, and *E. faecalis* for approximately one week[77, 131]. In light of the prevalence of enterococci in urinary catheters as determined in Chapter 2, and the increase in MDR bacteria, this chapter will further assess the spectrum of activity of the AUC. The drug content of the AUCs after 12 weeks of constant flow will be measured by high-performance liquid chromatography (HPLC).

This chapter is divided into studies of the ability of the catheter to resist encrustation and the ability of the catheter to prevent colonisation by MDR organisms with some similar methods.

3.1.1 Mineral encrustation of urinary catheters

Mineral encrustation results from the deposition and crystallisation of minerals on the urinary catheter lumen, balloon, or eyelet holes[12]. The deposition of crystals on the catheter surfaces can cause blockage of the catheter resulting in bypassing of urine or retention, and may also cause damage to the bladder or urethra when the catheter is removed[129, 210]. Bacteria that produce the enzyme urease are generally held responsible for

catheter blockage as the urease enzyme breaks down urea into ammonia and carbon dioxide; the ammonia causes the pH to become alkaline thus resulting in minerals precipitating out of the urine[211]. Infection with the urease-producer *Proteus mirabilis* is considered the main culprit for the formation of crystalline biofilms, mineral encrustations, and blockages of urinary catheters [212].

Mineral encrustation is a substantial problem for long-term catheter users and its management requires significant resources from the community and hospitals. A study of long-term catheter users over two years (457 patients in year one and 467 patients in year two) showed that there were 506 emergency referrals to local hospitals and district nurses, predominantly for the management of catheter blockage[213]. There are several strategies to minimise mineral encrustation and blockage including drinking lemon juice to increase the pH at which crystallisation occurs (nucleation pH), to a more alkaline pH[211, 214]. However, trials in patients with kidney stones, who have a similar urinary mineral composition, demonstrated that lemon juice did not improve the pH of the urine[215, 216]. Simple solutions such as increasing fluid intake to dilute the minerals in the urine, and acidifying the urine by cranberry juice or ascorbic acid have not had clinically successful outcomes[217]. Catheter maintenance solutions, which can be instilled into the bladder through the catheter, may help to break up pre-formed crystals if the solution is acidic. However, the instillation process, if not done gently, can cause pressure on the bladder lining and the acidity of the solution can cause tissue irritation and inflammation[217, 218].

In terms of catheter technology, there are no commercially available catheters that can reduce or prevent mineral encrustation. In a study of the time to blockage of 18 different catheter types from various manufacturers, all catheters perfused with artificial urine inoculated with *P. mirabilis* blocked within 56 hours, although some blocked quicker than others [78]. One experimental anti-encrustation urinary catheter was electrified by running a current across silver probes relying on iontophoresis and the introduction of metal ions into cells using an electrical current, to delay encrustation to 156 hours as compared to 24-48 hours of encrustation for the control [219].

One strategy which modifies the composition of the balloon inflation fluid is to inflate the balloon with triclosan as a method of delivering triclosan to the bladder. At a concentration of 10 mg/mL (1.0%) of triclosan, the silicone catheters inoculated with *P. mirabilis* did not block and the pH of the urine surrounding the balloon in the bladder model did not become alkaline during the seven day test period[220]. A 0.3% triclosan solution in 10 mL syringes is now being marketed and sold under the name Farco-fill® Protect by Clinimed Ltd, for use as a balloon inflation solution. They claim Farco-fill® Protect will extend the lifetime of the catheter by 14-24 days, but the average catheter length in situ using Farco-fill® is 23 days[90], so this may still not be suitable for those who would like to keep their catheter for three months.

There is a clinical need for a catheter that resists encrustation over the life of a long-term urinary catheter. It is important to confirm firstly, that this antimicrobial-impregnated urinary catheter's surface characteristics does not predispose to mineral encrustation. Secondly, it is important to understand if it is also able to resist mineral deposition in the presence of urease-producing bacteria, particularly *P. mirabilis.*

3.1.1.1 **Surface roughness and mineral encrustation**

Mineral encrustation of urinary catheters is a multi-faceted event, which is influenced by the presence of bacteria, as discussed above, but is also influenced by urinary pH, the concentration of solutes in the urine, length of exposure, and the material of the catheter[221]. The novel antimicrobial urinary catheter described herein aims to reduce mineral encrustation by reducing the presence of bacteria in the catheter, particularly urease-producing bacteria which may influence the urinary pH. The impregnation process of adding the antimicrobials to the catheter material should ideally not increase the surface roughness as this is correlated with increased mineral deposition and bacterial attachment. An increased surface roughness offers an increased surface area for greater attachment of bacteria and may protect bacterial cells from shear forces of passing fluid[222].

Likewise, studies of materials used for making urinary catheters and ureteral stents, both prone to mineral encrustation, have shown that silicone is less prone to encrustations than other biomaterials such as polyurethane

and latex [78, 79, 223]. Furthermore, silicone remains less prone to bacterial attachment by urinary pathogens than other biomaterials[224]. Scanning electron microscopy (micro-scale imaging) images of silicone catheters show the silicone surface and eyelet holes to be smoother than latex catheters [225]. However, in the previous work by Fisher, atomic force microscopy (nano-scale imaging) studies indicated that the impregnation process may have increased surface roughness of the AUC after two weeks of soaking in solution[77]. One possible explanation is that the release of the antimicrobial molecules from the silicone matrix might leave behind nano-size pores.

The increase in surface roughness or number or size of depressions left behind as the antimicrobials eluted from the catheter could increase bacterial attachment and biofilm formation. The size and depth of depressions is an important factor when considering surface roughness that predisposes to mineral attachment, as nanorough structures are intentionally manufactured to create mechanical stress on the bacterial cell wall as an antimicrobial biomaterial strategy[97, 98]. The nano-projections of nanorough black silicon are approximately 500 nm tall and spaced approximately 200 nm apart. Bacterial cells are approximately 1 μm and are stressed by the projections as they sit on top rather than fitting into the depressions[98]. These intentionally created structures are unlikely to be found naturally on biomaterials and depressions greater than 1 μm provide increased surface area for bacterial attachment.

An increase in surface roughness or depressions may also increase the number of nuclei available to initiate the crystallisation process. On an atomic or molecular level, nucleation is the first step in the process in which the initial atoms of a crystal arrange into a cluster, known as the nucleus, and then grow larger irreversibly as additional atoms are incorporated into the lattice structure of the crystal[226, 227]. The initial size of the nuclei is approximately 100-1000 atoms[227], so they are just the correct size to fit in nano-sized indentations. This hypothesis is supported by Santin et al., in which they perfused hydrogel-coated ureteral stents with concentrated urine and commented that the hydrogel-coating was degraded by the urine and that this '…[favoured] the formation of irregularities on the surface which may represent preferential sites for the massive deposition of organic matter for the nucleation of crystals[228].

The driving forces of nucleation are supersaturation of the solution, where the critical supersaturation value is the value at which nucleation begins, and the ability to overcome the energy barrier to initiate nucleation. The amount of energy needed decreases as supersaturation increases, so that the more supersaturated the solution is, the greater the rate of nucleation. Furthermore, the presence of a foreign body such as bacteria, drug molecules, or an uneven surface decreases the energy needed to overcome the energetic barrier and reduces the critical supersaturation value making the conditions for nucleation more favourable even in lower saturated solutions[226].

Another factor that may affect crystallisation onto the urinary catheter surface is the nucleation pH, which is the pH at which the precipitation of crystals begins[229]. This value is decreased, meaning that crystallisation begins at a lower pH and the pH is less alkaline, in catheter users labelled as ‘blockers’ or ‘rapid encrusters’ indicating that crystals can begin to precipitate out of solution, and are supersaturated, at a pH closer to the pH of normal urine. Patients with an increased nucleation pH need a more alkaline urine before the crystals in the urine begin to precipitate out of the urine[229, 230]. Again, this is related to supersaturation in which simply diluting the urine, by drinking more fluids will increase the nucleation pH[231]. As in simple crystallisation chemistry, nucleation is influenced by supersaturation and it is possible that supersaturation may be influenced by the catheter surface as it is influenced by solute concentration.

The impregnation process and the release of antimicrobials of the AUC influences surface roughness, and in turn, whether this increases bacterial attachment and mineral encrustation will be investigated by several assays. Bacterial attachment will be assessed by a Time Kill 100 assay and the surface roughness will be quantified by atomic force microscopy.

3.1.1.1.1 Time Kill 100 assay

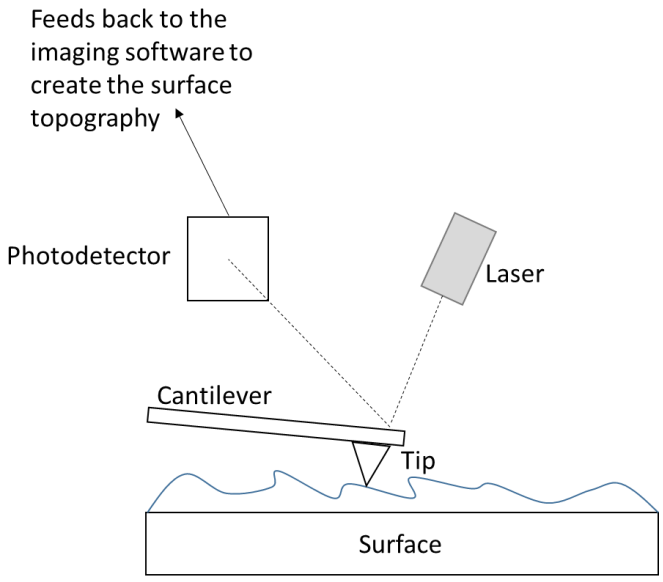
Bacterial attachment, as an indicator of surface roughness and surface area available, were assessed by a Time Kill 100 assay (TK100), which assesses the ability of a biomaterial to kill 100% of attached bacteria within 96 hours[130, 232]. Therefore, it is able to measure bacterial attachment over 96

hours. *P. mirabilis, S. saprophyticus*, and *E. coli* will be used as the test organisms for encrustation experiments with *P. mirabilis* as a urease positive organism with the greatest activity, *S. saprophyticus* with lesser urease activity, and *E. coli* as a urease negative control[211].

3.1.1.1.2 Atomic force microscopy

Atomic force microscopy (AFM) quantifies surface roughness changes of the silicone catheter surface at the nanometre scale. AFM is a type of scanning probe microscopy, in which a tip/probe interacts with the surface of the sample and then can provide an atomic-level resolution of the topography of the sample[233]. Specifically, the tip is mounted on a piezoelectric element, on a cantilever, which oscillates. The piezoelectric element converts the force between the tip and surface to energy to maintain the tip at a constant distance from the surface. The tip can then move sideways along the surface to trace the elements of the surface[234]. The movements of the tip are detected by the changes in the amplitude of the oscillations of the cantilever. A laser constantly fires on the head of the cantilever and the deflections of the light correlating to the deflections of the cantilever are read by a photo-detector. The measured deflections are processed by imaging software which then prepares a topographical image (Figure 3-1).

Figure 3-1 Simple schematic of the essential components and set-up of an atomic force microscope.



Within AFM there are three main modes: contact mode, non-contact mode, and tapping mode[235]. Contact mode is not suitable for delicate surfaces as the tip essentially is dragged across the surface and the repulsive force between the surface and tip is measured. The non-contact mode is useful for more delicate samples, in which the tip maintains a constant height above the surface, and the adjustment of the probe to maintain that height is measured[236]. In tapping mode, the cantilever oscillates at a constant frequency and amplitude, so that as the forces at the sample surface interact with the probe, the oscillation of the cantilever changes. When the probe intermittently contacts with the surface the change in oscillation amplitude is used to detect the surface heights[235].

3.1.1.1.3 Effect of soaking in artificial urine (AU)

Bacterial attachment and mineral encrustation were assessed according to not only whether the catheter was an all-silicone control or antimicrobial-impregnated, but also according to exposure to AU over three time points. Soaking in AU and its effect on the surface is important for several reasons. Firstly, soaking in AU will cause diffusion of antimicrobial molecules from the catheter to the surrounding solution, possibly leaving behind nano-sized pores, which could encourage bacterial attachment or encrustation, as alluded to above. Secondly, previous work showed that one hour soaking in AU produced a conditioning film[77], which may predispose to increased mineral encrustation[228] and bacterial attachment[237, 238].

The conditioning film is a protein layer composed of proteins derived from the urine that may attract other proteins and crystals[239]. A flow model of ureteral stents perfused with supersaturated urine demonstrated that differences in the composition of the urine and therefore the protein conditioning film, did not influence the composition of crystallisation; the presence of an organic conditioning film is fundamental for crystallisation, not its composition[228]. The proteins of the conditioning film provide receptor sites for bacterial attachment to act as a bridge between the biomaterial surface and the bacterial cells[240]. In this way, a conditioning film could facilitate mineral deposition and also bacterial attachment.

3.1.1.2 Quantifying mineral encrustation in the antimicrobial catheter

Several models have been proposed for detecting and measuring mineral encrustation in catheters including an in vitro bladder model, in which AU supplied to the 'bladder' can collect in a bladder chamber and then flow through the catheter[225]. Another flow model perfuses AU over biomaterial disks in a Modified Robbins device[241]. Encrustation can be visually examined by scanning electron microscopy or crystal composition can be identified and quantified by atomic absorption spectroscopy, infrared spectroscopy or dispersive x-ray analysis[241, 242].

In this study, the ability of the AUC to prevent or reduce the amount of mineral encrustation was tested by a modified in vitro flow challenge model[109, 130] in which AU was perfused through the plain control and AUC in the presence and absence of *P. mirabilis*. Time to blockage and quantification of the crystals (phosphate) were the two main outcome measures. Additionally, scanning electron microscopy was used to visualise the encrustations and X-ray photoelectron spectroscopy of the lumens was used to identify and quantify the composition of the crystals.

3.1.1.2.1 In vitro flow challenge model

The in vitro flow challenge model has been previously described to examine the efficacy of antimicrobial biomaterials to resist weekly bacterial challenges under flow conditions[109, 130]. This same model will be repurposed to examine the ability of biomaterials to resist mineral encrustation and blockage.

Artificial urine[243] was chosen as the perfusion fluid as its constituents are standardised unlike human urine, which varies by donor, diet, time of day and many other factors. AU has been shown to produce crystals similar to those in normal urine[244]. While the minerals for crystallisation are present in AU the many crystallisation promoters and inhibitors are absent[245]. However, having a standardised medium that can be made up regardless of donations was more advantageous to this experiment, which required upwards of 200L.

Mineral encrustation was quantified by detection and quantification of phosphate. Phosphate was chosen as an indicator of mineral encrustation as

the crystals associated with alkaline urine are magnesium ammonium phosphate crystals ($NH_4MgPO_4 \cdot 6H_20$) and as stated previously phosphate promotes crystalluria[10]. Phosphates are commonly monitored in water, detergent, and juice samples for quality assurance purposes and therefore there are several existing spectrophotometric methods available[246-248], which could be adapted for these experiments. A few samples of interest were also analysed by SEM and x-ray photoelectron spectroscopy (XPS). Therefore the aim of this study was to use a spectrophotometric method to quantify the amount of phosphate deposited on control and AUC lumens in the absence and presence of bacteria when incubated with AU as a quantifiable method of crystal formation.

3.1.1.2.2 Scanning electron microscopy

SEM provides a magnified (between 10-10,000x) three-dimensional image of a sample's surface topography by irradiating the surface with a fine electron beam[249]. When the electron beam interacts with the atoms on the surface of the sample x-rays, secondary electrons, and backscattered electrons are produced. Typically, secondary electrons are the most useful for observing surface topography as they have weak energy and therefore are emitted only close to the sample surface[250].

The sample is scanned by the electron beam in a vacuum chamber and therefore biological specimens need to be preserved by fixation and dehydration to prevent deformation and charging of the sample. Non-conductive materials, such as silicone, need to be made conductive to prevent decay by the electron beam and enhance the contrast of the surface[249, 251]. A thin layer of metal is sputter-coated onto the non-conductive samples, and it is important that the metal coating is thin so that the structures within the sample are not distorted or obscured. Gold and platinum are common choices, with carbon, iridium, and tungsten useful for more high-resolution imaging[251].

The fixative cross-links the proteins and lipids in the samples to maintain the structural integrity of the sample during dehydration, and such fixative agents include glutaraldehyde and osmium tetroxide[251] as well as

cold acetone. Typical dehydrating agents include ethanol, acetone, ether, chloroform, and tetramethylsilane[252].

3.1.1.2.3 X-ray photoelectron spectroscopy (XPS)

XPS is a useful tool for identifying and quantifying the elemental composition of the topmost 10 nm of a sample's surface[253]. All elements, except hydrogen and helium, and information about the binding of the elements can be detected. To detect and quantify this sensitive information, x-rays irradiate the surface and interact with the core electrons of the surface elements[254]. By the photoelectric effect, photoelectrons are emitted as a result. The energy needed to displace the electron can be detected and analysed. The energy identifies the element and a peak is generated by the total photoelectrons of that energy to quantify the element[254, 255]. The binding energy may vary slightly when atoms are bonded to one another so information as to the structure of the material can also be obtained.

3.1.2 Efficacy of the AUC in preventing colonisation

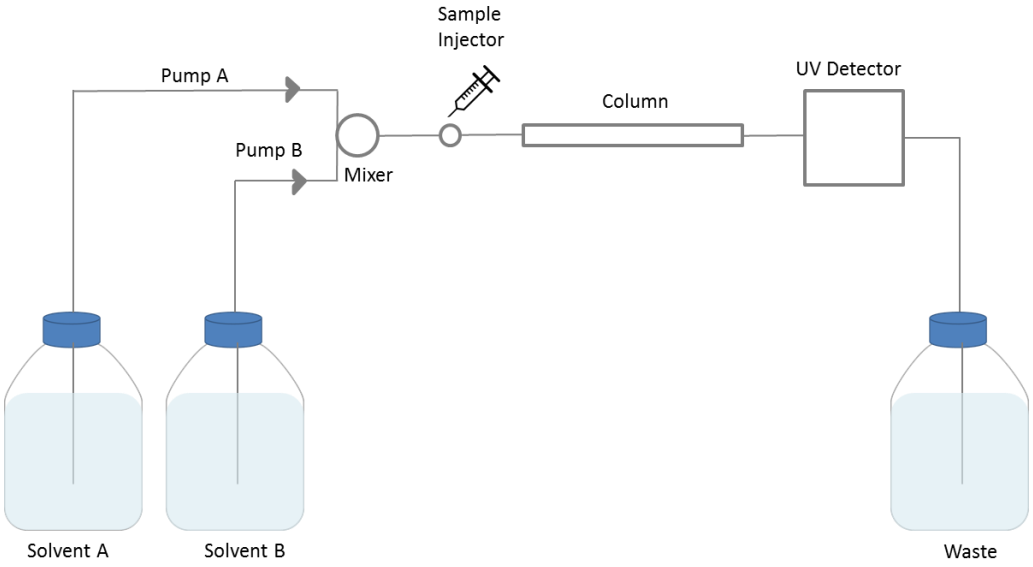
The efficacy experiments employ a TK100 assay as a screening assay to determine the ability of the AUC to kill attached microorganisms. The efficacy was confirmed by the in vitro flow challenge model perfused with 20% tryptone soya broth (TSB) for up to 12 weeks as opposed to AU for use in the encrustation experiments. The AUCs used in the in vitro flow challenge model will be saved for drug content analysed by high-performance liquid chromatography (HPLC) to determine drug content after 12 weeks of constant flow.

3.1.2.1 Drug content quantification-HPLC

Chromatography uses solvents to separate molecular components of a sample[256]. In liquid chromatography, the molecular analytes are separated as they are transferred between a mobile and stationary phase. The sample is dissolved in a solvent (compatible with the mobile phase) and then mixed with the mobile phase and forced through a column (stationary phase)[257]. As the mobile phase and sample pass through the column the molecular components are separated according to size and charge. As they exit the column they are detected by ultraviolet absorption[256]. The mobile phase

choice is dependent on the type of column and solubility with the solvent, and in reversed-phase high-performance liquid chromatography (referred herein simply as HPLC), the type used in these experiments, a mixture of two solvents can be used (Figure 3-2).

Figure 3-2 Schematic diagram of high-performance liquid chromatography set-up



Mobile phases can be pure water, methanol, or ethanol, acetonitrile or other salts, acids, or bases can be added to provide conditions at a specific pH. A gradient, in which two solvents are mixed, is used to increase certain concentrations in the mobile phase and can be used to help achieve separation and prevent the mobile phase from interfering with the measurements. In reversed-phase HPLC the stationary phase is a column packed with silica particles bound with hydrocarbons, and the retention time in the stationary phase is determined by hydrophobic interactions. A molecular component with a larger hydrophobic surface area will be retained longer and therefore have a higher retention time[256]. The type of hydrocarbon sidechain bound to the silica in the stationary phase will be determined by whether the molecular components of interest are polar or non-polar. The more hydrocarbons the more non-polar the stationary phase, and therefore non-polar compounds will be retained better than those that are polar[257].

3.1.3 Chapter aims

This chapter aims to determine:

1. The ability of the AUC to prevent or resist mineral encrustation in the presence of encrustation-inducing bacteria and also in their absence based on the surface characteristics of the AUC. This includes studies to understand if the impregnation process and release of antimicrobials affect surface roughness and the rate of mineral encrustation statically and under flow conditions.
2. The spectrum of preventative activity of the AUC especially against MDR organisms and also enterococci and the drug content of the AUCs after 12 weeks of perfusion and bacterial challenge

3.2 Materials and methods

3.2.1 Characterisation of isolates

3.2.1.1 Isolates for encrustation studies

Several isolates each of *P. mirabilis*, *S. saprophyticus*, and *E. coli* were collected from clinical cases for use in the TK100. All isolates screened for use in these experiments were clinical strains from CAUTI cases at Nottingham University Hospitals Trust. Clinical isolates were chosen instead of isolates from a strain bank due to their increased clinical relevance and their representation of the local microbial population. Several of each species were initially selected and their identifiers can be found in Table 3-1. The characterisation process selected for one representative isolate from each species.

Table 3-1: Isolates selected for screening for use in encrustation experiments including identifiers to separate strains of each species

Identifier	Organism name
F2636	*S. saprophyticus*
F2637	*S. saprophyticus*
F2627	*P. mirabilis*
F2629	*P. mirabilis*
F2647	*E. coli*
F2648	*E. coli*

All strains had colony morphology and gram stain reactions recorded. The staphylococci were further characterised by the catalase test. The DNase test was used in lieu of the coagulase test as the majority of *S. aureus* isolates hydrolyse DNA and the majority of other staphylococci do not. For further identification separating *S. saprophyticus* from other coagulase-negative staphylococci, the four isolates were tested for novobiocin resistance [258]. *E. coli* and *P. mirabilis* strains had their oxidase activity assessed by oxidase test strips (Fluka, Buchs, Switzerland). To further identify the isolates, their biochemical profiles were determined by API ID strips (API Staph and API 20E, Biomérieux, Marcy l'Etoile, France).

3.2.1.2 **Isolates for efficacy experiments**

All enterococci, MRSE, MSSA, and ESBL *E. coli* were isolated from the lumens of urinary catheters collected at NUHT. The MRSA isolate was provided by the Clinical Microbiology Department at NUHT and the isolate was from a mid-stream urine sample. The NDM-1 *E. coli* isolates were provided by NHS Dumfries and Galloway from patients with UTI. Of interest, NDM-1 *E. coli* is typically associated with travel abroad, particularly to India and Pakistan, but none of the patients involved had a history of travel or underlying disease. These isolates are the first in Scotland from a patient with no obvious travel or risk factors[62].

3.2.1.3 **Susceptibility testing**

It is important that the isolates selected from the screening process are representative of the species, not only in their biochemical profiles but also antimicrobial susceptibilities. The screening isolates were assessed for their susceptibility to common antibiotics and their susceptibility to the antimicrobials in the AUC.

3.2.1.3.1 Antibiogram

An antibiogram is a useful screening tool for determining the susceptibility of an isolate to a panel of antibiotics and relies on the disc diffusion method. Briefly, a 0.5 McFarland suspension, verified spectrophotometrically (Jenway UV/Vis), of the isolate in phosphate buffered saline (PBS) was swabbed onto Iso-Sensitest agar (ISA). Antimicrobial discs

(Oxoid, Basingstoke, Hampshire, UK), which reflect those that may be used as treatment, were placed on the seeded plate and incubated overnight at 37°C. After incubation, the zone of inhibition diameter around each disc was measured using digital callipers (Digimatic Caliper, Mitutoyo, Andover, Hampshire).

3.2.1.3.2 Methicillin-resistance testing of staphylococci

The staphylococci were screened for methicillin resistance using a 10µg cefoxitin disc (Oxoid) and incubated overnight at 35°C. To verify that the isolates did not also confer glycopeptide resistance, their sensitivities to vancomycin were determined by a MIC gradient test (Etest, bioMérieux) according to the EUCAST guidelines[180].

3.2.1.3.3 Detecting vancomycin resistance in enterococci

According to EUCAST guidelines, all enterococci were screened for susceptibility to vancomycin (using a 5µg disc) by disc diffusion. Vancomycin resistance was confirmed by MIC gradient test (Etest, bioMérieux).

3.2.1.3.4 ESBL production by *E. coli*

ESBL screening was carried out by disc diffusion to cefotaxime (5µg), ceftazidime (10µg), and cefpodoxime (10µg) discs. If resistant to any of the three, then a confirmatory test was performed. Of the four confirmatory tests Etest ESBL strips are the most accurate and precise[259, 260].

Etest ESBL CT/CTL and TZ/TZL (biomérieux) contain the cephalosporin at one end of the strip, and the cephalosporin and clavulanic acid at the other. Briefly, a 0.5 McFarland solution in PBS was swabbed onto Mueller-Hinton agar (MHA). Excess moisture was allowed to soak into the agar for 15 minutes before E-test strip application. It was incubated at 35°C for 16-20 hours. Results were interpreted according to the manufacturer's instructions[261].

3.2.1.3.5 Carbapenemase production

The initial screening test was a disc diffusion test using meropenem (10 µg) and imipenem (10 µg, Oxoid). If the zone diameter was less than the screening cut-off provided by EUCAST, the isolates were confirmed for specific carbapenemase production using phenotypic detection methods.

Specifically, metallo beta-lactamase activity was detected using the Total Metallo-beta-lactamase Confirm Kit: MBLs (Rosco Diagnostica, Taastrup, Denmark) and the procedure was carried out according to the manufacturer's instructions. Briefly, for each isolate, a 0.5 McFarland suspension in PBS was prepared and swabbed onto two MacConkey agar (Oxoid) plates. One plate, a synergy plate, had a dipicolinic acid (DPA) tablet placed in the centre of the plate with an imipenem (10 µg) tablet placed approximately 10 mm to the side (edge to edge) and a meropenem (10 µg) tablet placed approximately 10 mm to the other side of the DPA tablet. On the second plate an imipenem (10 µg) tablet, an imipenem + DPA, and an imipenem + EDTA are evenly placed. Plates were incubated overnight at 35°C. Results were interpreted according to the manufacturer's instructions and EUCAST MBL diagnostic criteria.

The manufacturer's instructions for interpreting the results for Enterobacteriaceae can be found in Table 3-2. Simply, the EUCAST diagnostic criteria for a MBL is a zone diameter of <25 mm for meropenem (10 µg) and then synergy with DPA and EDTA compared to the zone diameter of the 10 µg meropenem disc[180]. Synergy was defined as enhancement of the zone diameter towards the central DPA disc.

Table 3-2 Criteria for interpreting results of metallo-beta lactamase confirmatory testing according to the manufacturer's instructions for the Total Metallo-beta-lactamase Confirm Kit: MBLs (Rosco Diagnostica) [262]

Criteria	**Result**
Zone diameters of imipenem (10 µg), imipenem + DPA, **and** imipenem + EDTA discs are within 3 mm of each other	MBL negative
Difference in zone diameter of ≥5 mm between imipenem (10 µg) and Imipenem + DPA	MBL positive
Synergy between DPA **and** imipenem (10 µg) **and** meropenem (10 µg)	MBL positive

3.2.1.3.6 Susceptibility to antimicrobials in the impregnated catheter

The disc diffusion method provides quick and easy results of susceptibility of an organism, but it does not provide the MIC, which is a defined value as to the minimum amount of drug needed to inhibit growth of an organism. The MIC can be determined by E-test strip, which contains a predefined gradient of antibiotics, or by broth microdilution assay in which

growth is examined in wells of increasing antibiotic concentrations. Broth microdilution assay was chosen as MIC gradient strips for sparfloxacin and triclosan were not commercially available.

For the broth microdilution assay, again a 0.5 McFarland suspension was prepared using bacteria grown overnight on blood agar. Then, 100 µL of cation-adjusted Mueller-Hinton Broth (MHB, Sigma-Aldrich) was added to each well of a 96 flat-bottomed well plate (Nunclon Delta Surface, Thermo Fisher Scientific, Roskilde, Denmark). Mueller-Hinton broth supplemented (MH-F) with 5% lysed horse blood and 20 mg/l β-nicotinamide adenine dinucleotide (β-NAD hydrate, Sigma-Aldrich) was used for the enterococci. Initial broth microdilution assays with MHB of the enterococci did not consistently support a clear visible growth. MH-F broth was prepared by preparing a 20 mg/mL β-NAD solution in sterile deionised (DI) water and filtering through a 0.2 µm membrane (Minisart, Sartorius Stedim Biotech, Göttingen, Germany) into a sterile Bijou bottle. This was stored at -20°C ± 2°C until use. Mechanically defibrinated horse blood (Oxoid) was diluted 1:2 in sterile DI water. It was then frozen at -20°C ± 2°C overnight and then defrosted at room temperature, and this was repeated an additional six times. After it had been defrosted for the last time, it was centrifuged at 14,000 rpm for 10 minutes. The clarified blood was added to pre-prepared and cooled MHB (Sigma). To prepare the 300 mL of MH-F needed, 30 mL of clarified lysed horse blood was added to 270 mL of MHB which had been prepared with enough powder for 300 mL. The 20 mg/mL β-NAD solution was defrosted and 300 µL was added to the broth to give a final concentration of and 20 mg/L[263]. MH-F broth was stored at 4-8°C until use.

In triplicate, 100 µL of the drug standard was added to the first well. The drug standard for triclosan (Irgasan, BASF Schweiz AG, Basel, Switzerland) and rifampicin (Sigma) was 64 mg/L and 12.8 mg/L for sparfloxacin (Sigma). Then, 100 µL from the first well was transferred to the second well to give a 1:2 dilution. This was continued for 11 wells, and the twelfth well contained only TSB (positive control). Ten microliters of the bacterial suspension were added to each well and incubated overnight at 37°C.

MIC was determined by reading the well before where turbidity was first noticed, showing the lowest concentration capable of inhibiting growth. The minimum bactericidal concentration (MBC) was determined by removing 10 µL from non-turbid wells and plating onto blood agar for all isolates except *P. mirabilis*, which was spotted onto CLED agar. CLED was chosen for *P. mirabilis* as it prevents swarming to allow quantification of individual colonies. After overnight incubation at 37°C, the MBC was determined by the lowest concentration that did not grow.

Susceptibility to rifampicin of the staphylococci was determined by EUCAST clinical breakpoints[264]. EUCAST breakpoints were not available for enterococci to rifampicin. However, current Clinical and Laboratory Standards Institute (CLSI) guidelines do provide breakpoints of ≤1.0 mg/L as susceptible to rifampicin and ≥4.0 mg/L as resistant for enterococci[265]. Gram-negative rods are intrinsically resistant to rifampicin.

The MIC breakpoint for sparfloxacin was described by Fuchs et al. of ≤1.0 µg/mL, which is in agreement with the current EUCAST MIC breakpoint of ≤1.0 mg/L for staphylococci to ciprofloxacin. The current EUCAST MIC breakpoint for enterococci to ciprofloxacin and levofloxacin is 4.0 mg/L[264], which is in agreement with the CLSI breakpoint for ciprofloxacin, but not levofloxacin whose breakpoints are one-fold higher[265]. The EUCAST ciprofloxacin breakpoint for Enterobacteriaceae to ciprofloxacin, ofloxacin, and moxifloxacin is 0.25 mg/L and 0.5 mg/L for levofloxacin.

Triclosan is not orally or systemically administered, even though it will be present in the serum due to exposure to medical devices and cosmetic products. Triclosan MIC breakpoints are therefore not standardised as such. Morrissey et al. provide epidemiological cut-off values (ECOFFs), which are based on the normal distribution of MICs. All MICs within the normal distribution are considered to be the wild-type, meaning resistance has not been acquired. This is slightly different from the clinical breakpoints, which are based on the likelihood of treatment failure to define resistance. The ECOFFs provided in the paper by Morrissey et al. (Table 3-3) for staphylococci, enterococci, and *E. coli* will be used to interpret the results of the microtitre assay for triclosan in the absence of clinical breakpoints[266].

Table 3-3 Epidemiological cut-off values (ECOFFs) for the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) provided by Morrissey et al, 2014 [266]for *S. aureus*, *E. coli, E. faecalis, and E. faecium*

Organism	MIC ECOFF	MBC ECOFF
S. aureus	0.5	2
E. coli	2	16
E. faecalis	16	32
E. faecium	32	64

3.2.2 Biomaterial colonisation ability

The extent of biofilm formation was initially measured using crystal violet. This model is well-described by O'Toole[267] with modifications by Stepanović et al., of the assay described here[268]. The biofilm was grown by inoculating bacteria cultured overnight into TSB and incubating at 37°C with shaking at 200 rpm for four hours. The bacterial suspension was standardised spectrophotometrically by adjusting the optical density to 0.6-0.7 at 490 nm. Two hundred microliters were added to wells in three columns of a polystyrene, flat-bottomed, tissue culture-treated, 96-well microtitre plate (Nunclon Delta Surface, Thermo Fisher Scientific). A positive control (known biofilm former) and a negative control (uninoculated TSB) were also added accordingly. The plates were incubated at 37°C for 24 hours.

Biofilm was quantified by rinsing the 96-well plate in PBS. Then, 150 µL of methanol (Fisher Scientific) was added to each well and left for 20 minutes. Adding 150 µL of methanol as opposed to 200 µL prevents the pellicle that forms on the surface from being measured. The methanol was removed by flicking the plate which was left to air dry. The wells were stained with 150 µL of 2% crystal violet for 15 minutes and rinsed in PBS until the wells ran clear. After drying in air, 150 µL of ethanol was added to solubilize the crystal violet. This was transferred to a clean 96-well plate and the optical density was measured at 630 nm. The biofilm forming ability of each organism was classified according to the criteria described by Stepanović et al[268].

Bacteria that were unable to form biofilm according to the crystal violet assay above were examined for their ability to attach to a biomaterial as this is possible in the absence of biofilm formation. To determine attachment to

the catheters a modified TK100 assay was carried out. Isolates were grown overnight at 37°C on blood agar. A loopful of each isolate was placed in 20 mL TSB and incubated at 37°C with shaking at 200 rpm for four hours. This provides an ideal environment for log-phase growth. After incubation, the bacterial-TSB suspension was spectrophotometrically adjusted to an optical density of 0.6-0.7 at 490 nm. The adjusted solution was centrifuged at 3000 rpm for five minutes, the supernatant was discarded, and the pellet was resuspended in fresh TSB. 1.0 mL of this solution was added to a sterilised 1.5 mL microcentrifuge tube containing a 1.0 cm longitudinally cut segment of all-silicone tubing (1.0 mm x 3.0 mm, Versilic). This was done in triplicate for all isolates. The microcentrifuge tubes were incubated at 37°C for one hour. After incubation, the silicone segments were removed, rinsed in PBS, and placed into a second microcentrifuge tube containing 1.0 mL 2% TSB (as a maintenance broth) and sonicated for five minutes at 20kHz. The sonicate and its dilutions were spread onto blood agar and incubated overnight at 37°C before counting the colonies.

3.2.3 Antimicrobial impregnation of catheters and catheter segments

The balloon and connection ports of Bard All-Silicone long-term Foley catheters were removed. For the TK100 assay, the remaining tubing was cut longitudinally into 1.0 cm long segments. For the in vitro flow challenge model the catheter was cut in half to create two shorter catheters each approximately 16 cm long each. Those segments of catheters serving as the all-silicone controls were autoclaved. Internal laboratory studies of AUC segment controls, autoclaved segments, and segments sterilised by ethylene oxide produced equivalent zones of inhibition when serially plated onto a bacterial lawn (unpublished data) and it appears that the drugs are stable throughout sterilisation.

The remaining catheter segments were impregnated with a solution of 0.2% w/v rifampicin (Sigma), 1.0% w/v triclosan (Irgasan, BASF), and 1.0% w/v sparfloxacin (Sigma-Aldrich), dissolved in 1 L chloroform (chloroform analytical reagent grade, Fisher Scientific). The catheter segments were

immersed in the antimicrobial-chloroform mixture for one hour. Catheter segments were removed and the chloroform was evaporated over 24 hours after which the segments and catheters were rinsed in ethanol to remove excess antimicrobials that may have aggregated on the surface during drying. The segments were sterilized by autoclaving at 121°C for 15 minutes.

For the chloroform-only control segments, the above process was repeated excluding the addition of the antimicrobials to the chloroform.

3.2.4 **TK100 assay**

The TK100 assay is designed to investigate the ability of a biomaterial to kill 100% of attached bacteria within 72 hours. The catheter segments were soaked in a bacterial suspension that contained a percentage of TSB that allowed bacterial maintenance but not growth. To determine the most appropriate TSB concentration for each strain, the TK100 procedure was carried out (see below), with at least two concentrations of TSB for each isolate. The choice of concentrations of 0.1%, 1.0%, and 2.0% TSB was chosen based on previous TK100 assays[77]. These concentrations of TSB were not sufficient for the enterococci and 4.0% was added for the *E. faecalis* isolates and 8.0%, 25%, and 50% TSB were added for *E. faecium* isolates. The TSB concentration that allowed for survival in the plain control over the 72 hour test period, without an increase or decrease in bacterial numbers, was chosen and used in the following TK100 experiments.

Firstly, the isolates were grown in neat TSB for four hours at 37°C in a shaker incubator at 200 rpm. This phase stimulates early log phase growth in which bacteria actively replicate. Post-incubation, the bacteria-TSB suspension was centrifuged at 3000 rpm for five minutes. The supernatant was drained off and the bacterial pellet was resuspended in the previously determined TSB concentration. This bacterial suspension was adjusted spectrophotometrically to A_{490} 0.6 – 0.7, and 1.0 mL was aliquoted into 1.5 mL sterile microcentrifuge tubes (Sarstedt). A previously processed all-silicone or AUC segment was added to the bacterial suspension and incubated at 37°C for one hour.

Three of the catheter segments of each series were sampled at 0, 24, 48, and 72 hours, in which the segment was removed using sterile forceps,

rinsed in an appropriate TSB concentration without bacteria, placed in a new microcentrifuge tube containing 1.0 mL PBS, and sonicated for five minutes at 50 Hz. Viable attached bacteria were enumerated by spreading 200 µL onto blood agar for all isolates except *P. mirabilis*, which was plated on to CLED. The plates were incubated at 37°C overnight. Those segments not being sampled at that time point were removed, rinsed, replaced in fresh TSB of the appropriate concentration, and incubated at 37°C.

3.2.4.1 Soaking of AUC segments for TK100 investigating mineral encrustation

The experimental groups included all-silicone and AUC segments each soaked for one hour, one week, and two weeks in AU, including a silicone - only control with no soaking. A chloroform - only control that was impregnated without the antimicrobials was also used, as the chloroform acts as a swelling agent and may alter the surface alone in the absence of antimicrobial molecules. The chloroform was removed from antimicrobial impregnated and chloroform - only controls by evaporation under constant flow before TK100 testing. Please refer to Table 3-4 for the control and experimental groups used in each TK100 assay per isolate.

Table 3-4: Description, abbreviation, and purpose of the control and experimental groups used in the TK100 assay. AU: artificial urine

Experimental Groups	Abbreviation	Purpose
All-silicone catheter segments not soaked in AU for any length of time	Control (no soaking)	To control for the effect of soaking for the plain and AUC segments
All-silicone catheter segments which underwent the impregnation process in the absence of any antimicrobials and were not soaked in AU	Chloroform only (no soaking)	To control for the effect of the impregnation process on the AUC segments
All-silicone catheter segments soaked in AU for 1 hour	Plain – 1 hour	1 hour of soaking will form a conditioning film on the catheter surface
All-silicone catheter segments soaked in AU for 1 week	Plain – 1 week	1 week soaking in AU to imitate exposure of the catheter to urine
All-silicone catheter segments soaked in AU for 2 weeks	Plain – 2 weeks	2 weeks soaking in AU to imitate exposure of the catheter to urine
All-silicone catheter segments impregnated with antimicrobials and soaked in AU for 1 hour	AUC – 1 hour	1 hour of soaking to form a conditioning film and to imitate antimicrobials lost to urine after 1 hour
All-silicone catheter segments impregnated with antimicrobials and soaked in AU for 1 week	AUC – 1 week	1 week of soaking to imitate antimicrobials lost to urine after 1 week
All-silicone catheter segments impregnated with antimicrobials and soaked in AU for 2 weeks	AUC – 2 weeks	2 weeks of soaking to imitate antimicrobials lost to urine after 2 weeks

For consistency, AU was employed to soak the catheter segments to simulate exposure to urine. The recipe for AU can be found in Table 3-5.

Table 3-5 Recipe for artificial urine [131].

Chemical	Amount (g/L)
Ammonium chloride	1.0
Calcium chloride	0.49
Potassium chloride	1.6
Sodium chloride	4.6
Magnesium chloride hexahydrate	0.65
Sodium sulphate	2.3
Trisodium citrate dihydrate	0.65
Disodium oxalate	0.02
Potassium dihydrogen phosphate	2.8
Gelatin	5.0
Urea	25.0

The above chemicals (Sigma) were weighed out and dissolved in 1.0 litre of deionised (DI) water. The pH was adjusted to 6.1 using 1M sodium hydroxide (Fisher Scientific). The AU was sterilised by membrane filtration

using 0.2 µm cellulose acetate filters (Whatman, GE Healthcare Life Sciences, Buckinghamshire, UK), and pulled through the filter using a dry vacuum pump (25100 dry vacuum pump/compressor, Welch, Skokie, IL).

After sterilisation of the AU, the segments were added to the AU and incubated at 37°C for either one hour, one week, or two weeks with rocking to ensure all surfaces were exposed.

3.2.4.2 **Extended TK100**

To determine how closely the TK100 may predict the success of the AUC in the in vitro flow challenge model, a novel extended TK100 was developed. The TK100 protocol as described remained essentially unchanged except that at each time point, the AUC catheter segments that were sonicated and quantified, were kept in the correct TSB percentage rather than disposed of. The saved segments were transferred into fresh TSB at each time point to ensure the antimicrobials did not build-up in the TSB to allow continuous diffusion from the AUC catheter segment. If at the end of the 72 hours, 100% of the attached bacteria had been killed, the saved segments and a new set of control catheter segments were reinoculated the following week and tested in the same way. This was continued until the AUC catheter segments failed to kill 100% of attached bacteria with 72 hours. This was only carried out for the enterococci.

3.2.5 **Atomic force microscopy**

Using the same experimental and control groups as the TK100 (Table 3-4), plus additions of AUC non-soaked and AUC segments soaked in DI water for one week, the catheter segments were processed and soaked according to the methods in Section 3.2.4.1.

For imaging by AFM, a small section of the 1.0 cm longitudinally cut catheter segment was cut away, placed on a microscope glass slide with the lumen side of the segment facing up. The slide was pre-coated with Araldite glue and dried. The samples were imaged using a Bruker ICON FastSCan Bio microscope in Peakforce in air mode. Tap150A tips with an 8nm radius were used in the microscope. Analysis of the micrographs and calculations of

the surface roughness of samples was carried out using the Nanoscope Analysis 1.5 software (Bruker Corporation).

3.2.6 Static model of mineral encrustation

To quantitatively measure the ability of the AUC to reduce or delay mineral encrustation, a colourimetric assay to measure phosphates precipitating out of artificial urine was developed. This assay is based on the method developed by Mahadevaiah et al., in which phosphate concentration correlates to a blue colour intensity which can be measured spectrophotometrically[247].

3.2.6.1 Reagent preparation

The preparation of the reagents can be found in the method by Mahadevaiah et al.[247], but the stock solution concentrations are described below:

- 5.528×10^{-3} M ammonium molybdate (Hopkin and Williams)
- 2.0833×10^{-3} M sodium sulphide (Scientific Laboratory Supplies, Nottingham, UK). Due to the instability of aqueous sodium sulphide, the solution was prepared fresh each day.
- 0.25 N sulphuric acid (Fisher Scientific)
- 8.066×10^{-4} M disodium hydrogen phosphate (BDH Laboratory Supplies) stock solution. The working solution was created by adding 5.0 mL of this solution to 50.0 mL of water.

3.2.6.2 Creating the calibration plot

The calibration plot was created using disodium hydrogen phosphate. 0.5 mL of ammonium molybdate solution was added first, then 3.0 mL of 0.25 N sulphuric acid, and then the disodium hydrogen phosphate in increasing volumes (with parts per million (ppm) predetermined), and finally 1.0 mL of sodium sulphide solution. The solutions were left to stand at room temperature for 20 minutes and absorbance was measured against MilliQ water. The samples were measured spectrophotometrically at 715 nm.

The phosphate concentrations in each standard for the calibration plots were determined in mg/L of phosphate ion (PO_4^{3-}). Disodium hydrogen

phosphate (Na_2HPO_4) was used to prepare the standards (Figure 3-3). In an effort to not measure the additional hydrogen and sodium added with the phosphate, the molar mass of phosphate (94.9714 g/mol) was divided by the total molar mass of disodium hydrogen phosphate (141.96 g/mol) to determine that 66.9% of each standard is composed of phosphate. This was taken into account when determining phosphate concentration in the calibration plot standards.

Figure 3-3 Calibration plot phosphate standards showing the effect of dilution on the intensity of the colour blue

3.2.6.3 **Method development of experimental samples**

The phosphate concentration in sterile AU was determined by adding 1.0 mL of AU to the solution of 0.5mL ammonium molybdate and 3.0 mL 0.25 N sulphuric acid in triplicate. One millilitre of sodium sulphide was added and left at room temperature for 20 min. All experiments were carried out in triplicate and the mean was interpolated from the standard curve.

To determine if precipitated phosphate could be detected sensitively by this assay, AU was incubated with either *S. saprophyticus* F2637, *P. mirabilis* F2629, or *E. coli* F2647 for 24 hours in microcentrifuge tubes. After incubation, they were centrifuged for five minutes at 3000 rpm. The supernatant was poured off the solution and the pellet containing precipitate was resuspended in 1.0 mL sterile DI water by pipetting. The suspended pellet was added to the reagents and measured spectrophotometrically.

3.2.6.4 **Phosphate precipitation in the absence of bacteria**

AU was prepared aseptically and adjusted to pH of 6.1, 7.1, or 8.3 using 1 M sodium hydroxide. In triplicate, 1.0 mL AU was added to a sterile

microcentrifuge tube for each of the eight sampling time points. The tubes were incubated at 37°C for 0, 4, 24, 48, 72, or 96 hours. Post-incubation, the microcentrifuge tubes were centrifuged at 3000 rpm for five minutes. The supernatant was removed by pipetting and the pellet of precipitate was resuspended in 1.0 mL sterile DI water. The 1.0 mL of the resuspended pellet was added to the reagents as described in Sections 3.2.6.1-2.

3.2.6.5 Phosphate precipitation in the presence of bacteria

S. saprophyticus (F2637), *E. coli* (F2647), and *P. mirabilis* (F2629) were subcultured on blood agar overnight at 37°C. Each strain was inoculated into 20.0 mL of TSB and incubated at 37°C for four hours with shaking at 200 rpm. Post-incubation, the TSB suspensions were standardised to an optical density of 0.6-0.7 at A_{490}. 10.0 µL of the bacterial suspension was added to pre-prepared filter-sterilised AU (adjusted to pH of 6.1) in triplicate at eight time points and sampled as previously described in Section 3.2.6.1-2. At each time point, the pH of the inoculated AU was determined by pH indicator strips (spectral 5.0-9.0, Hydrion, Micro Essential Laboratory Inc, New York, USA).

3.2.6.6 Static model of phosphate attachment to catheter segments

2.0 cm long segments were cut from all-silicone long-term urinary catheters (12Ch, Bard Medical). One set was individually placed in 1.5 mL microcentrifuge tubes and autoclaved at 121°C for 15 minutes to sterilise. The second set was impregnated according to the methods in Section 3.2.3, individually placed in 1.5 mL microcentrifuge tubes, and sterilised.

F2629 *P. mirabilis*, F2627 *S. saprophyticus*, and F2647 *E. coli* were subcultured overnight on blood agar at 37°C. After overnight incubation, a loopful of the fresh culture was mixed in 20.0 mL TSB. The bacterial broth suspension was incubated for four hours at 37°C with shaking at 200 rpm. The bacterial suspension was then adjusted to an optical density of 0.6-0.7 at 490 nm. 5.0 µL of the bacterial suspension was added to 50.0 mL filter-sterilised AU (adjusted to pH of 6.1) in triplicate. An additional 3 X 50.0mL filter-sterilised AU was also prepared and then adjusted to a pH of 6.1, 7.1, or 8.3 using 1M sodium hydroxide.

1.0 mL of the AU solution was added to the catheter segment in the 1.5 mL microcentrifuge tube and incubated at 37°C with shaking at 200 rpm for 0, 4, 24, 48, or 72 hours. At the end of the incubation period, the catheter segments were removed and placed into a new 1.5 mL microcentrifuge tube containing 1.0 mL autoclaved milliQ water. The segments in water were then sonicated for five minutes. After sonication, the 1.0ml of sonicate was added to the reagents and measured spectrophotometrically as discussed in Section 3.2.6.1.

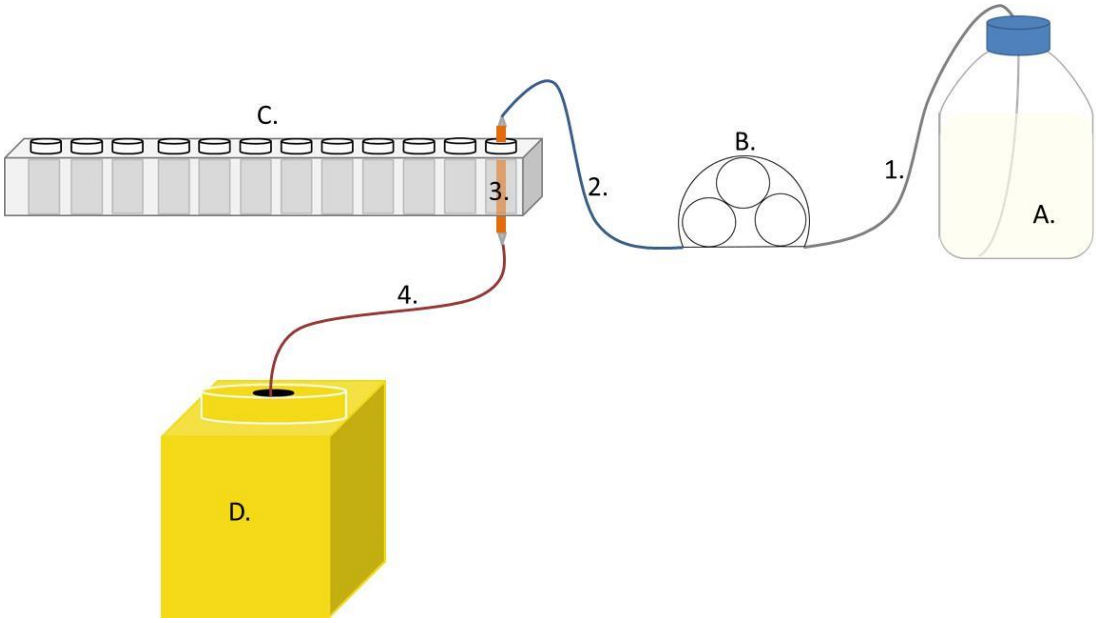
3.2.7 In vitro challenge model

The in vitro challenge model has been described previously in the literature[130 232 269]. It is a clinically predictive model to determine the protective lifetime of an antimicrobial biomaterial against weekly bacterial challenges. This model was employed in this manner to determine the ability of the AUC to resist colonisation by MDR organisms and enterococci. It was also adapted to perfuse AU through all-silicone catheters and AUC to determine the time to blockage and phosphate deposition on the catheter lumens in the presence and absence of *P. mirabilis.*

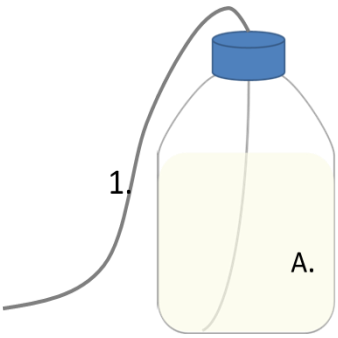
3.2.7.1 Set-up of the perfusion model

A detailed set-up of the model is described in the diagram (Figure 3-4) and panels below. Briefly, the catheters were aseptically inserted into a multi-chamber water jacket. The water jacket was maintained at 37°C by a heating circuit. Media of either AU or 20% TSB was perfused from a 2 L bottle via a series of pump tubing that passed through a peristaltic pump which regulated flow and connected to the catheters. The waste AU was then collected in a 30 L waste collection container.

Figure 3-4: Diagram of the set-up of the in vitro flow model and as adapted to serve as a perfusion model of encrustation. Tubing is labelled numerically and apparatus is labelled alphabetically. A. artificial urine reservoir and TSB reservoir *depending on the experiment, B. peristaltic pump, C: multichannel water jacket, D: waste collection container. 1: Reservoir tubing, 2: Pump tubing, 3: urinary catheter (either all-silicone or all-silicone and antimicrobial impregnated), 4: Outlet tubing



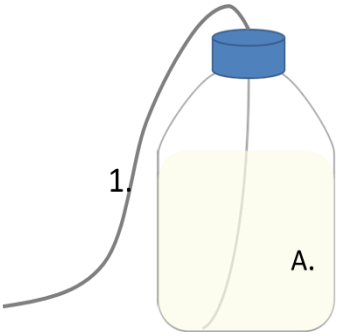
Preparation and Assembly: Artificial Urine Reservoir (Encrustation assay)*



1. The ingredients, with the exception of urea and potassium dihydrogen phosphate, for 2.0 L of AU were weighed out according to the recipe in Section 3.2.4.1. 1.5 L of DI water was added to these ingredients
2. The partially complete AU was sterilised by autoclaving at 121°C for 15 minutes
3. A 28g/L solution of potassium dihydrogen phosphate was prepared and sterilised by vacuum filtration using 0.2 µm cellulose nitrate filters (Sartorius Stedim Biotech GmbH, Göttingen, Germany)
4. A 150 g urea/900 mL DI water was prepared and sterilised by vacuum filtration using 0.2 µm cellulose nitrate filters
5. 1.0 L of 1M sodium hydroxide solution was prepared and autoclaved at 121°C for 15 minutes
6. After sterilisation of the above solutions and the autoclaved solutions had cooled below 50°C, 300 mL of the filtered urea solution and 200 mL of the potassium hydrogen phosphate solution were added to the 1.5 L of partially complete AU for a total volume of 2.0 L.
7. The pH of the AU was adjusted to 6.1 ± 0.05 with the sterile 1M sodium hydroxide. The pH probe was sterilised with a rinse with 99.0% ethanol, a rinse with a 1:10 concentrated solution of Distel

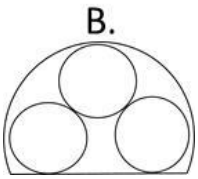
Disinfectant (Distel High-Level Laboratory Disinfectant, Tristel, UK), and two rinses with sterile DI water to remove the cleaning agents.

Preparation and assembly: TSB reservoir (Efficacy assay)*



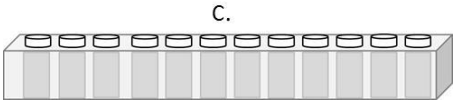
1. 20% TSB was prepared according to the manufacturer's instruction
2. A hole was drilled into the lid of 2L Duran bottles to allow enough silicone tubing 1 mm x 3mm (Scientific Laboratory Supplies, Nottingham, UK) to go through the lid and touch the bottom of the reservoir.
3. The other ending of the tubing (1.) was covered with aluminium foil and clamped with surgical clamps
4 The clamps were taped to the outside of the reservoir and a piece of aluminium foil covered the top of the reservoir and most of the tubing except the end covered in aluminium foil. The sheet was taped to the reservoir
5. The assembled reservoir was autoclaved at 121°C for 15 mins. It needed 24 hours of cooling before it could be used in the model

Preparation and assembly: peristaltic pump



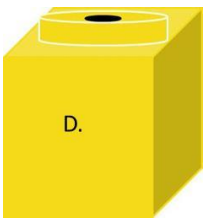
A multichannel cassette pump (Watson-Marlow Ltd, Falmouth, UK) regulated the flow of the artificial urine. The flow rate for these studies was 20 mL/hour for 8 hours Monday – Friday and 4 mL/hour overnight and on the weekends. This was to mimic decreased urine flow at night and to prevent major spillage of artificial urine in the event of catheter blockage and subsequent leakage.

Preparation and Assembly: Multichannel Water Jacket



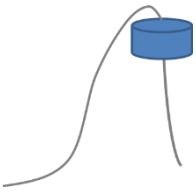
1. A specially made water jacket, consisting of 14 channels, was made by the University of Nottingham Medical Engineering department.
2. The water jacket was filled with distilled water and maintained at 37°C by a heating circuit (FH16-D, Grant Instruments, Cambridge, UK).

Preparation and assembly: waste collection container



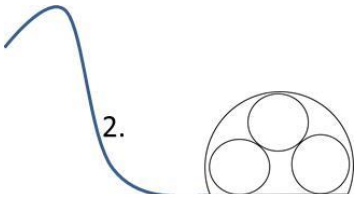
1. A 30 L sharps container was used to collect the waste artificial urine
2. Distel (Tristel, Snailwell, UK) was added to the sharps container to kill remaining bacteria in the spent perfusion medium.
3. The ends of the outlet tubing were spacing evenly and taped in place to prevent cross-contamination between the ends.

Preparation and assembly: reservoir tubing



1. A 3 mm hole was drilled into the lids to the 2 L glass Duran bottles serving as the AU reservoirs
2. Silicone tubing 1 mm x 3 mm (Scientific Laboratory Supplies) was inserted through the hole with enough tubing to reach the bottom of the AU reservoir.
3. To seal the space between the hole of the lid and the silicone tubing aluminium foil was wrapped around the tubing and lid and taped in place.
4. A glass connector (1mm diameter) was inserted into the end of the tubing to connect to the pump tubing.
5. Both ends were covered with aluminium foil and autoclaved to sterilise.

Preparation and assembly: pump tubing

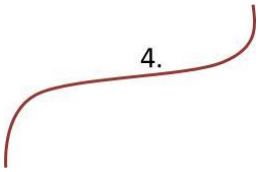


1. Marprene tubing for peristaltic pumps (Watson-Marlow Ltd.) was connected to a long section of 1 mm x 3 mm silicone tubing by a 1 mm diameter glass connector
2. The ends of the entire piece of tubing were covered with aluminium foil and autoclaved.

Preparation and assembly: catheter tubing

1. The balloon and connection ports were removed from 100% all-silicone, 16 Ch long-term urinary catheters (BARDIA® AQUAFIL®, Bard Medical) by scalpel and discarded.
2. Nine catheters were impregnated with antimicrobials
3. A hole of sufficient size was made through 14 x 22 mm silicone bungs (SLS, Nottingham UK). These bungs fit and seal the channels within the water jacket.
4. Non-impregnated catheters and AUC were inserted into the hole in the bungs. The bungs were glued around the catheter using silicone rubber compound-flowable fluid (RS components Ltd., Corby, UK), at a distance approximately 4.0 cm from the end of the catheter.
5. At the ends of the catheter tubing, a plastic connector (Watson-Marlow Ltd.) was inserted.
6. The ends were covered in aluminium foil and sterilised by autoclaving at 121°C for 15 minutes.

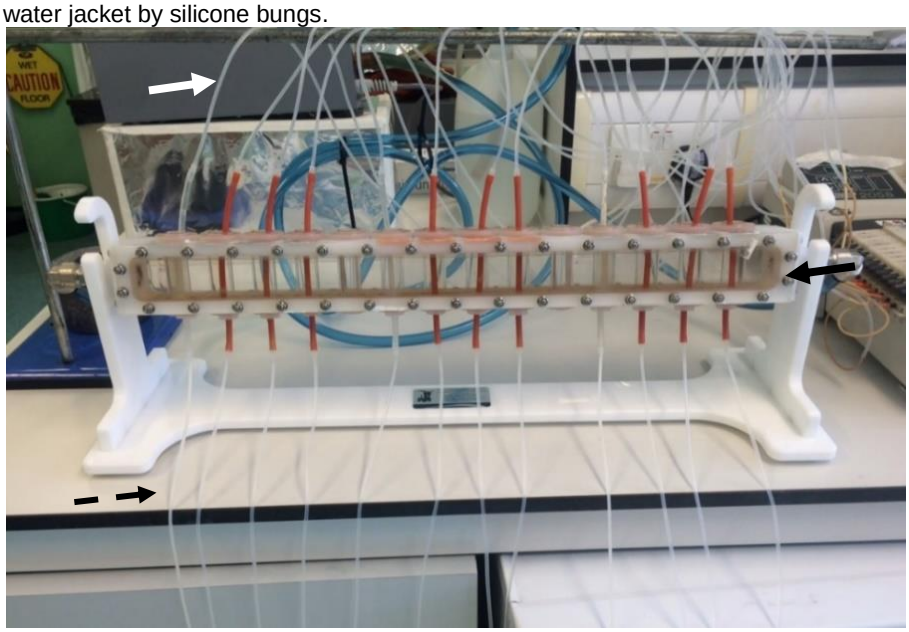
Preparation and Assembly of Outlet Tubing



1. 1 mm x 3 mm silicone tubing was cut into lengthy sections.
2. The ends of tubing were covered with aluminium foil and autoclaved.

Three antimicrobial catheters per bacteria were each aseptically inserted into a channel in a multi-channel water jacket (solid black arrow in Figure 3-5). One all-silicone catheter (16 Ch, Bard Medical) control per bacteria of interest was placed aseptically into the model alongside the antimicrobial ones. Water maintained at 37°C by a heating circuit was circulated through the water jacket. The channel surrounding each catheter was filled with DI water and replaced weekly to prevent a build-up of antimicrobials in surrouding water in the channel.

Figure 3-5 The set-up of the in vitro challenge model. In this figure, the first four catheters on the left were inoculated with MSSA, the middle four with MRSE, and the last four on the right with MRSA. In the MRSA all-silicone control there is visible bacterial growth**.** The solid black arrow indicates the water jacket, the dashed black line indicates outlet tubing, and the white arrow indicates the inlet tubing. The red-orange catheters are those that have been antimicrobial impregnated and they are held in the water jacket by silicone bungs.



The outlet tubing (dashed arrow in Figure 3-5) was connected to the base connector of the catheter tubing and drained into a waste collection container. The inlet tubing (white arrow in Figure 3-5) was connected to the top connector of the catheter tubing and threaded into a cassette of the peristaltic pump. The inlet tubing was connected to the reservoir tubing by a glass connector. Artificial urine was pumped from the reservoir at 20.0 mL/hour during the day and at 4.0 mL/hour at night and over weekends.

The catheters were challenged weekly with a bacterial inoculum by filling a sterile 2.0 mL syringe with a 10^5 CFU/mL bacterial suspension. The catheter tubing (part 3) was disconnected from the inlet tubing (dashed arrow) and it was tubing was clamped. The inoculum was injected into the

catheter tubing and the outlet tubing (dashed arrow) was clamped. The inoculum was given one hour to attach and then the flow was restarted for an hour. At this point, the connector at the top of the catheter tubing and the inlet tubing were replaced to remove any bacteria that may have entered this section during the inoculation step.

Bacterial growth was monitored after inoculation and then daily to quantify bacterial colonisation. To do so, the outlet tubing was disconnected and the effluent was collected into a sterile bijou bottle. The collected effluent and its dilutions were plated and spread onto CLED agar in 200 µL aliquots for *P. mirabilis* and onto blood agar for the other isolates. The plates were incubated overnight and colonies were enumerated.

For the encrustation studies using AU, the in vitro challenge model ran for 28 days or until blockage of the catheter. Blockage of the catheter was determined by leaking of the system proximal to the test catheter and then subsequent examination by microscope of the catheter lumens for an occlusion.

For the efficacy studies, the in vitro flow challenge model ran for 12 weeks until the AUC failed to eradicate 100% of attached challenge bacterial within the week. The catheters were then saved in the fridge for drug content analysis by HPLC.

3.2.7.2 **Determining phosphate concentration in lumens**

At the end of the 28 day perfusion period or blockage of the catheter, whichever came first, the catheter tubing was removed from the in vitro challenge model. The bung attached to the catheter tubing was cut away and the top of the bung was marked as the section above is the section exposed to ideal conditions inside the 37°C water jacket. A 1.0cm long segment of the catheter tubing was cut away above the top of the bung marking by sterile scalpel and placed in 2.0 mL of cold acetone and refrigerated for future SEM. Another 1.0cm long segment above the top of the bung was cut away by sterile scalpel and placed in a clean glass bottle and refrigerated for future x-ray photoelectron spectroscopy analysis.

The remaining section of the catheter tubing was placed onto a sterile, empty polystyrene petri dish and dried at 40°C for 24 hours for analysis of

phosphate content by the previously described phosphate colorimetric assay. The lid of the dish was lightly lifted to allow any humidity to escape. Excess AU was dried off in this way and only the attached crystals remained in the catheter lumen. After drying, the catheter tubing was filled with approximately 1.0 mL of DI water and the ends were clamped. The clamped tubing was placed in a sterile resealable bag and sonicated for five minutes at 20 kHz. The sonicate was drained into a sterile bijou bottle and was added to 0.5 mL ammonium molybdate and 3.0 mL 0.25N sulphuric acid. One millilitre of sodium sulphide was added and left at room temperature for 20 mins before being read spectrophotometrically at 715 nm.

3.2.7.3 Scanning electron microscopy of catheter segments from the in vitro challenge model

The catheter segments were fixed in cold acetone as described previously until the day of carrying out the microscopy. The 1.0cm long catheter segments were cut longitudinally by sterile scalpel to expose the inner lumen. The lumen samples were dehydrated by tetramethylsilane (Sigma-Aldrich) and then fixed onto specimen stubs (12.5mm diameter, Agar Scientific, Essex, United Kingdom). The samples were gold sputter-coated for 300 seconds and imaged used a Jeol JSM-6060 scanning electron microscope.

3.2.7.4 X-ray photoelectron spectroscopy of catheter segments from the in vitro challenge model

The catheter segments were stored in clean glass vials until the day of processing by a Kratos AXIS ULTRA instrument with a monochromated Al kα X-ray source (1486.6eV). Before analysis, the 1.0 cm catheter segments were cut longitudinally using a clean, sterile scalpel. The samples were mounted lumen side-up on a Kratos sample bar by double-sided tape (Sellotape). The sample bar was inserted into the airlock of the instrument and the pressure was pumped to approximately 3×10^{-7} Torr overnight. It was then transferred to the analysis chamber where the pressure remained at 5×10^{-9} Torr or less. Two areas of approximately 700 x 300 µm from the lumens of the catheter segments were analysed by a wide scan and a high-resolution scan. The data were collected using Kratos VISIONII software and

processed with CASAXPS software (version 2.3.17) with Kratos relative sensitivity factors. Wide scan data were used to calculate the elemental atomic percentage and the high-resolution scan data were used to look more in-depth at small differences of peak shifts.

3.2.8 **HPLC**

AUCs were removed from the in vitro flow challenge model at failure or at the end of 12 or 13 weeks after challenge with MDR organisms and kept in sterile universal containers in the freezer until the drug extraction process. Three 1.0 cm long segments were cut from the middle of each AUC using a sterile scalpel. The segments were weighed.

3.2.8.1 **Drug extraction**

Each segment was placed in a clean, glass vial and 2.0 mL of chloroform was added to each segment and left for an hour to extract the drug. The tops of the vials were covered with aluminium foil to prevent evaporation of the chloroform. After one hour, the segments were transferred to an empty, clean, glass vial to dry for one hour. The drug-containing chloroform was re-covered with aluminium foil. Then 2.0 mL of chloroform was added to the catheter segments and the vials were covered to prevent chloroform evaporation. This was repeated twice for each catheter segment. The drug-containing chloroform was pooled together for each segment and the chloroform was left to evaporate under constant air flow for 24 hours. The drug residue remained in the freezer until 24 hours before analysis by HPLC.

3.2.8.2 **Preparation of standards for analysis**

A solution containing 1000 mg/L of sparfloxacin, rifampicin, and triclosan was prepared in 100% methanol (LC-MS grade, Fisher Scientific) and filtered through a sterile, single-use Minisart filter with a pore size of 0.2 µm (Sartorius Stedim Biotech) into a clean, glass bottle. Dilutions were prepared in 100% methanol and 1.0 mL of each dilution of 1, 5, 10, 50, 100, 200, 300, 400, 500, 600, 700, 800 mg/L was added to a 2.0 mL amber robotic 9mm thread vial with a clear short-thread silicone cap 9 mm with PTFE septa (Chromatography Direct Ltd., Cheshire, UK). Standards were refrigerated in any remaining time before analysis.

3.2.8.3 **Preparation of samples for analysis**

No more than 24 hours before analysis, the drug residues were removed from the freezer and left at room temperature until the condensation was removed from the outside of the container. 1.0 mL of methanol was added to reconstitute the drug residue. When fully re-solubilised each sample was individually filtered through a sterile, single-use Minisart filter with a pore size of 0.2 µm into a 2.0 mL amber robotic 9 mm thread vial with a clear short-thread silicone cap 9mm with PTFE septa. Samples were refrigerated for any remaining time before analysis.

3.2.8.4 **HPLC method**

Analysis was performed by an Agilent 1100 HPLC machine with a variable wavelength UV detector (Agilent Technologies, Berkshire, UK) connected to a Chemstation operating system. The chromatographic separations were performed on an Eclipse XDB-C8 (5µm, i.d. 4.6mm x 150 mm) column (Agilent Technologies). The mobile phase was a mixture of 10% acetonitrile (HPLC grade, Fisher Scientific) and aqueous sodium dihydrogen phosphate (Sigma-Aldrich) adjusted to 15mM and pH 2.5. The organic phase was 100% methanol (LC-MS grade, Fisher Scientific).

A gradient method, in which the percentage of methanol increases over the time of the run, was developed as the solvent front coincided with the peak for sparfloxacin. The gradient method maintained a flow rate of 1.0 mL/min and an injection volume of 5.0 µL with each run lasting eight minutes. The starting solvent concentration was 90% aqueous phase, 10% methanol. The gradient timetable can be found in Table 3-6. Eluted drugs were read at a wavelength of 254 nm.

Table 3-6 Gradient method timetable and percentage of methanol with the converse being the aqueous solvent

Time (min)	% methanol
0:00	10
1:00	90
3:00	90
4:00	10
8:00	10

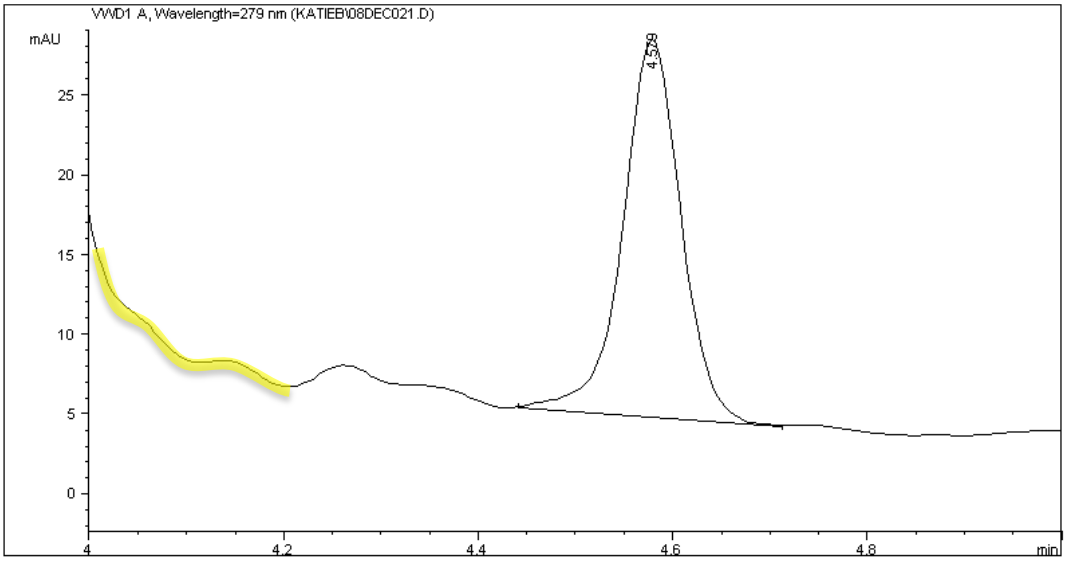
The retention times were approximately 3.0, 3.6, and 4.5 minutes for sparfloxacin, rifampicin, and triclosan respectively. Data were reprocessed by the Chemstation software after integrating the peaks and manually checking for accuracy by scanning each chromatogram by batch analysis. Unknown concentrations of the samples were interpolated from the standard curve by GraphPad Prism 7.01 software. Quantifying the peaks was determined by the signal:noise ratio, which should be greater than 10.0 for each peak to be reliably quantified. The noise determination baseline was determined from 4.00 – 4.20 minutes per chromatogram and was chosen as this is an area close to the peaks of interest.

3.2.8.5 **HPLC method to increase triclosan detection**

The HPLC method described above is more sensitive to sparfloxacin and rifampicin than triclosan as seen by the greater peaks for those two as compared to triclosan at the same concentration. The amount of triclosan remaining in the catheters after 13 weeks and then extracted was a small amount and was originally detected but not quantifiable by the method described previously. Therefore, a more triclosan - sensitive method was employed to quantify triclosan.

The samples were run a second time (24 hours later, during which time they were refrigerated) using the described gradient method (Table 3-6) A 10µL injection volume was used instead of 5.0 µL to increase the drug content detected. 10 µL was the greatest injection volume that could be used as anything beyond this created large sparfloxacin peaks that could overload the equipment. The wavelength was changed from 254 nm to 279 nm as used previously by Fisher, 2011[77] to detect triclosan. The noise:signal baseline was moved from 4.00-4.20 minutes to 4.90 – 5.10 minutes, which is after the triclosan peak due to rifampicin degradation product peaks at 3.7 and 3.9 minutes, which did not resolve completely before 4.00-4.20 minutes and therefore interfered with the noise:signal baseline (Figure 3-6). This was not originally detected in the standards as there were no peaks from rifampicin degradation products as the standards were freshly prepared.

Figure 3-6 Chromatogram of triclosan peak at 4.57 from catheter MRSA 1.2 with the highlighted line indicating the unresolved peak of rifampicin degradation products at 3.7 and 3.9 minutes that interfered with the noise:signal ratio that was originally set from 4.00-4.20 minutes. Read at 279 nm



The drug extracts from the catheters perfused throughout the in vitro flow challenge model were analysed by HPLC in two batches. The first batch consisted of the drug extracts from the AUCs inoculated with MRSA, MSSA, and MRSE in the in vitro flow challenge model. The second batch consisted of the drug extracts from the AUCs inoculated with *S. saprophyticus*, ESBL *E. coli*, and NDM-1 *E. coli*. Both batches were initially read at 254 nm, refrigerated and read again at 279 nm within 24 hours. Rifampicin and sparfloxacin drug content was calculated using the areas under the peak determined at 254 nm. Triclosan drug content was calculated using the areas under the peak determined at 279 nm.

3.2.8.6 **Calculating drug concentrations**

Two values of interest were calculated from the HPLC results after interpolating the sample peak values from the standard curves to give the drug content per catheter segment: total drug content per catheter, and proportional drug content per catheter.

Firstly, to get the total drug content per catheter:

$$\frac{\text{total weight of catheter (g)}}{\text{weight of 1.0 cm segment (g)}} = \text{z}$$

$$\text{z} \times \text{drug content per catheter segment} = \text{total drug content (g)}$$

To then calculate the proportional drug content per catheter the following equation was then employed:

$$\frac{\text{total drug content (g)}}{\text{total weight of catheter (g)}} \times 100 = {}^{\text{w}}/_{\text{w}}\,\%$$

Each drug 1.0 cm drug segment was weighed individually.

3.2.9 **Statistical analysis**

Data were analysed for normality by plotting a histogram of the control group values (GraphPad Prism 7). As there are several groups of experiments, this was done for each control group. Non-parametric data of the TK100 experiments of the encrustation studies and efficacy studies were not normalised due to values of zero which could not have been transformed. These data were analysed by Mann-Whitney U or Kruskal-Wallis depending on the number of comparisons. However, the data from the TK100 studies of the enterococci were normalised by log transformation as the control values were greater than zero and could be transformed. Normal data were analysed by unpaired t-test and one-way ANOVA depending on the number of comparisons. Matched data, such as the matched all-silicone catheters and AUCs in the in vitro flow model, were analysed by paired t-test. To determine unknown sample concentrations determined by HPLC, standard curves were created, and unknown values were interpolated by the software to determine the sample values.

3.3 Results

3.3.1 Characterisation of isolates for encrustation experiments

All isolates displayed characteristics common to their species and all isolates were obtained from ureteral stents. *S. saprophyticus* isolates were positive for catalase, negative for DNase, resistant to novobiocin, and sensitive to cefoxitin.

The Enterobacteriaceae showed characteristics common to each species such as *E. coli* was capable of producing indole and *P. mirabilis* was able to swarm on blood agar. The gram-negative isolates were resistant to ampicillin, which is of interest because *P. mirabilis* and *E. coli* are typically sensitive [270] and all were sensitive to ciprofloxacin. The three *E. coli* isolates had intermediate sensitivity to ceftazidime and cefotaxime, two ESBL production screening antibiotics. Confirmatory testing by MIC gradient strip showed that neither isolate was an ESBL producer.

All isolates were capable of biofilm formation and F2637 *S. saprophyticus* produced moderate biofilms according to the classification by the Stepanović Modification[268]. These isolates were collected from urinary catheters and ureteral stents and therefore are from urological biomaterials infections. It was not surprising that all were able of forming biofilm as this would be the predominant mode of growth on a biomaterial.

3.3.1.1 Susceptibility to antimicrobials in the AUC

The staphylococci were sensitive to rifampicin according to EUCAST clinical breakpoints. *S. saprophyticus* and *E. coli* were sensitive to sparfloxacin according to the EUCAST breakpoints for staphylococci and Enterobacteriaceae to ciprofloxacin, respectively. Using the same criteria *P. mirabilis* had intermediate susceptibility to sparfloxacin and resistance to rifampicin. All displayed wild-type sensitivity to triclosan (Table 3-7). The ECOFFs provided for *E. coli* are also used to infer the sensitivity of *P. mirabilis* as no ECOFFs are available for *P. mirabilis* to triclosan.

Table 3-7 Minimum inhibitory concentration (MIC) of the organisms for use in the encrustation experiments against the three antimicrobials in the antimicrobial urinary catheter. Susceptibility was determined by 1.) EUCAST Clinical Breakpoints for Bacteria v7.1 for staphylococci to rifampicin and 2.) EUCAST Clinical Breakpoints for Bacteria v7.1 of staphylococci and Enterobacteriaceae to ciprofloxacin, 3.) Epidemiological cut-off values (ECOFFs) provided by Morrissey et al., 2014 [266]. WT: wild-type meaning not likely resistance mechanisms acquired; AR: acquired resistance mechanisms possible

		Rifampicin		Sparfloxacin		Triclosan		
F Number	**Organism ID**	**MIC (mg/L)**	**Susceptibility?[1]**	**MIC (mg/L)**	**Susceptibility? [2]**	**MIC (mg/L)**	**Susceptibility?[3]**	**Number of sensitive antimicrobials**
F2636	*S. saprophyticus*	0.016	susceptible	0.8	susceptible	0.25	susceptible (WT)	3/3
F2637	*S. saprophyticus*	0.012	susceptible	0.8	susceptible	0.25	susceptible (WT)	3/3
F2627	*P. mirabilis*	6	resistant	0.8	resistant	1	susceptible (WT)	1/3
F2629	*P. mirabilis*	4	resistant	0.4	intermediate	1	susceptible (WT)	1/3
F2647	*E. coli*	6	resistant	0.025	susceptible	0.5	susceptible (WT)	2/3
F2648	*E. coli*	6	resistant	0.1	susceptible	0.5	susceptible (WT)	2/3

3.3.2 Characterisation of isolates used in efficacy studies

The basic characterisation of bacteria used throughout this chapter can be found in Table 3-8 for the staphylococci, Table 3-9 for *E. coli,* and Table 3-10 for the enterococci. The staphylococci all exhibited typical characteristics of *S. aureus* and *S. epidermidis,* respectively, including colony morphology, catalase production, susceptibility to novobiocin, DNAse production by *S. aureus* isolates, and coagulase production by *S. aureus* isolates. All staphylococci were tested for their antimicrobial activity by antibiogram (Table 3-8) which included detection of methicillin resistance by cefoxitin disc. F4001 MRSE and F4142 MRSA demonstrated methicillin resistance and F3991 MSSA did not. All staphylococci were susceptible to vancomycin.

Table 3-8 Staphylococcal characterisation and susceptibilities for efficacy experiments. MSU; mid-stream urine specimen, MIC; minimum inhibitory concentration, R; resistant, S; susceptible, Vanc,; vancomycin

F Number	Organism ID	Isolated from	Biofilm-Forming Ability	API Profile	Antibiogram										Vanc. MIC
					Penicillin	Tetracycline	Chloramphenicol	Erythromycin	Cefoxitin (methicillin screen)	Trimethoprim	Gentamicin	Clindamycin	Rifampicin	Ciprofloxacin	
F4142	MRSA	MSU	Not a biofilm-former	6726153 97.7% *S. aureus*	R	R	S	S	R	R	R	R	R	R	1.5 mg/L S
F4001	MRSE	lumen of urinary catheter	weak	6706013 93.1% *S. epidermidis*	R	S	S	S	R	R	R	I	S	R	2.5 mg/L S
F3991	MSSA	lumen of urinary catheter	weak	6336153 94.1% *S. aureus*	S	R	S	S	S	S	R	R	S	S	1.5 (mg/L) S

The *E. coli* isolates demonstrated typical characteristics of *E. coli* including colony morphology, indole production and no oxidase activity (Table 3-9). F3802 was incapable of forming biofilm in the crystal violet assay, which is not to say that it is incapable of attaching to a material. The attachment assay showed that the attachment of F3802 NDM-1 *E. coli* to silicone was no different to that of F3986 ESBL- E*. coli*, which was able to form a weak biofilm according to the crystal violet assay (p=0.5388, unpaired t-test).

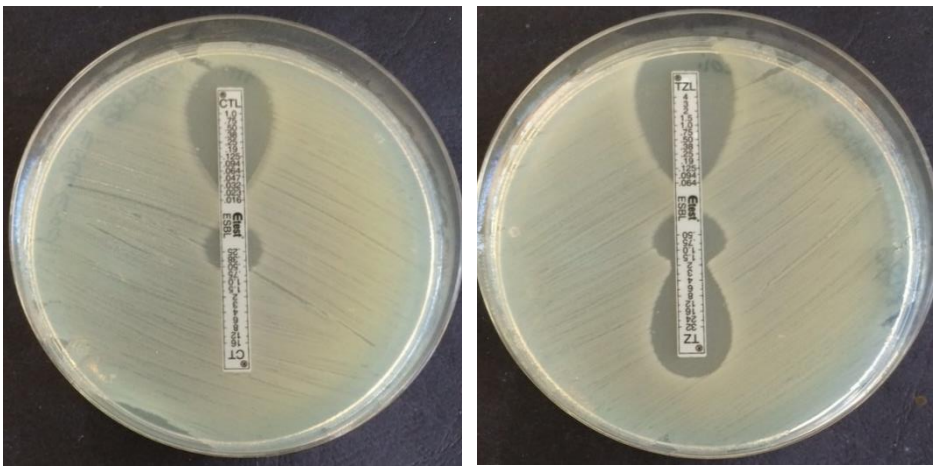
Table 3-9 *E. coli* characterisation and susceptibilities for use in the efficacy experiments. CTX (5µg): cefotaxime, CAZ (10µg): ceftazidime, CPD (10µg): cefpodoxime, MEM (10µg): meropenem, IPM (10µg): Imipenem, R: resistant, S: sensitive

F No.	Org ID	Isolated from	Biofilm-forming ability	API Profile	ESBL Screen			Carba-screen	
					CTX	CAZ	CPD	MEM	IPM
F3802	NDM-1 *E. coli*	MSU	Not a biofilm-former	5144572 99.5% *E. coli*	R	R	R	R	R
F3986	ESBL *E. coli*	lumen of urinary catheter	weak	1144572 69.2% *E. coli*	R	R	R	S	S

F3986 *E. coli* was resistant to all three ESBL screening cephalosporins by disc diffusion and ESBL production was inferred by the ‘phantom zone’ seen in the CT/CTL MIC gradient strips and ‘deformation of the ellipse’ seen in the TZ/TZL MIC gradient strips (Figure 3-7).

Figure 3-7 Images of the Etest ESBL confirmatory testing plates for F3986. The gradient strips contain the cephalorsporin on one end and the cephalosporin with clavulanic at the other. ESBL production can be inferred from a larger zone produced in the presence of clavulanic acid which inhibits the ESBL enzyme and/or by the appearance of phantom zones. CT: cefotaxime, CTL: cefotaxime + clavulanic acid, TZ: ceftazidime, TZL: ceftazidime + clavulanic acid

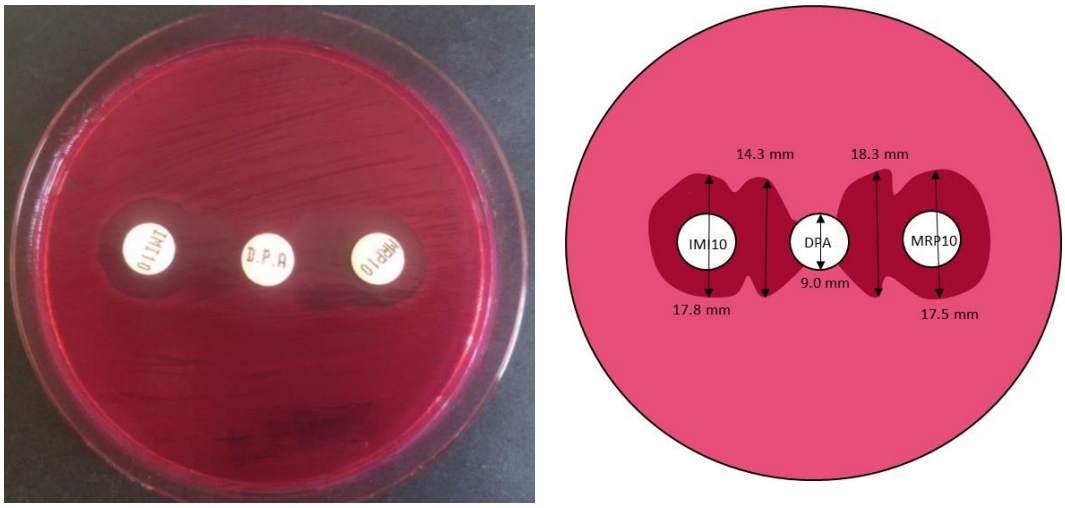
CT/CTL TZ/TZL



Isolate F3802 met the EUCAST and manufacturer's instructions for detection of the MBLs. F3802 was resistant to meropenem and imipenem according to the EUCAST screening guidelines and had zone diameter differences greater than 3.0 mm between Imipenem (10µg), imipenem +DPA, and imipenem + EDTA, which if the zones had been within 3.0 mm would confirm no MBL activity. Whereas isolate F3802 had a zone size slightly slightly under 5.0 mm (≥4.6mm), it clearly demonstrated synergy with DPA as seen in Figure 3-8. There was no zone around the DPA zone alone, but there was an enhancement of the meropenem and imipenem zones between the two discs. F3802, therefore, meets the EUCAST and manufacturer's guidelines for confirmation of MBL production.

Figure 3-8: F3802 *E. coli* (A) synergy plate and (B) diagram of zone sizes of the same plate of the metallo-beta-lactamase confirmatory test according to the manufacturer's instructions (Rosco Diagnostica). DPA: Dipicolinic acid, IMI10: Imipenem (10µg), MRP10: meropenem (10µg**)**

A B



The enterococci showed characteristic traits of enterococci as all were all non-haemolytic on blood agar, were negative for catalase, and were positive for bile aesculin hydrolysis (Table 3-10). F4291 *E. faecalis* was resistant to vancomycin and this was confirmed by its MIC. The biofilm forming ability of the enterococci was variable according to the crystal violet assay.

Table 3-10 Characterisation and susceptibility to vancomycin of enterococci. MIC; minimum inhibitory concentration; S: susceptible; R: resistant

F Number	Organism ID	Isolated from	Biofilm-forming ability	API Profile	Vanc. Disc diffusion	Vanc MIC
F3946	*E. faecalis*	lumen of urinary catheter	weak	5143311 99.3% *E. faecalis*	S	-
F3950	*E. faecalis*	lumen of urinary catheter	Not a biofilm former	5143711 97.0% *E. faecalis*	S	-
F4076	*E. faecalis*	lumen of urinary catheter	Not a biofilm former	514331 99.3% *E. faecalis*	S	-
F4291	*E. faecalis*	lumen of urinary catheter	Not a biofilm former	514331 99.3% *E. faecalis*	R	12.0 mg/L
F4117	*E. faecalis*	lumen of urinary catheter	weak	5143711 99.7% *E. faecalis*	S	-
F4261	*E. faecalis*	lumen of urinary catheter	weak	5143711 99.7% *E. faecalis*	S	-
F3947	*E. faecium*	lumen of urinary catheter	weak	7556110 94.6% *E. faecium*	S	-
F4081	*E. faecium*	lumen of urinary catheter	Not a biofilm former	7556110 94.6% *E. faecium*	S	-
F4206	*E. faecium*	lumen of urinary catheter	Not a biofilm former	5357710 99.8% *E. faecium*	S	-

3.3.2.1 **Susceptibility to the antimicrobials in the AUC**

All *E. coli* isolates were resistant to rifampicin, as expected as they are intrinsically resistant, and all staphylococci were susceptible. Susceptibility of enterococci to rifampicin and sparfloxacin was variable. Interestingly, for the *E. faecalis* isolates if it was resistant/intermediate to sparfloxacin then it was susceptible to rifampicin, with the converse also being true. Of interest, F3802 demonstrated greater susceptibility to sparfloxacin (MIC: 0.8 mg/L) compared to the ESBL-producing *E. coli* isolate (F3986), which had a MIC < 6.4 mg/L (Table 3-11).

Triclosan susceptibility was based on whether it is showing wild-type susceptibility or has acquired resistance mechanisms. The MIC's of F4142 MRSA and the *E. faecalis* isolates indicated that they may have acquired resistance. As the ECOFF values related to the normal distribution of susceptibilities to triclosan rather than the likelihood of clinical cure, it was

possible that despite F4142 MRSA having a non wild-type susceptibility it could still have activity against the organism as its MIC was 1.0 mg/L. This may be less likely for the *E. faecalis* isolates as their MICs were significantly higher at 32.0 mg/L. Alternatively, *E. faecium* isolates with a MIC of 32.0 mg/L and less were considered to be the wild-type without acquired resistance, but this is a high MIC that may not have any efficacy clinically.

Table 3-11 Minimum inhibitory concentration (MIC) of the organisms for use in the efficacy experiments against the three antimicrobials in the antimicrobial urinary catheter. Susceptibility was determined by 1.) EUCAST Clinical Breakpoints for Bacteria v7.1 for staphylococci to rifampicin and CLSI breakpoints for enterococci to rifampicin 2.) EUCAST Clinical Breakpoints for Bacteria v7.1 of staphylococci, enterococci and *E. coli* to ciprofloxacin, 3.) Epidemiological cut-off values (ECOFFs) provided by Morrissey et al., 2014 [266]. WT: wild-type meaning not likely resistance mechanisms acquired; AR: acquired resistance mechanisms possible

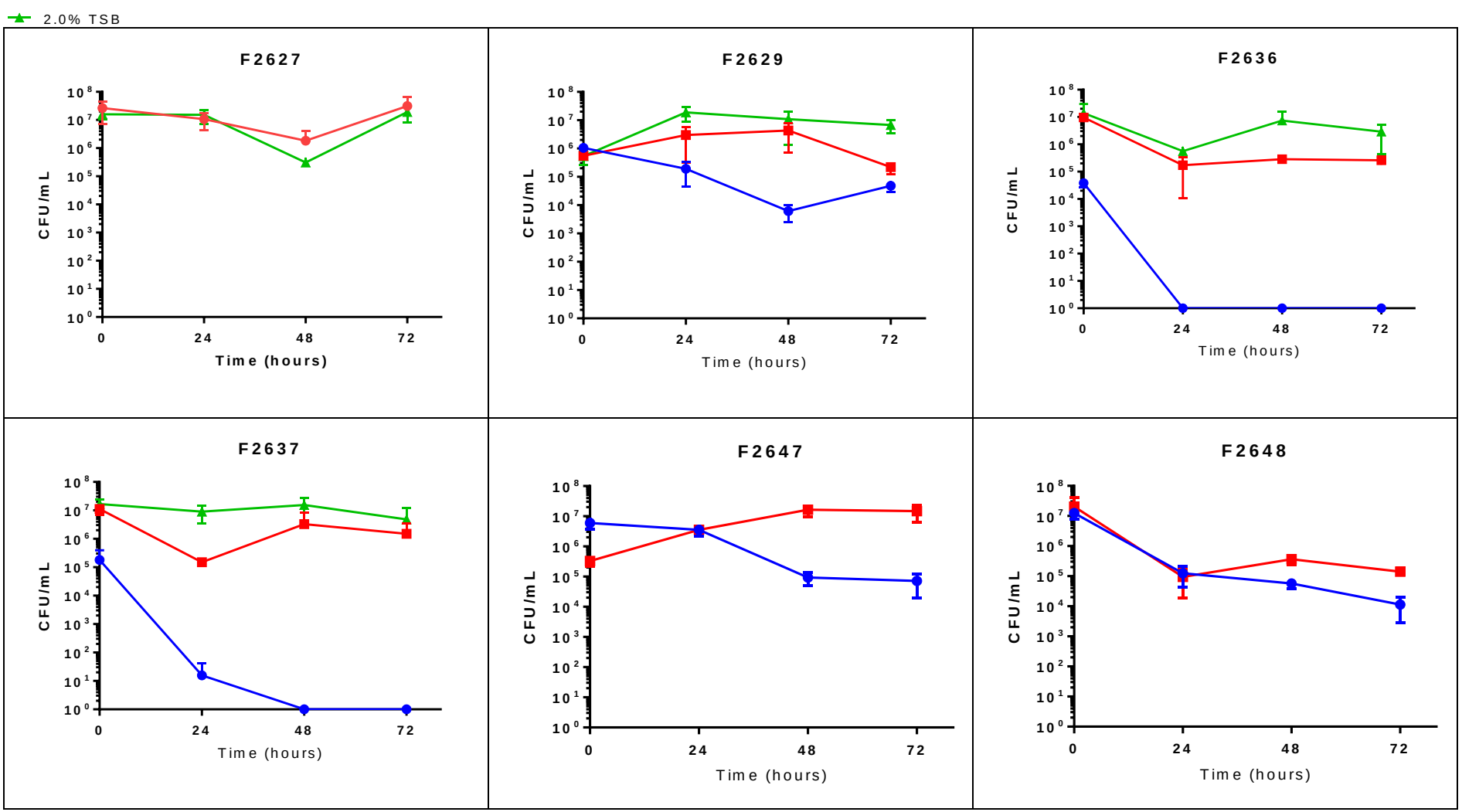
		Rifampicin		Sparfloxacin		Triclosan		**Susceptible to antimicrobials in AUC**
F Number	**Organism ID**	**MIC (mg/L)**	**Susceptibility?[1]**	**MIC (mg/L)**	**Susceptibility? [2]**	**MIC (mg/L)**	**Susceptibility?[3]**	
F3986	ESBL *E. coli*	>2.0	resistant	>6.4	resistant	0.5	susceptible (WT)	1/3
F3802	NDM-1 *E. coli*	>2.0	resistant	0.8	intermediate	0.5	susceptible (WT)	1/3
F3991	MSSA	0.016	susceptible	0.2	susceptible	0.063	susceptible (WT)	3/3
F4001	MRSE	0.016	susceptible	6.4	resistant	0.125	susceptible (WT)	2/3
F4142	MRSA	0.008	susceptible	>6.4	resistant	1.0	resistant (AR)	1/3
F3946	*E. faecalis*	4.0	resistant	0.05	susceptible	32.0	resistant (AR)	1/3
F3950	*E. faecalis*	2.0	intermediate	0.4	susceptible	32.0	resistant (AR)	1/3
F4076	*E. faecalis*	4.0	resistant	0.2	susceptible	32.0	resistant (AR)	1/3
F4291	*E. faecalis* (VRE)	0.25	susceptible	>6.4	resistant	32.0	resistant (AR)	1/3
F4117	*E. faecalis*	2.0	intermediate	0.4	susceptible	32.0	resistant (AR)	1/3
F4261	*E. faecalis*	2.0	intermediate	0.4	susceptible	32.0	resistant (AR)	1/3
F3947	*E. faecium*	0.06	susceptible	0.4	susceptible	32.0	susceptible (WT)	3/3
F4081	*E. faecium*	8.0	resistant	>6.4	resistant	32.0	susceptible (WT)	1/3
F4206	*E. faecium*	8.0	resistant	>6.4	resistant	32.0	susceptible (WT)	1/3

3.3.3 TK100 assay

3.3.3.1 TSB percentage determination

A preliminary TK100 assay was carried out with plain catheter segments in varying TSB concentrations to determine the concentration that maintains growth over 72 hours for the encrustation study organisms (Figure 3-9) and the efficacy study organisms (Figure 3-10). Maintenance of growth was defined as a colony count at 72 hours within one log of the initial colony count at 0 hours. If two TSB percentages met this definition, then the higher concentration was chosen as using a higher concentration is more conservative.

Figure 3-9 Determination of the most appropriate tryptone soya broth (TSB) concentration to maintain growth of *P. mirabilis* (F2627 and F2629), *S. saprophyticus* (F2636 and F2637), and *E. coli* (F2647 and F2648) over 72 hours. Each point represents the mean and the standard deviation

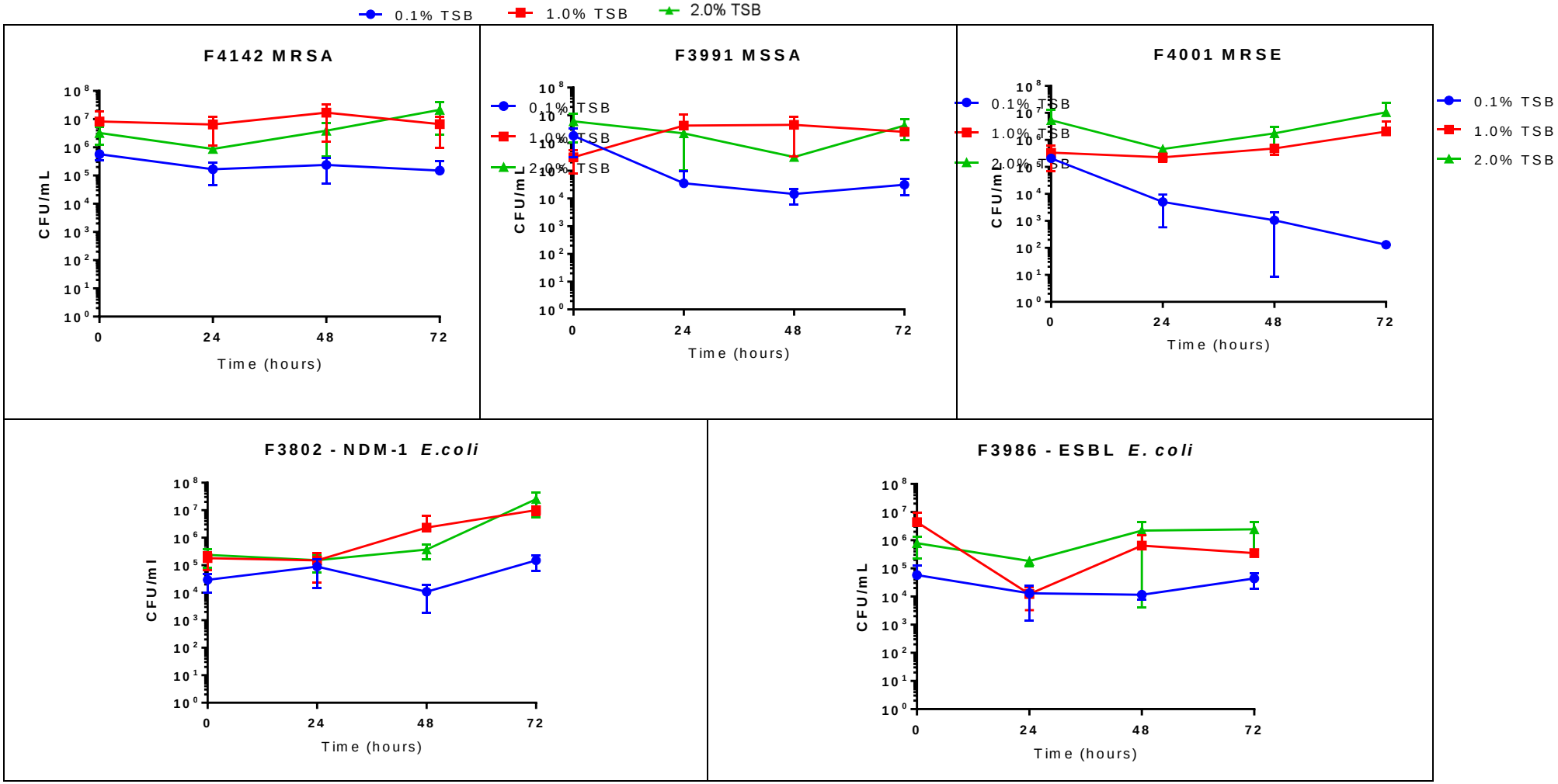


For F2647 *E. coli* and F2648 *E. coli*, neither TSB concentration tested was within one log of the original count at 0 hours. Consequently, 1% TSB was chosen for both isolates as 0.1% TSB decreased bacterial counts over 72 hours and would perhaps facilitate killing by the biomaterial. At least two TSB concentrations for each isolate supported bacterial survival. The chosen concentration is shown in Table 3-12.

Table 3-12 Percentage of tryptone soya broth (% TSB) chosen for each isolate to use in future TK100 assays

Isolate	% TSB concentration chosen
F2627	1.0%
F2629	1.0%
F2636	2.0%
F2537	2.0%
F2647	1.0%
F2648	1.0%

Figure 3-10 Determination of the tryptone soya broth (TSB) for TK100 of methicillin-resistant *S. aureus* (F4142 MRSA), methicillin-susceptible *S. aureus* (F3991 MSSA), methicillin-resistant *S. epidermidis* (F4001 MRSE), F3802 New Delhi metallo beta-lactamase producing *E. coli*, and F3986 extended spectrum beta-lactamase producing *E. coli* over 72 hours. Each point represents the mean and the standard deviation.



The TSB percentage chosen for each isolate for use in the experimental TK100 assays can be found below in Table 3-13. Although F4142 MRSA and F3802 NDM-1 *E. coli* were not capable of producing biofilm according to the crystal violet assay, this initial TK100 demonstrates they are capable of attaching and remaining attached to the relevant biomaterials.

Table 3-13 The percentage of tryptone soya broth (% TSB) chosen for each isolate to use in future TK100 assays

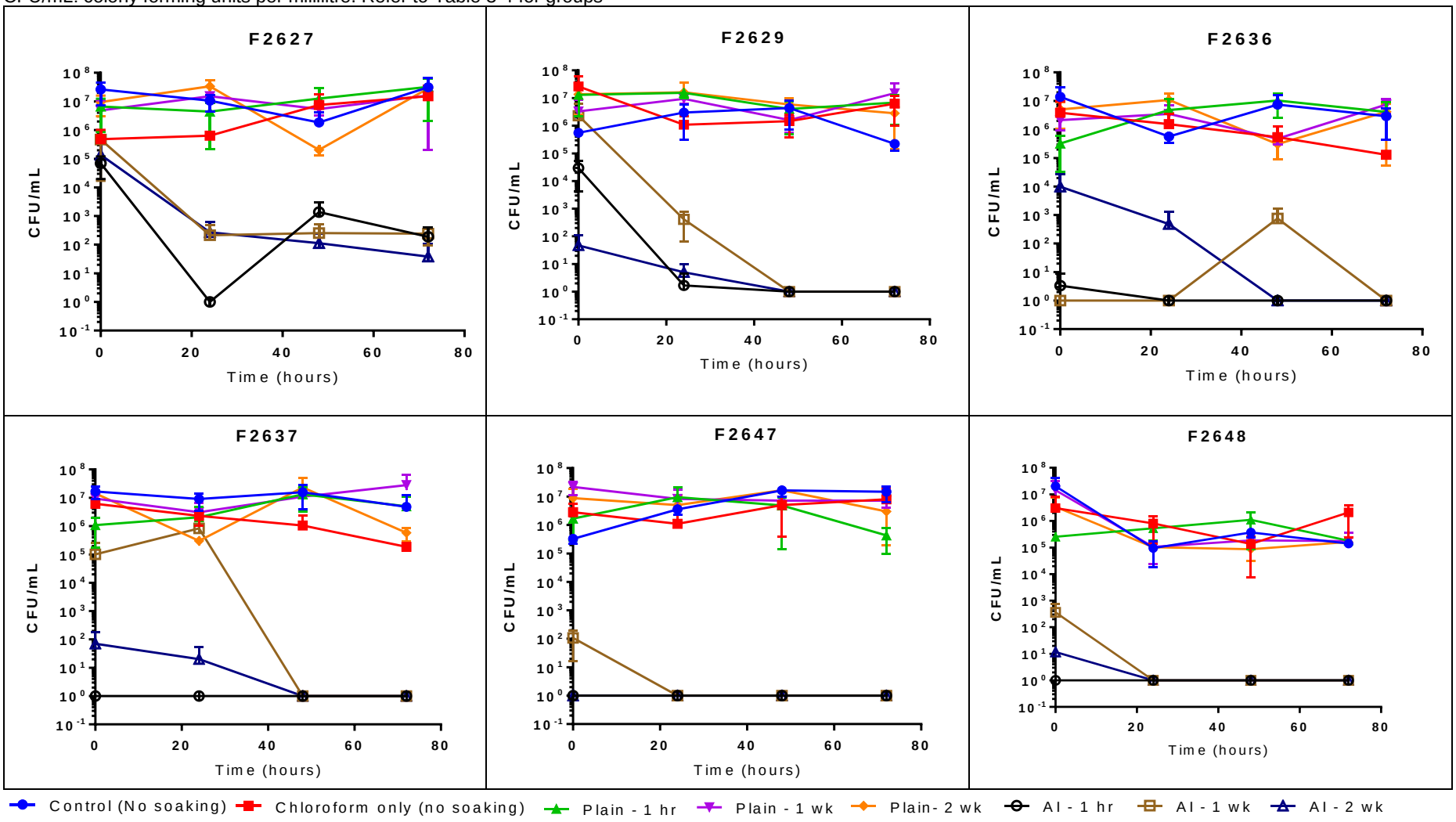
Isolate	% TSB concentration
F4142 MRSA	1.0%
F4001 MRSE	1.0%
F3991 MSSA	1.0%
F3802 NDM-1 *E. coli*	0.1%
F3986 ESBL *E. coli*	2.0%

3.3.3.2 TK100 assay – encrustation studies

All all-silicone controls including those segments without soaking and segments soaked over the three time points had bacterial attachment at all time points. All soaked AUC segments killed 100% of bacteria for five of the six isolates by 72 hours. In fact, 100% of *E. coli* isolates attached to the soaked AUCs were killed by 24 hours (Figure 3-11).However, AUCs incubated with F2627 had reduced bacterial counts at 72 hours, but none of the soaked AUCs killed 100% of this strain. This is consistent with what was found in previous TK100 studies with *P. mirabilis,* that a few bacteria are able to persist[77].

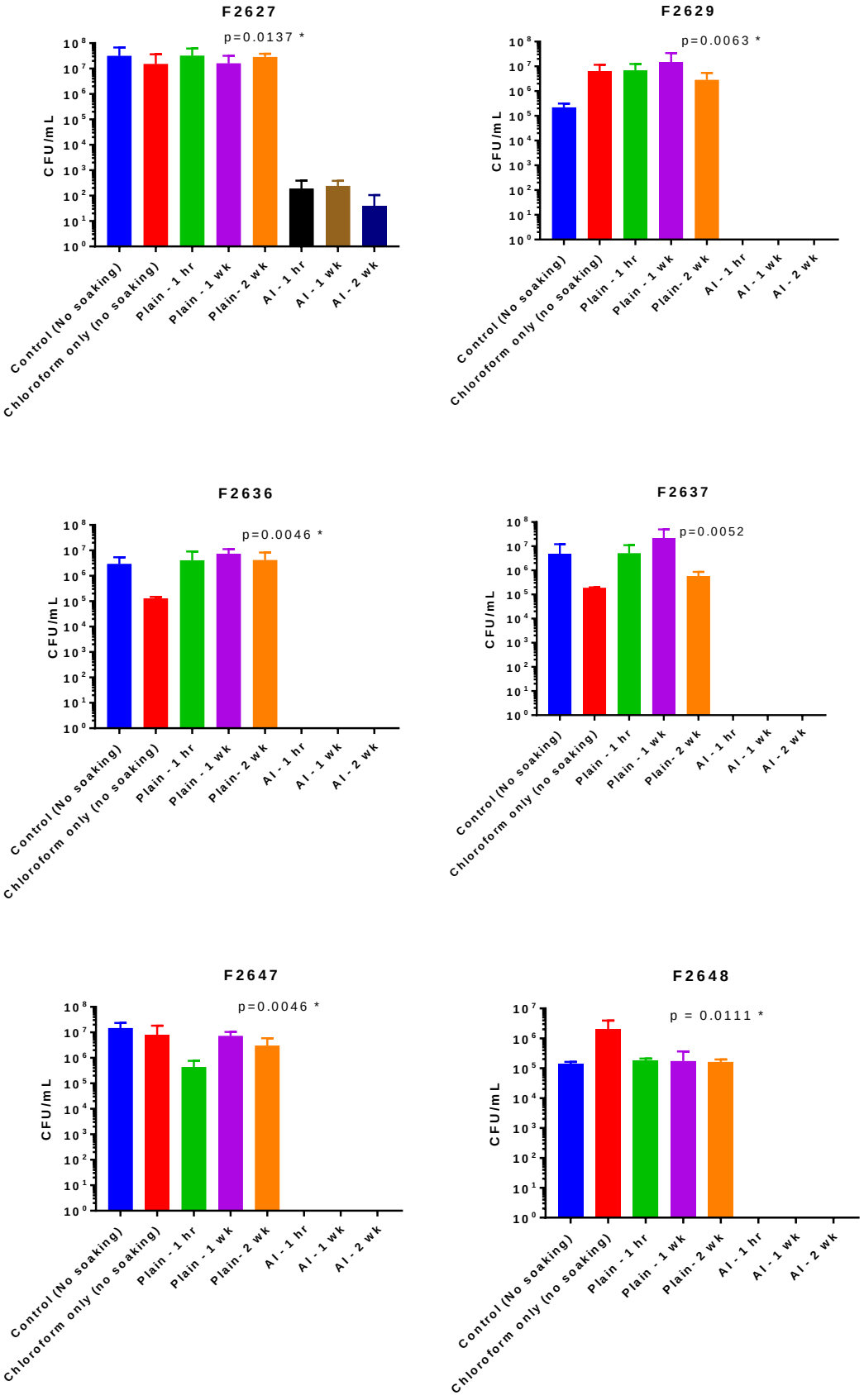
The general trend amongst all isolates in the TK100 is that the AUC soaked in AU for one hour, one week, and two weeks have reduced bacterial loads compared to the non-soaked all-silicone controls and soaked all-silicone controls.

Figure 3-11 TK100 assay of antimicrobial impregnated(AI) and silicone only (plain) catheter segments soaked for 1 hour (1hr), 1 week (1 wk) or 2 weeks (2 wk) against *P. mirabilis* (F2627, F2629), *S. saprophyticus* (F2636, F2637), and *E. coli* (F2647, F2648). Each data point represents the mean and the standard deviation. CFU/mL: colony forming units per millilitre. Refer to Table 3-4 for groups



Kruskal-Wallis test between the eight control and experimental groups show that for all six organisms there is a significant effect of the conditions of the catheter segments on bacterial attachment (Figure 3-12) at 72 hours. As there are two variables which may be responsible for the significant differences between the groups, post-hoc multiple comparisons using Dunn's correction were used to elucidate whether soaking duration or antimicrobial impregnation was the most likely responsible factor.

Figure 3-12 Encrustation TK100 assay mean CFU/mL values with standard deviation at 72 hours. Kruskal-Wallis test p-value of one-way ANOVA data (all groups) is presented. P<0.05 indicates a significant difference between all groups and is represented by *. AI: Antimicrobial-impregnated



Multiple comparisons of the Control (No soaking) group to Plain-1hr, Plain–1 wk, and Plain–2 wk groups for all six organisms was not significant for any comparison. The p-values for between the soaked segments and Control (No Soaking) segments was p>0.9999 for all comparisons except Control (No soaking) vs Plain-1 hr for organisms F2647 with a p-value of 0.5610, which is still not significant. This corresponds to the graphs presented in Figure 3-11 in which it is clear that there is consistent bacterial attachment between the all-silicone non-soaked and soaked segments. The same is true of the soaked AUC segments in which the bacterial counts for 5/6 organisms at 72 hours is zero for all AUC segments over all three soaking periods. It is apparent that soaking is not responsible for any disparities in bacterial attachment.

Comparison of the Control (No soaking) vs Chloroform only (No soaking), which are the segments processed according to the antimicrobial impregnation process without the addition of the antimicrobials, demonstrates no significant difference (p>0.9999 for all comparisons) between the segments for all six organisms. This strongly suggests that the impregnation process does not predispose to increased bacterial attachment.

In looking at the graphs in the above figures, and after determining it is unlikely that soaking affects bacterial attachment, it is clear that it is the exertion of antimicrobial activity that is reducing bacterial attachment and is likely to be the condition responsible for the differences between groups seen by the Kruskal-Wallis test. To examine this statistically ‘control (no soaking)’ group was compared to the AUC - 2 week group, which are those catheter segments soaked for two weeks. As the AUC - 2 week group may have reduced antimicrobial content due to the release of molecules over the two week soaking period and the longest period of soaking, it is the ‘worst case scenario’ of the experimental groups and is, therefore, conservative compared to the control.

However, it was difficult to perform meaningful statistical comparisons between the controls and soaked AUC segments as there was no variance of the soaked AUC samples at 72 hours due to all values of bacterial counts on AUC segments being zero (with the exception of F2627 *P. mirabilis*) (Figure 3-12). The values of zero mean the data are not normally distributed,

and a Mann-Whitney test would asess between two groups, but the sample size is small with each group containing n=3. In GraphPad Prism 7 software if the total sample size is less than seven, the test will always produce a p-value greater than 0.05 due to the reduced power of the test [271]. Furthermore, it is also difficult to transform the data to produce normally distributed values to use tests that assume Gaussian distribution as zero doesn't transform to anything other than zero. If one is substituted for zero the data can be transformed on the log-scale. However, given that the Kruskal-Wallis test was significant and soaking was not responsible for the differences in bacterial attachment, and that the counts were consistently 0 CFU/mL for 5/6 organisms and 0 CFU/mL for two of the set of triplicate repeats for F2627 it seemed unnecessary to perform confounding statistics when the antimicrobial effect is clear.

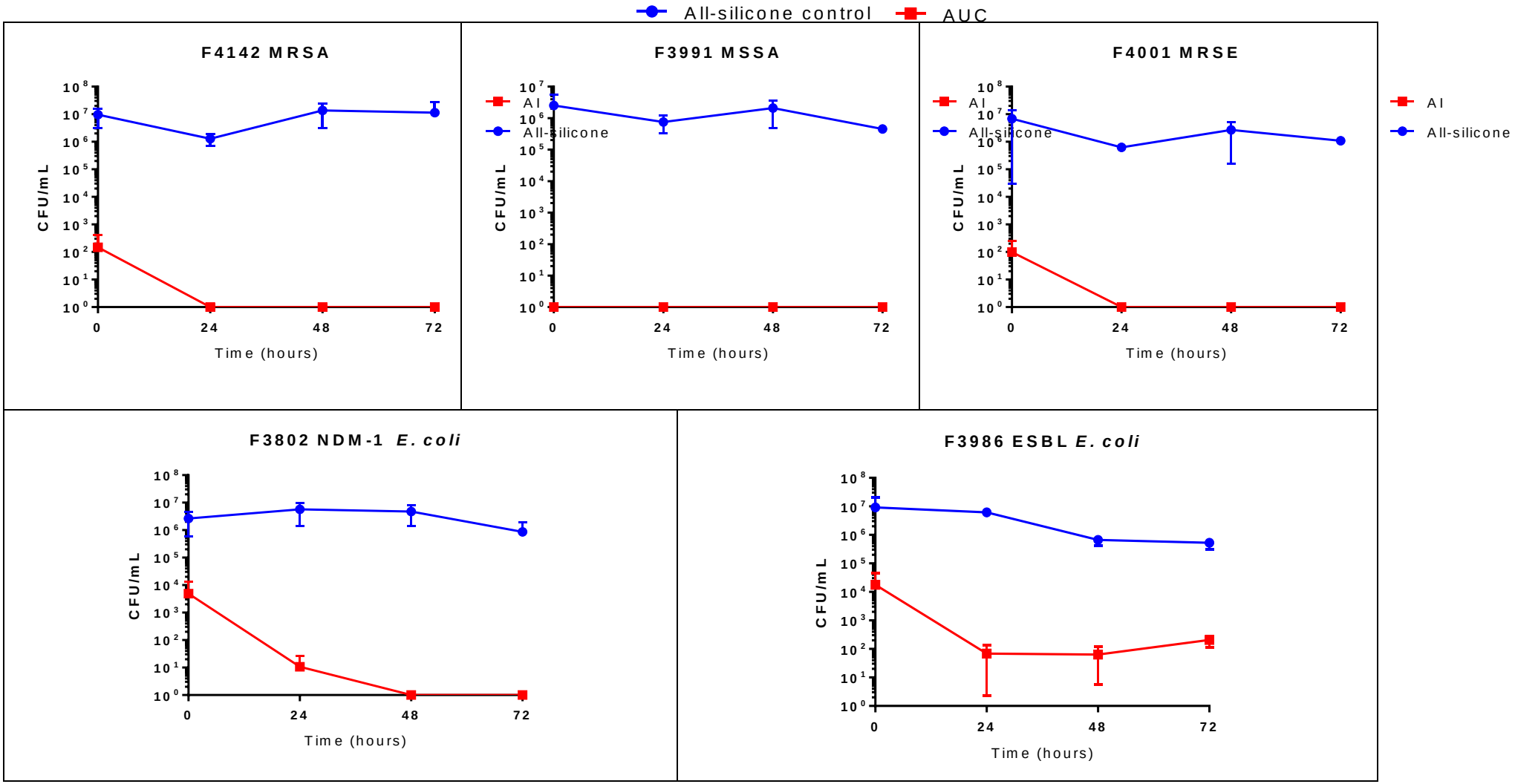
Length of soaking time does not increase bacterial attachment to the silicone - only catheters compared with the non-soaked controls. The same is seen with the AUC segments. The impregnation process does not affect bacterial attachment. Success of the TK100 assay is determined by eradication of all attached bacteria by 72 hours. Despite that two segments out of the set of three colonised with F2627 *P. mirabilis* were eradicated by the AUCs and the third segment had significantly reduced bacterial attachment, the TK100 was not considered a success due to a few remaining bacteria on one segment. The AUC segments were able to kill 100% of attached bacteria by 72 hours for *P. mirabilis* (F2629), *S. saprophyticus* (F2636 and F2637), and *E. coli* (F2647 and F2648) despite exposure to bacteria after soaking for one hour, one week, and two weeks.

3.3.3.3 **TK100 assay – efficacy experiments**

The results of the TK100 assay show that the AUC was able to kill 100% of four out of the five organisms within 72 hours (Figure 3-13). F3991 MSSA was unable to attach to the AUC at 0 hours, which was most likely due to its susceptibility to all of the antimicrobials in the AUC, and it was the organism susceptible to the most number of antimicrobials in the catheter. F4142 MRSA and F4001 MRSE were killed within 24 hours. Interestingly, F3802 NDM-1 *E. coli* was killed within 72 hours, whereas there was a

reduction of F3986 ESBL-producing *E. coli* compared to the control at 72 hours, but 100% of attached bacteria were not killed at 72 hours. This could be due to the differences in these two organisms' susceptibility to sparfloxacin. F3802 had intermediate susceptibility and F3986 was resistant. It may be that susceptibility to triclosan alone is not enough to prevent bacterial attachment. Susceptibility to the antimicrobials in the catheter determines the ability of the AUC to prevent colonisation, not its multi-drug resistance status.

Figure 3-13 TK100 assay of antimicrobial impregnated (AI) and all-silicone only (plain) catheter segments methicillin-resistant *S. aureus* (F4142), methicillin-susceptible *S. aureus* (F3991 MSSA), methicillin-resistant *S. epidermidis* (F4001 MRSE), New Delhi metallo beta-lactamase produce *E. coli* and extended-spectrum beta-lactamase producing *E. coli*. Each data point represents the mean and the standard deviation. CFU/mL: colony forming units per millilitre.

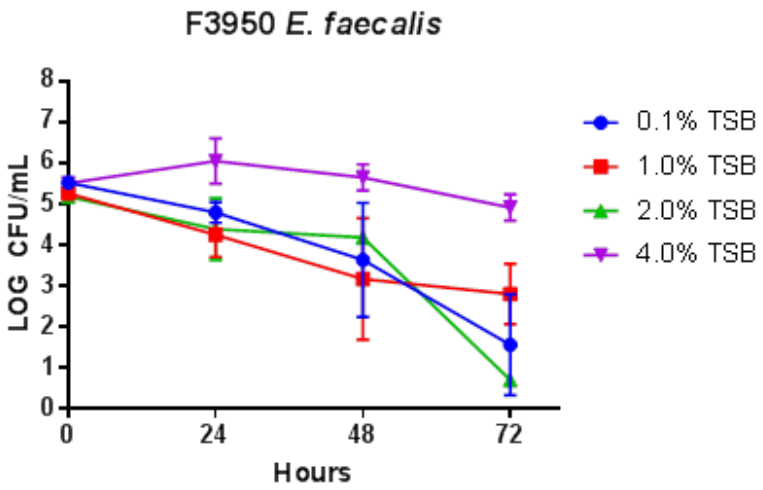


3.3.4 **Extended TK100**

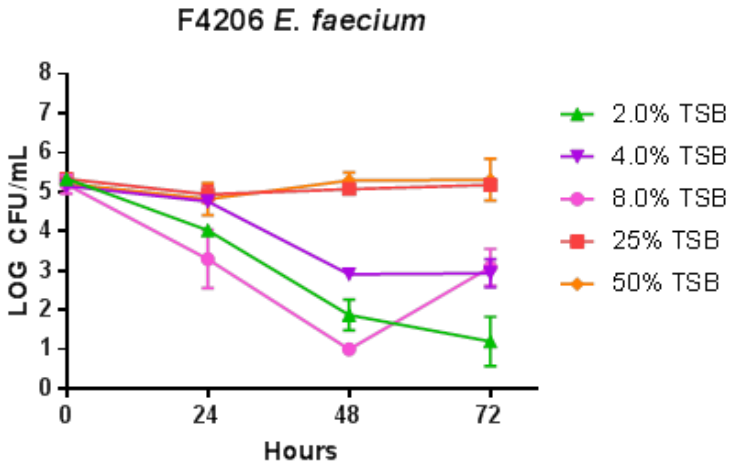
The TSB percentage that maintained growth of *E. faecalis* was 4.0% and 50% for *E. faecium* isolates consistently for each species (Figure 3-14).

Figure 3-14 Examples of TSB percentage determination assays for A.) one *E. faecalis* isolate and B.) one *E. faecium* isolate

A.)

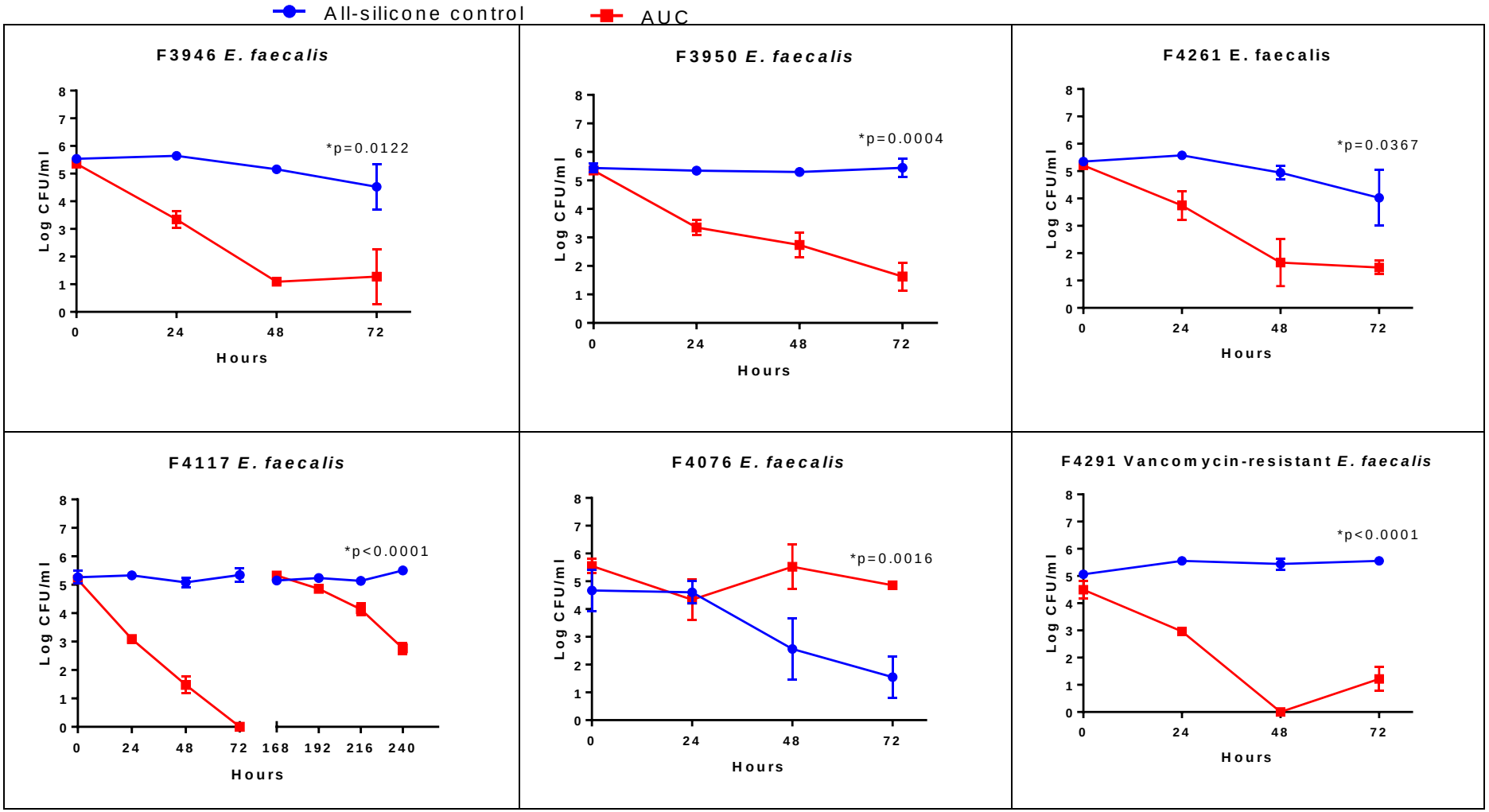


B.)



The AUC successfully eradicated 100% of attached F4117 *E. faecalis* and was extended to a second week, but the AUC was unable to kill all attached bacteria by the end of the second weekly challenge. Bacterial attachment to the AUC was significantly reduced at 72 hours after the second bacterial challenge ($p<0.0001$, unpaired t-test). The AUC did not successfully kill 100% of attached bacteria for five other *E. faecalis* isolates including the vancomycin-resistant isolate after the initial bacterial challenge (Figure 3-15). However, all AUC segments had significantly reduced bacterial attachment at 72 hours. The all-silicone controls for all TK100 assays colonised successfully and maintained growth over the test period.

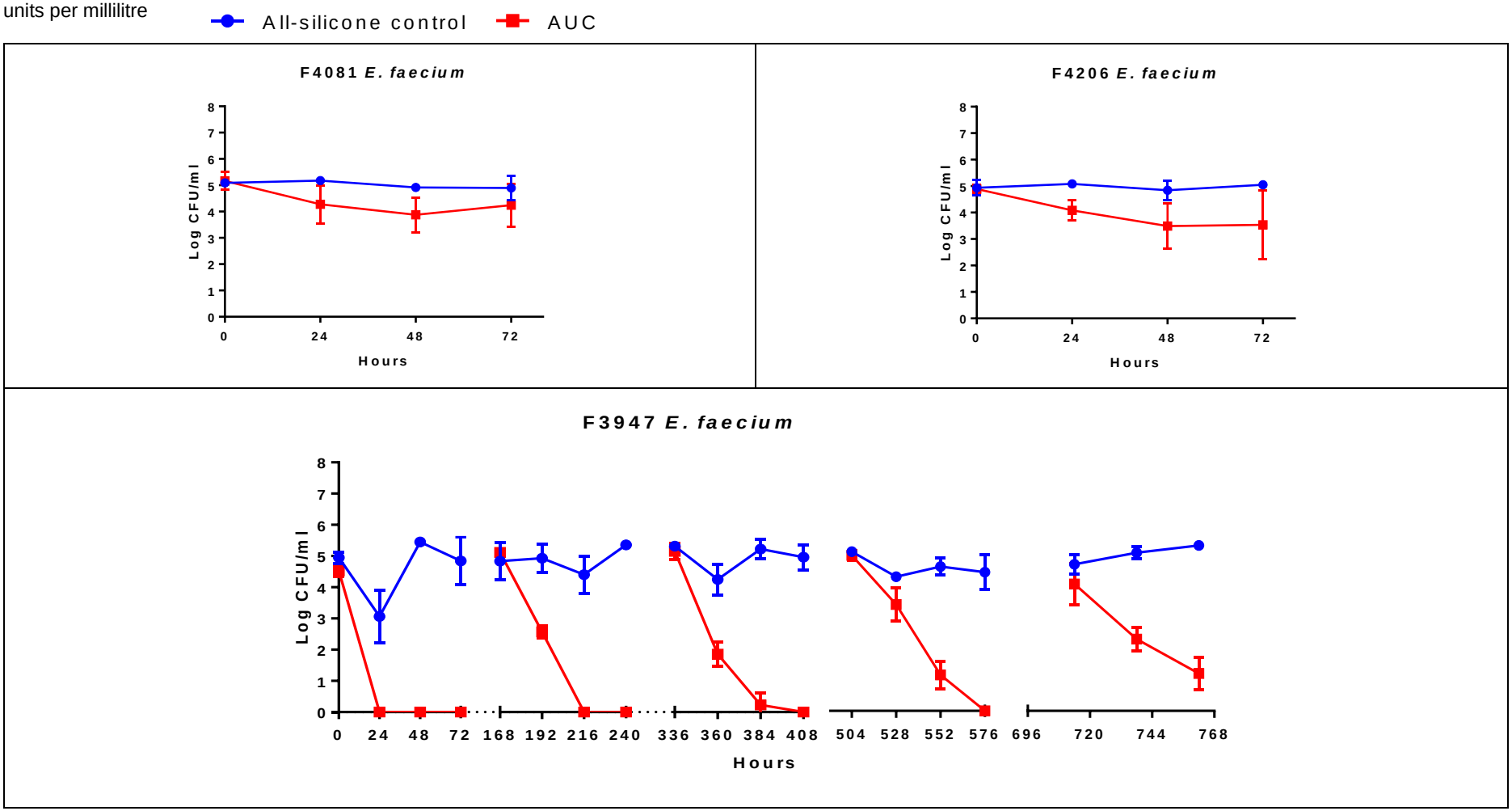
Figure 3-15 TK100 assay for *Enterococcus faecalis* isolates. The assay was extended for F4117 which was killed by the antimicrobial-impregnated catheter (AUC) at 72 hours Each data point represents the mean and the standard deviation. Log CFU/mL: colony forming units per millilitre



Of the three *E. faecium* isolates only one, F3947, was eradicated by the AUC after 72 hours and then continued to exert protective activity for three more weeks. The AUC killed 100% of attached *E. faecium* F3947 for four weeks and significantly reduced bacterial attachment at five weeks ($p=0.0002$). The other two *E. faecium* isolates, F4081 and F4206, were not eradicated within 72 hours nor significantly reduced ($p=0.2971$ and $p=0.1144$, respectively) (Figure 3-16). The difference in protective activity of the AUC against F3947 compared to F4081 and F4206 is most likely due to the suseptibility of F3947 to rifampicin and sparfloxacin, whereas F4081 and F4206 were resistant to rifampicin and sparfloxacin and showed wild-type susceptibility to triclosan but a MIC of 32.0 mg/L to triclosan is unlikely to be susceptible clinically.

The TK100 can be extended past 72 hours according to the method modification. It can show changes in the activity of the AUC segments as seen in Figure 3-15 in which F4117 *E. faecalis* was eradicated at 72 hours in week one and was not eradicated in week 2 but colony counts were reduced at 72 hours. Likewise, in Figure 3-16 in which during the first week F3947 *E. faecium* was eradicated at 24 hours, then at 48 hours in week 2, 72 hours in weeks three and four and then was not eradicated by the end of 72 hours in week five. As the antimicrobials are eluted the change in protective activity is detected.

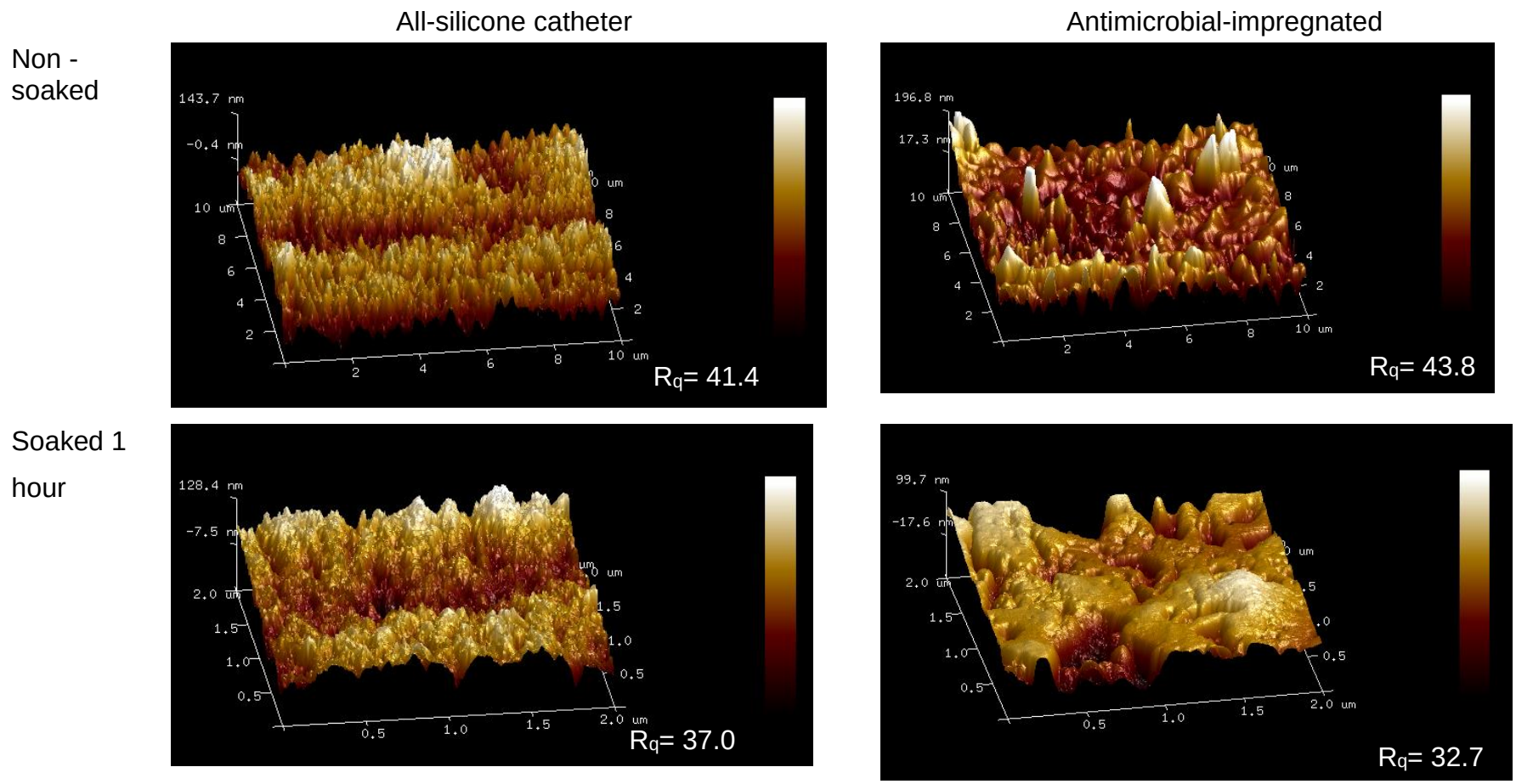
Figure 3-16 Extended TK100 assay for *Enterococcus faecium* isolates. The assay was extended for F3947 which was eradicated by the antimicrobial-impregnated catheter (AUC) at 72 hours. Sampling was continued for F3947 as it was eradicated at the end of each 72 hour challenge. New control segments were used with each challenge and the AUC segments were carried over to each consecutive challenge. Each data point represents the mean and the standard deviation. CFU/mL: colony forming units per millilitre

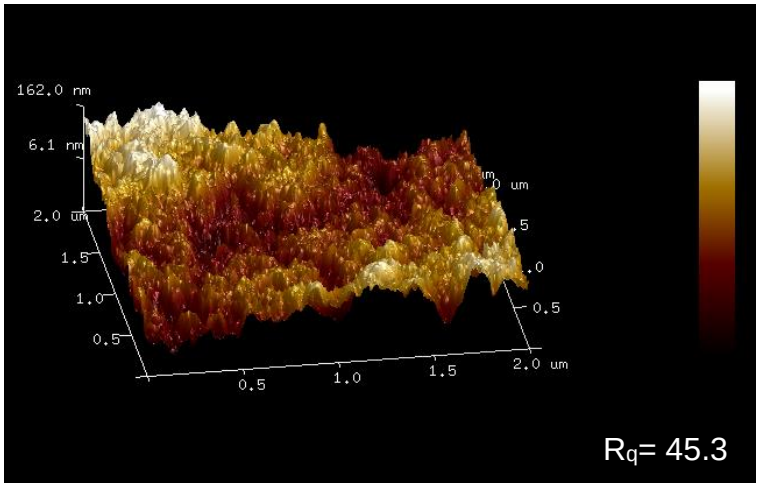
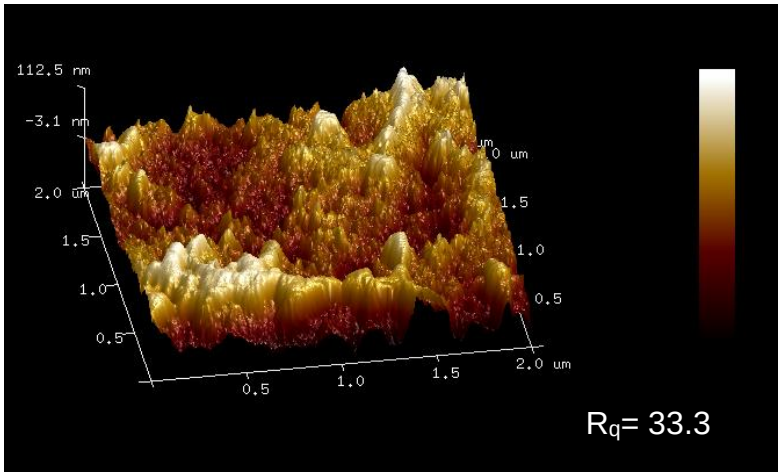
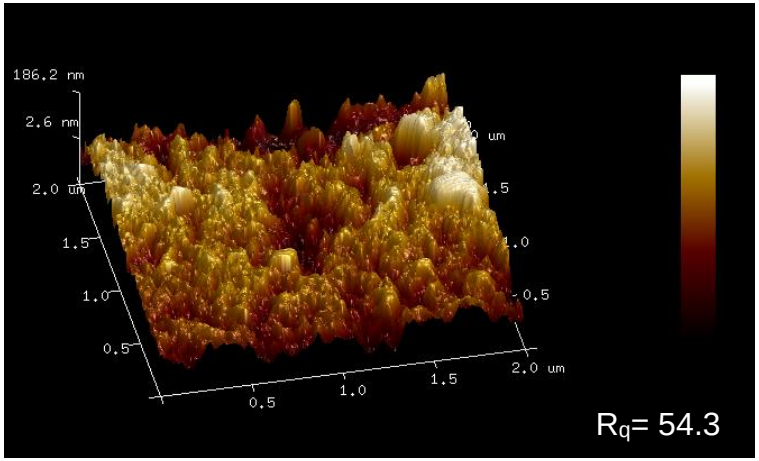
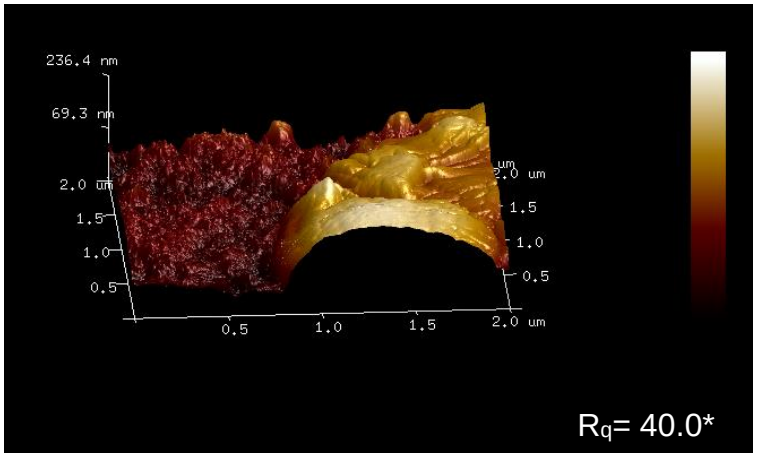


3.3.5 Atomic force microscopy

The surface of a silicone, non-soaked catheter appears to be composed of nano-sized peaks and spikes (see Figure 3-17 for examples of images generated by AFM analysis of controls and AUC segments). The spiky surface is apparent after soaking at all three time points. Of interest, the appearance of the AUC segments without soaking compared to the all-silicone catheters has a different appearance despite mean surface roughness values as represented by R_q values of 34.77 ± 5.95 and 56.6 ±14.09, respectively, which were not significantly different (Figure 3-20). Despite having non - significantly different R_q values to the all-silicone segments, the surface of the non-soaked AUC segments appears to have less numerous sharp spikes and instead fewer spikes, but wider, with flatter tops. The same can be said of those all-silicone and AUC segments soaked for one hour in which there is no significant difference between group means, but the topography of the AUC 1 hr soaked segments is composed of more plateaus and fewer spikes compared to the all-silicone 1hr soaked segment.

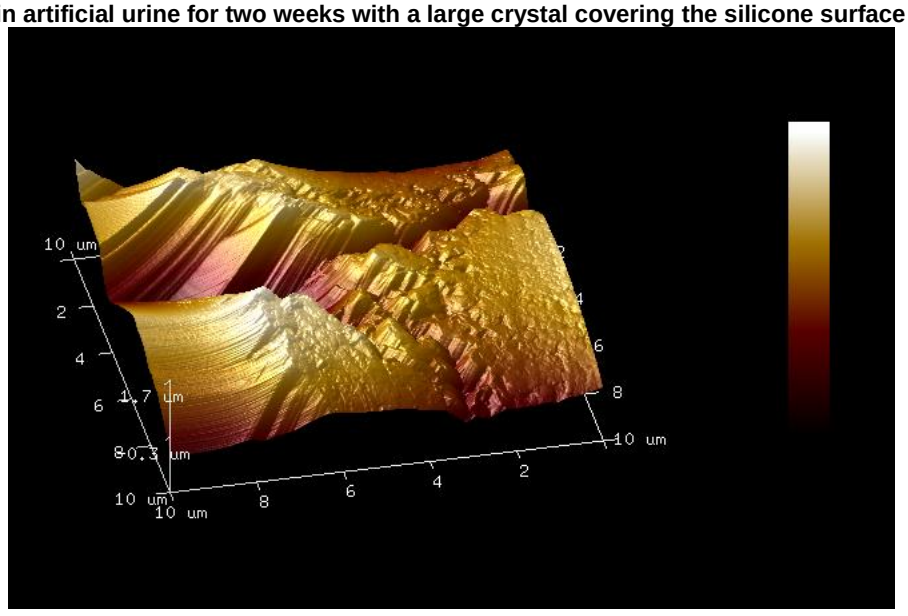
Figure 3-17 3D AFM images prepared with Nanoscope Analysis (v1.5) software and the respective R_q value of all-silicone and antimicrobial-impregnated catheter segments soaked in artificial urine for 1 hour, 1 week, or 2 weeks or not-soaked. The images chosen for this figure were the first of the set of triplicate samples for each experimental group. * The R_q value was determined from the section not containing a crystal



	All-silicone catheter	Antimicrobial-impregnated
Soaked 1 week		
Soaked 2 weeks		

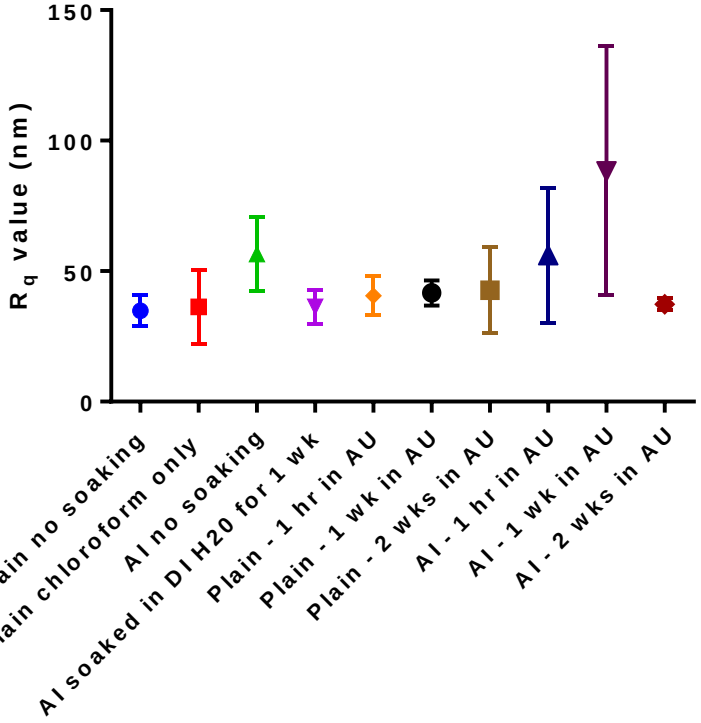
Crystals formed on four samples presumably from the AU and not from drug accretions as shown by no crystals forming on the water soaked catheter segments and that the crystals are present also on a plain catheter segment. An example of this can be seen in Figure 3-17 above, in which part of the sample of the AUC segment soaked in AU for two weeks is partly obscured by a crystal. Three of the four samples with crystals were AUC segments soaked in AU and the fourth was on a plain silicone catheter segment soaked in AU for two weeks. Since changes of surface roughness due to the impregnation process and/or soaking in AU were being investigated, it was important not to measure the surface roughness of the crystal on the surface as it masks the silicone. Therefore, for the four samples with crystallization, the R_q value is that of an area without crystal as opposed to the entire sample surface. However, for one sample of the plain silicone catheter soaked in AU for two weeks (Figure 3-18), a crystal encompassed the entire surface and it was not possible to get a R_q value for the surface independent of the crystal, and it was therefore excluded from the results.

Figure 3-18: 3D image prepared with Nanoscope Analysis software of a plain catheter segment soaked in artificial urine for two weeks with a large crystal covering the silicone surface



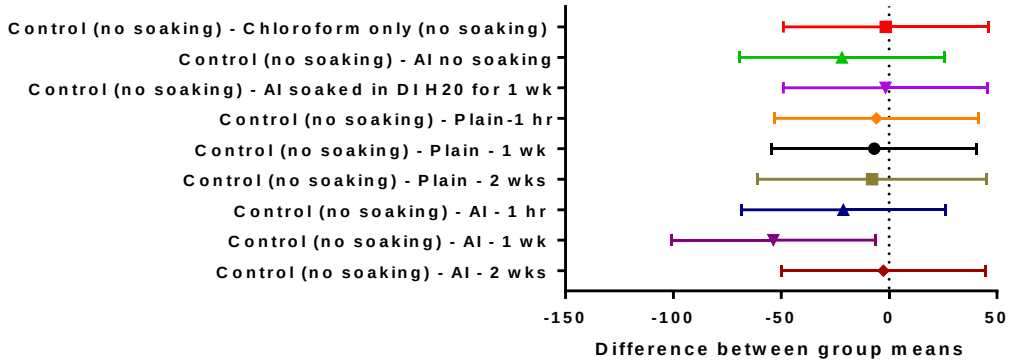
In Figure 3-19, it is apparent that surface roughness, as determined by the R_q values, remains mostly consistent between all-silicone catheters and over the three soaking periods.

Figure 3-19: Surface roughness expressed as the root of mean square (Rq)(nm) means and standard deviation of silicone catheters and antimicrobial-impregnated (AI) catheters soaked in artificial urine (AU) for 1 hour (1 hr), 1 week (1 wk), and 2 weeks (2 wks) in addition to no soaking controls, those impregnated in chloroform only, and those soaked in deionised (DI) water (H_20)



Ordinary one-way ANOVA was used to compare the surface roughness (R_q) of controls, soaked all-silicone catheters, and soaked AUCs. ANOVA shows the differences of all experimental means are not statistically significant (P=0.806). Despite the non-significance of the ANOVA, it is important to still investigate the group AUC– 1 wk in AU as the mean is noticeably greater and the standard deviation larger than the other groups. The mean R_q value is clearly different as seen by comparing the group means and 95% confidence intervals (Figure 3-20).

Figure 3-20 Difference in group means calculated by 95% confidence interval and corrected for multiple comparisons by Dunnett's multiple comparison test of antimicrobial-impregnated (AI) and all-silicone (plain) catheter segments soaked in artificial urine (AU) for one hour (1 hr), one week (1 wk), or two weeks (2 wks)



An important limitation of this method is that of the 1.0 cm longitudinally cut catheter segments, only a 2.0 µm x 2.0 µm area is measured and quantified so it is possible that chance would allow for rougher or smoother areas to be sampled, which may be responsible for the large standard deviation for the AUC – 1 wk group. This outlying group also does not correspond to the mean values of AUC – 1 hr and AUC – 2 wks, which were not significantly different from the Control (No soaking) mean, and in fact it would be expected that those segments soaked for two weeks would have a greater surface roughness than those soaked for one week. The R_q values for the all-silicone segments soaked for one week (Plain – 1 wk) were consistent with the control and all-silicone segments soaked for one hour and soaked for two weeks.

3.3.6 Colorimetric determination of the extent of encrustation

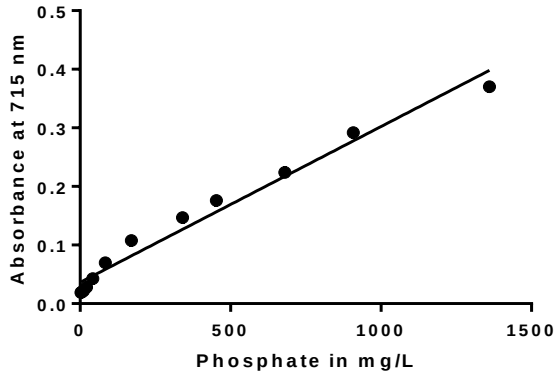
A method was developed to quantitatively measure phosphate precipitating out of artificial urine as a measure of mineral encrustation. The applicability of this model to detect mineral encrustation was determined in the presence of the three test bacteria and a range of pH-adjusted AU.

3.3.6.1 Calibration Plot

A final calibration plot composed of 13 standards was produced with a R^2 value of 0.9754 (Figure 3-21). This calibration plot was determined to be appropriate based on the R^2 value and was used to infer phosphate

concentrations of experimental samples. All experimental samples fell within the calibration plot and this was used to interpolate unknown experimental values.

Figure 3-21 Calibration plot of phosphate determination measured spectrophotometrically at 715nm and corresponding to phosphate concentration (mg/L). Slope is represented by y and goodness of fit is represented by R^2



$Y = 0.0002661*X + 0.03609$

$R^2 = 0.9754$

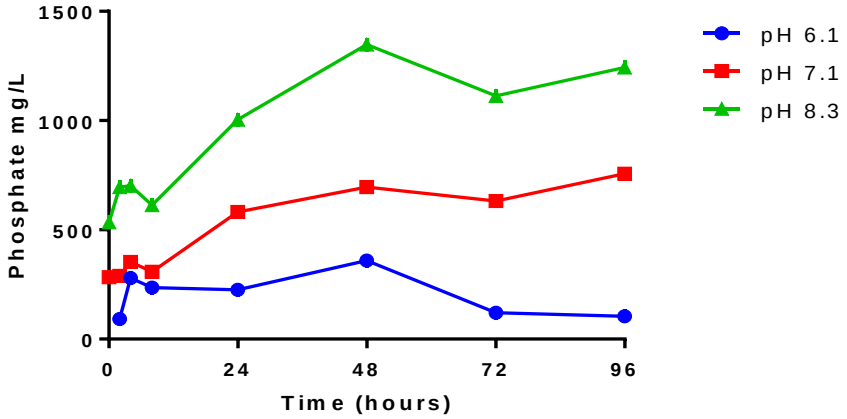
3.3.6.2 **Method Development**

This colorimetric assay for the determination of phosphate concentration was able to sensitively detect phosphate precipitated out of solution when AU was adjusted to different pHs and when inoculated with the three test organisms. The absorbances of colour intensity fall within the values of the calibration plot (Figure 3-21) so the phosphate values were able to be interpolated appropriately.

3.3.6.3 **Phosphate precipitation in the absence of bacteria**

The optical density values were averaged and the average was interpolated from the calibration plot in Figure 3-21 to give the amount of phosphate precipitated from the artificial urine adjusted to a pH of 6.1, 7.1, or 8.3. Figure 3-22 shows that pH alone has a dramatic effect on phosphates precipitating out of solution. At 48 hours, which is the peak of phosphate precipitation, 1347.876 mg/L, 696.089 mg/L and 359.067 mg/L of phosphate are precipitated from AU adjusted to 8.3, 7.1, and 6.1, respectively. This highlights the importance of pH-dependent phosphate precipitation in the absence of bacteria.

Figure 3-22 Phosphate precipitated (mg/L) from artificial urine adjusted to three different pHs in the absence of bacteria over 96 hours

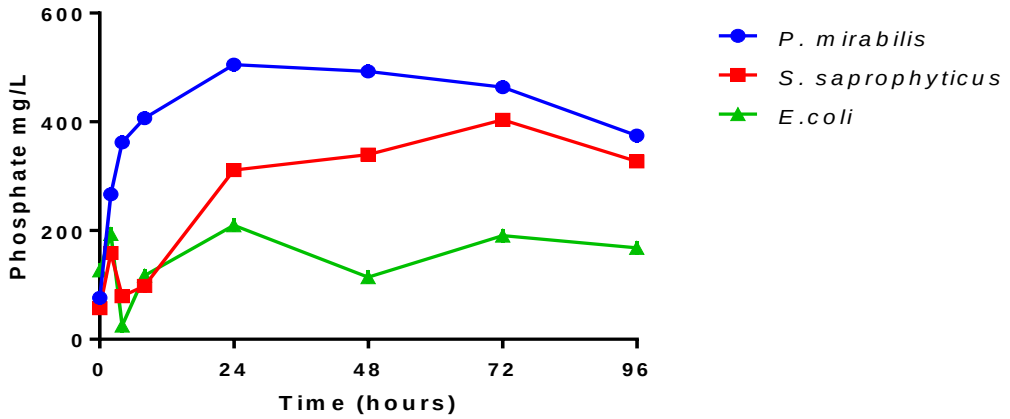


Of interest, at 48 hours AU adjusted to 7.1 and 8.3 more phosphate was precipitated out of solution than was measured in uncentrifuged AU adjusted to 6.1. This suggests that pH may play a role in the availability of phosphate and will be investigated further. Additionally, 1347.876 mg/L of phosphate was precipitated in AU at pH 8.3 which is 69.0% of the total phosphate ion added to the AU solution.

3.3.6.4 **Phosphate precipitation in the presence of bacteria**

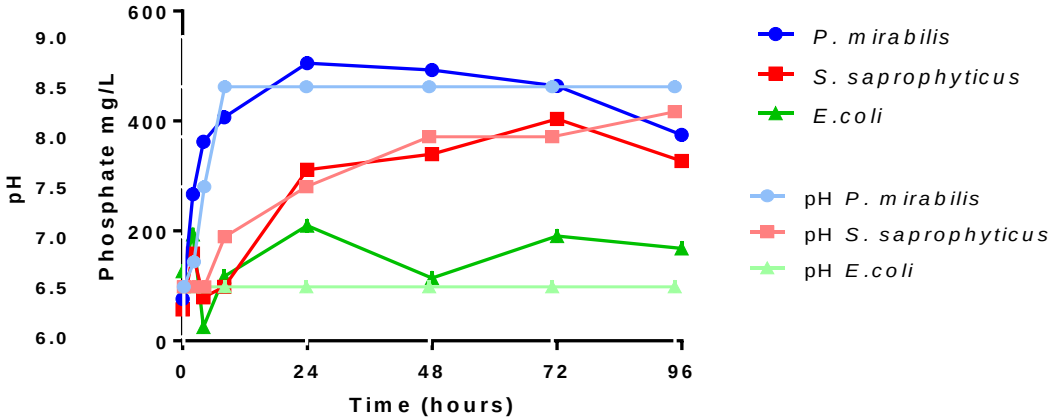
Phosphate precipitation varied between the three bacteria with *P. mirabilis* consistently precipitating more phosphate from solution than *S. saprophyticus* and *E. coli*, and *S. saprophyticus* precipitating more than *E. coli* (Figure 3-23). For *P. mirabilis* there is a rapid increase in phosphate precipitation between 0 and 24 hours after which phosphate precipitation slowly reduces. With *S. saprophyticus* phosphate precipitation slowly increases over 72 hours.

Figure 3-23 Phosphate precipitated (mg/L) from artificial urine inoculated with either *P. mirabilis*, *S. saprophyticus* or *E. coli* over a period of 96 hours



Phosphate precipitation in the presence of the three bacteria does appear to be influenced by the activity of the urease enzyme and its ability to change the pH of the urine (Figure 3-24). The rapid increase of phosphate precipitation by *P. mirabilis* is most likely a result of the rapid rise of the pH, which was adjusted to 6.1 before inoculation, and after four hours the pH rose to 7.5 then to 8.5 after eight hours where it remained for the duration of the test period. Likewise, the steady increase of phosphate precipitation by *S. saprophyticus* is likely to be related to its steady pH increase where it reached a pH of 7.0 at eight hours, 7.5 at 24 hours, 8.0 at 48 hours, and 8.25 at 96 hours.

Figure 3-24 Phosphate precipitated (mg/L) from artificial urine inoculated with either *P. mirabilis*, *S. saprophyticus* or *E. coli* overlayed with the pH of artificial urine inoculated with the three bacteria over the 96 hour test period

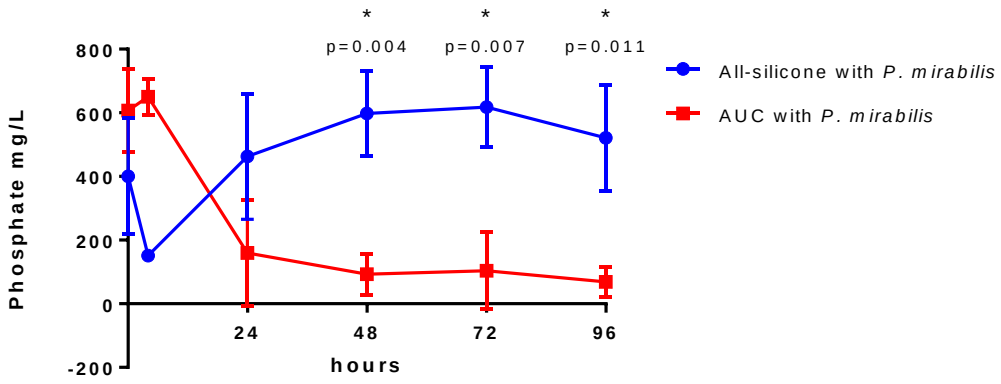


3.3.7 **Static model of mineral encrustation**

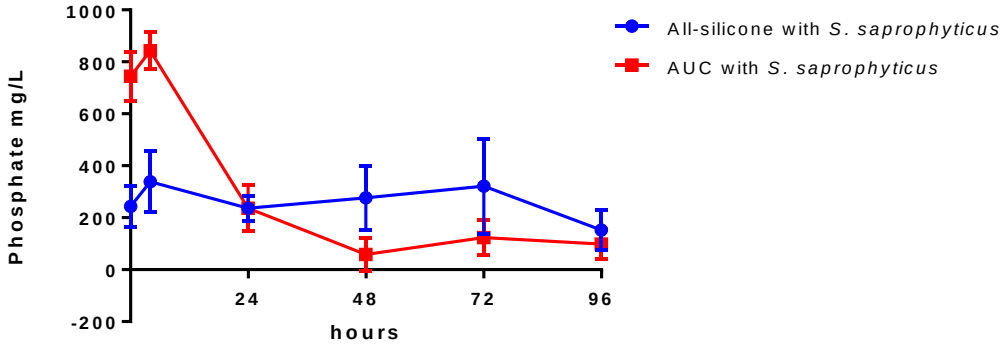
There was significantly less phosphate attached to the AUCs incubated with *P. mirabilis* at 48 hours (p=0.004), 72 hours (p=0.007), and 96 hours (p=0.011) as compared to the control catheter segments (Figure 3-25a). Phosphate attached to the all-silicone and AUCs was not significantly different for those catheter segments incubated in artificial urine with *S. saprophyticus* and *E. coli,* and AU adjusted to the three different pHs. No differences may be detected to a lesser extent for mineral precipitation in the absence of *P. mirabilis*; there may simply not be enough phosphate generated for this method to sensitively detect any smaller differences. As there are no significant differences in mineral deposition on the surface between the AUC and the all-silicone catheters this suggests that the processing of the catheters to impregnate them with antimicrobials does not predispose to mineral deposition on the catheter surface.

Figure 3-25 Phosphate (mg/L) attached the all-silicone and antimicrobial impregnated catheter (AUC) segments incubated statically with A) artificial urine (AU) inoculated with *Proteus mirabilis (*F2629*)*, B) AU inoculated with *Staphylococcus saprophyticus* (F2636), C) AU inoculated with *Escherichia coli* (F2647), D) AU adjusted to a pH of 8.3, E) AU adjusted to a pH of 7.1, and F) AU adjusted to a pH of 6.1. The data were analysed using unpaired multiple comparisons t-tests. The multiple comparisons were corrected for using the Holm-Sidak method and $p<0.05$ was significant. Error bars represent standard deviation.

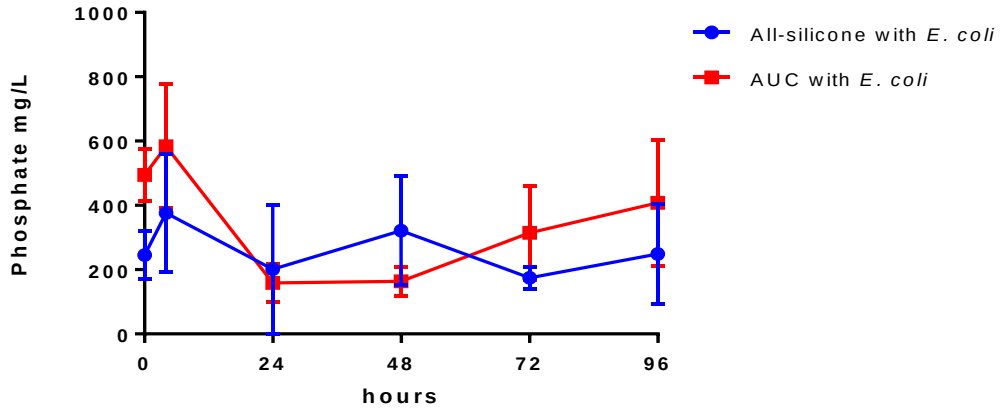
A



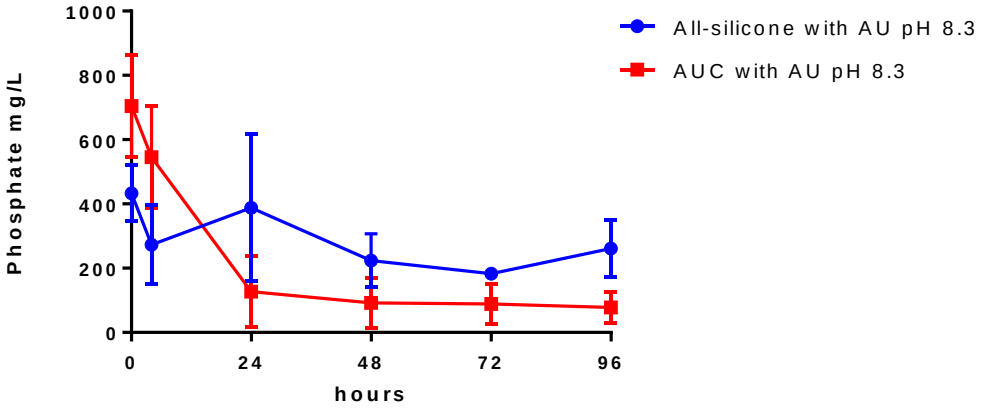
B



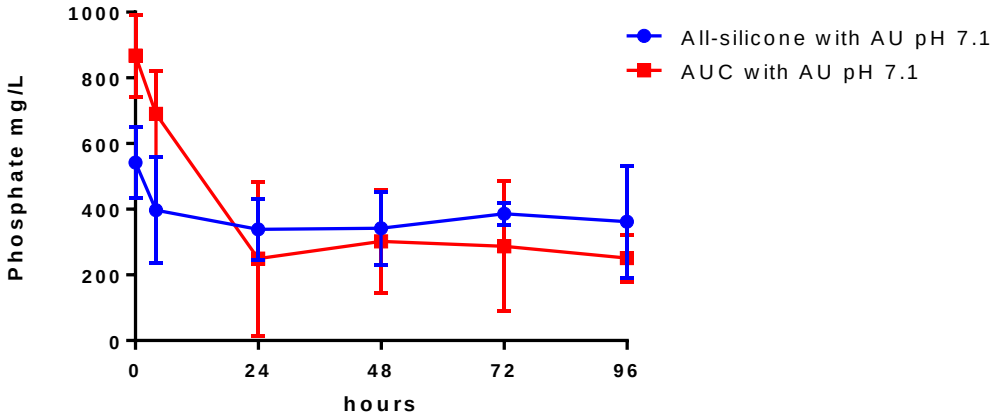
C



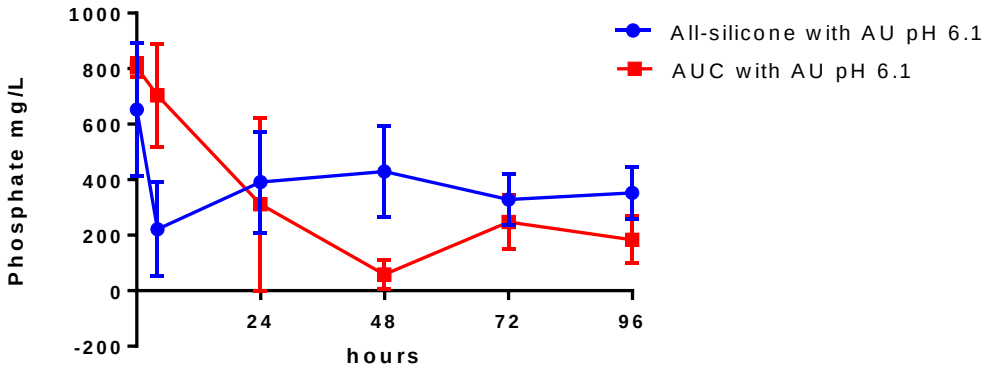
D



E



F



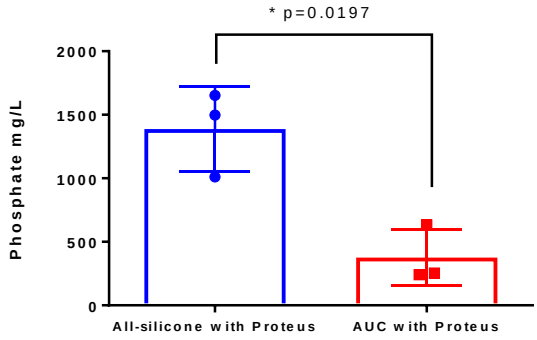
The AUC reduced phosphate deposition on the catheter surface under static conditions when incubated with AU inoculated with *P. mirabilis* over 96 hours compared to the all-silicone control. It is likely that the prevention of phosphate deposition is due to the killing effect of *P. mirabilis* by the AUC.

3.3.8 In vitro flow challenge model of mineral encrustation

As seen from the static model of encrustation, the AUC was able to reduce the amount of phosphate attached to the catheter surface over 96 hours in the presence of *P. mirabilis*. To determine if the AUC was able to prevent phosphate attachment over a longer period and under flow conditions, antimicrobial-impregnated and all-silicone catheters were perfused with AU with or without *P. mirabilis* for 28 days.

The control catheters inoculated with *P. mirabilis* all blocked before the end of the 28 days test period. The average time until blockage was 21.7 days (range 16-26 days). None of the AUCs blocked during this time period. The corresponding AUC was removed from the model for analysis at the time the control catheter blocked. After removal from the model, phosphate content was analysed and the amount of phosphate attached to the lumens of the AUCs perfused with AU inoculated with *P. mirabilis* was significantly less (p=0.0197) than the phosphate attached to the control catheters (Figure 3-26).

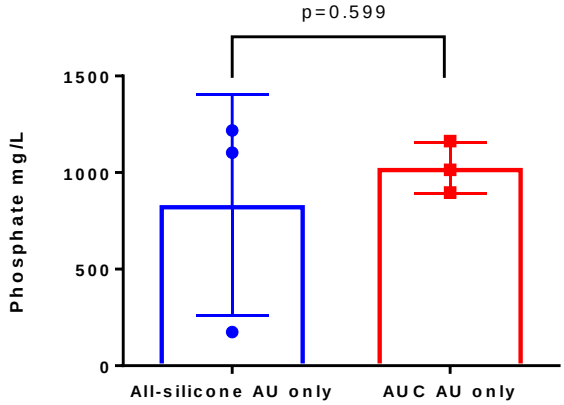
Figure 3-26 The phosphate (mg/L) attached to the all-silicone and antimicrobial-impregnated catheters (AUC) perfused with artificial urine and inoculated with *Proteus mirabilis*. Phosphate was quantified when a set of catheters was taken down from the model when they all-silicone controls become blocked. The data were analysed using a paired two-tailed t-test where p<0.05 is significant.



These results correspond to the findings of the static model, in that the AUC is able to reduce phosphate attachment in the presence of *P. mirabilis*. None of the AUC or all-silicone controls perfused with AU only (without *P. mirabilis*) blocked within the 28 day test period. In the absence of bacteria, there is no significant difference (p=0.599) between the amount of phosphate

attached to the AUC and all-silicone catheters after being exposed to flow conditions for 28 days (Figure 3-27). Again, this corresponds to the static model of encrustation as there was no significant difference in the phosphate attachment between the AUC and all-silicone control catheter segments in the absence of bacteria. This reinforces the AFM results that demonstrate that the impregnation process does not predispose to mineral encrustation.

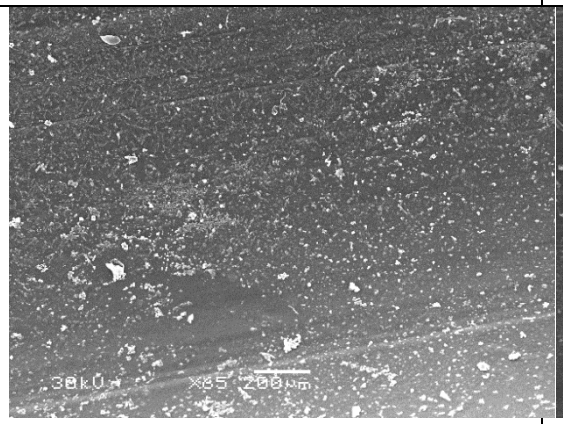
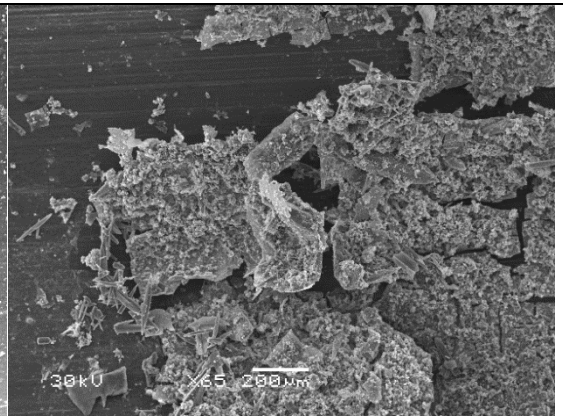
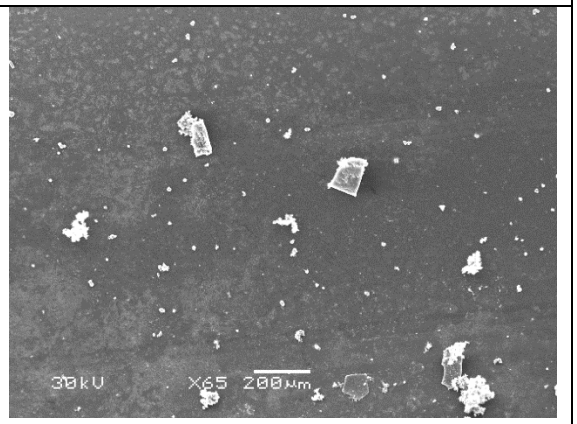
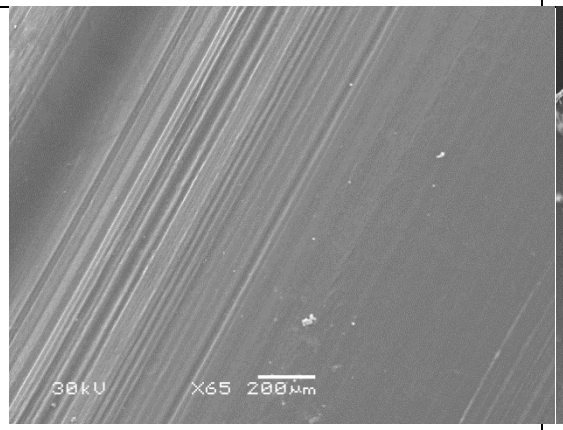
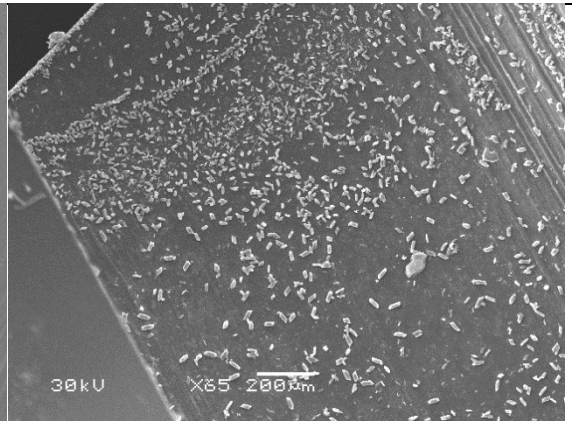
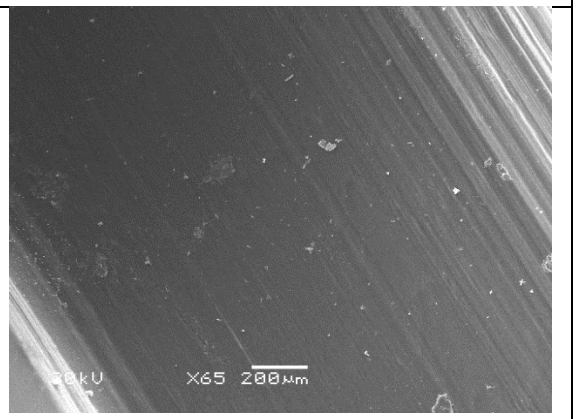
Figure 3-27 Phosphate (mg/L) attached to the all-silicone and antimicrobial-impregnated catheters (AUC) perfused with artificial urine (AU) only. Phosphate was quantified at the end of the 4 week period as none of these catheters blocked. The data were analysed using an unpaired two-tailed t-test where p<0.05 is significant.



3.3.8.1 **Scanning electron microscopy**

Segments cut away from the AUC and all-silicone catheters upon removal from the in vitro flow model were fixed immediately for imaging by SEM. It was clear that the AUC lumens compared to its matched all-silicone catheter had significantly fewer minerals on the catheter surface when inoculated with *P. mirabilis*. For example, the AUCs removed at 23 and 16 days when the all-silicone control blocked, were virtually clean with no mineral deposition. The catheters removed at 26 days had slightly more deposition on the catheter surface compared to the others removed earlier. However, the AUC had a single layer of widely spaced minerals, whereas the all-silicone matched control had much larger 3D clusters of minerals (Figure 3-28).

Figure 3-28: The lumens of all-silicone and antimicrobial-impregnated catheters (AUCs) perfused with artificial urine and inoculated with *P. mirabilis* visualised at x65 magnification using a Jeol 6060LV variable pressure scanning electron microscope. All-silicone catheters and AUCs are matched and were removed from the model at the same time as the control catheters.

	23 days before blockage of control and removal from the model	26 days before blockage of control and removal from the model	16 days before blockage of control and removal from the model
All – silicone catheters inoculated with *P. mirabilis*			
AUCs inoculated with *P. mirabilis*			

While SEM is not a quantitative method, it does provide a strong qualitative supporting statement for the reduction of mineral deposition of the AUC surfaces when inoculated with *P. mirabilis*. The straight lines/ridges on the catheter surfaces apparent in most SEM images are most likely an artefact of the extrusion process during manufacture.

3.3.8.2 **X-ray photoelectron spectroscopy**

Identical segments to those in the SEM experiments were removed from the catheters at the end of in vitro flow model challenge, and the lumens were analysed by XPS to quantify mineral content. XPS quantifies the topmost 10 nm of the surface so most likely the silicone surface was quantified as part of this. Silicone is composed of alternating silicon (Si) and oxygen (O) molecules, with two carbon groups attached to each Si atom [272], with an approximate atomic composition of 25% Si, 25% O, and 50% C. There are hydrogen atoms associated with the carbon groups, but these are not detectable by XPS. The percent atomic concentrations per catheter segment can be seen in

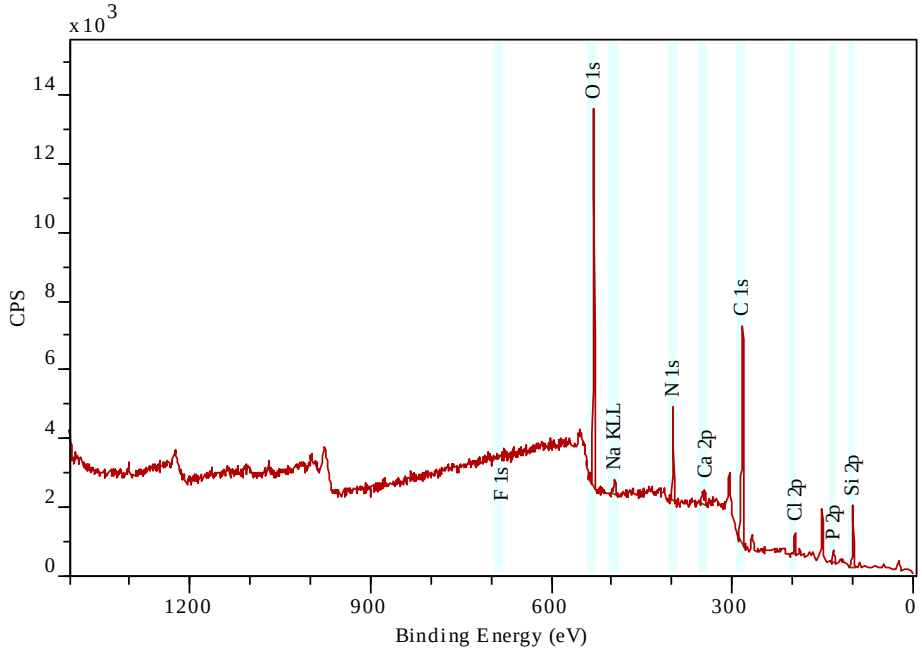
Table 3-14 in which the samples were composed of between 50.0% - 59.5% C, 20.1% - 25.3% O, and 8.9%-24.2% Si. These values are relatively consistent with the composition of silicone, with there being 50.0% and greater C in all samples, the extra carbon being a likely result of deposition of cells and organic material. The O and Si are slightly less than 25% consistently and this is probably due to some of the surface being covered by cells and mineral deposits that prevents much of the catheter surface from being detected.

Table 3-14 Percent (%) atomic composition of all-silicone (plain) and antimicrobial-impregnated urinary catheter (AUC) catheter segments perfused with artificial urine alone (AU) or artificial urine inoculated with *Proteus mirabilis* (Proteus). C: Carbon, O: oxygen, Si: Silicon, N: nitrogen, Ca: calcium, Na: sodium, P: phosphorus, Cl: chlorine, F: fluorine

	C 1s %	O 1s %	Si 2p %	N 1s %	Ca 2p %	Na KLL %	P 2p %	Cl 2p %	F 1s %
AUC Proteus 1	59.5	20.1	15.7	2.9	0.0	0.1	0.0	0.7	0.8
AUC Proteus 2	56.7	20.8	17.6	3.1	0.0	0.0	0.0	0.3	1.4
AUC Proteus 3	53.0	24.3	18.3	2.8	0.1	0.2	0.0	0.2	1.0
Plain Proteus 1	52.2	23.8	21.9	1.5	0.2	0.0	0.0	0.3	0.0
Plain Proteus 2	54.7	23.9	8.9	8.1	0.9	0.8	1.3	1.4	0.0
Plain Proteus 3	53.1	24.6	18.2	1.9	0.5	0.6	0.6	0.5	0.0
AUC AU 1	55.2	21.6	18.0	2.4	0.2	0.4	0.3	0.4	1.5
AUC AU 2	53.0	25.2	18.4	2.2	0.1	0.0	0.0	0.1	1.0
AUC AU 3	52.4	24.4	21.2	1.3	0.0	0.0	0.0	0.2	0.5
Plain AU 1	50.0	25.3	24.1	0.2	0.1	0.3	0.0	0.0	0.0
Plain AU 2	54.2	24.1	20.1	1.3	0.1	0.1	0.0	0.0	0.0
Plain AU 3	52.8	24.8	20.3	1.6	0.2	0.1	0.1	0.1	0.0

Of interest is the sample ‘Plain Proteus 2’; its spectrum (Figure 3-29) shows a markedly reduced atomic concentration of Si and markedly increased calcium (Ca), nitrogen (N), phosphorous (P), and chlorine (Cl) atomic concentrations. ‘Plain Proteus 2’ sample is taken from the same catheter with heavy visible mineral encrustation seen by SEM (Figure 3-28) which blocked after 26 days in the in vitro flow challenge model. The atomic concentration of Si is only 8.9% which is noticeably less than the all other samples. It is possible that the deposition of minerals on the surface masked more of the silicon so it could not be detected by this method.

Figure 3-29 X-ray photoelectron generated spectrum of sample Plain Proteus 2, an all-silicone catheter inoculated with *P. mirabilis*. The spectrum produced by the CASAXPS software shows the binding energy (eV) of the detected elements with peak heights represented by counts per second (CPS) as a measure of intensity.



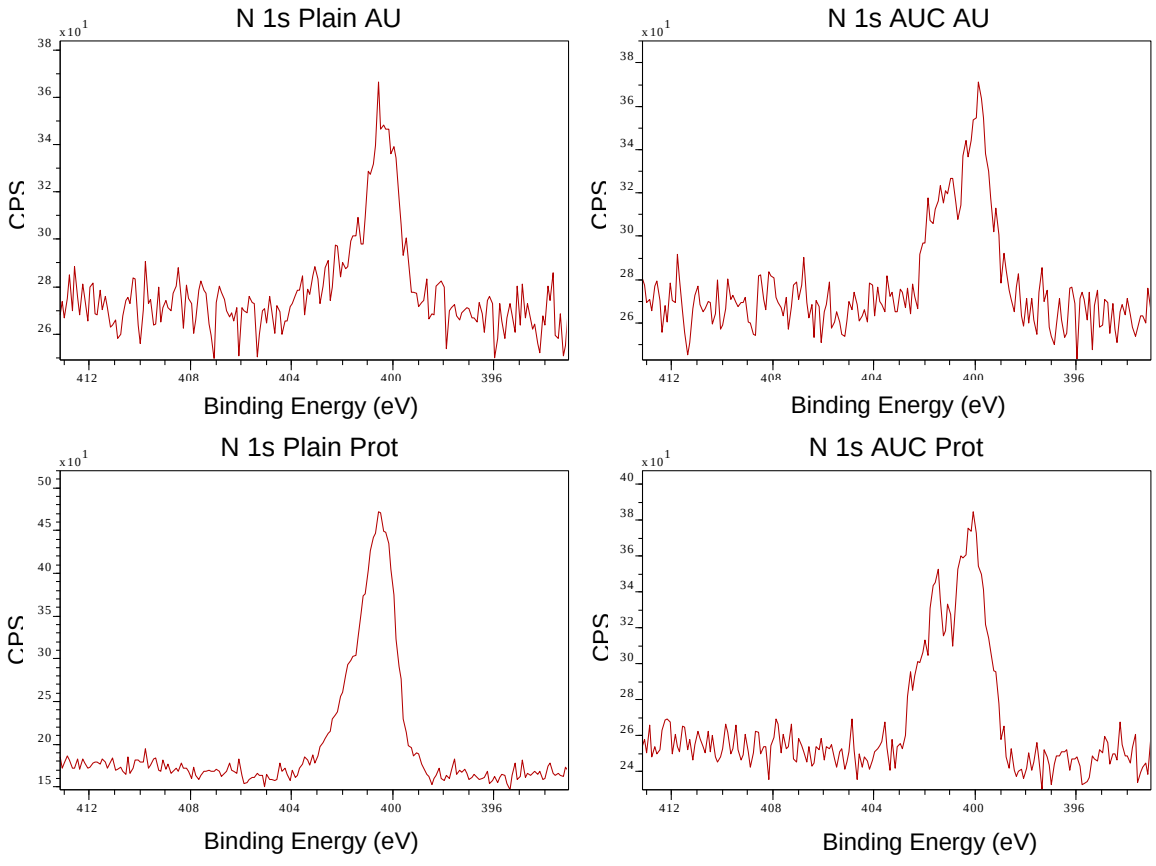
N is a component of rifampicin and sparfloxacin, and Cl is a component of triclosan so these elements could be present on the catheter surface due to the impregnation process. The source of these elements could also be from the artificial urine coating the surface as sodium chloride, calcium chloride, urea, and potassium hydrogen phosphate are present in many of the samples. However, artificial urine alone would not explain the outlying atomic concentrations of these elements in this sample.

The possible elemental markers of mineral deposition include P as a component of phosphate, and Ca as a component of other crystals such calcium oxalate. One-way ANOVA between the groups identifies a significant

difference (p=0.0421) in the amount of Ca between the groups, with post-hoc tests showing there is a significant difference (p=0.0453) between AUC and all-silicone catheters inoculated with *P. mirabilis*. However, of interest, there was no significant difference (p=0.1514, one-way ANOVA) in the amount of P between sample groups, despite quantification of the samples by the spectrophotocolorimetric method showing a clear significant difference between phosphate on all-silicone and AUCs inoculated with *P. mirabilis*.

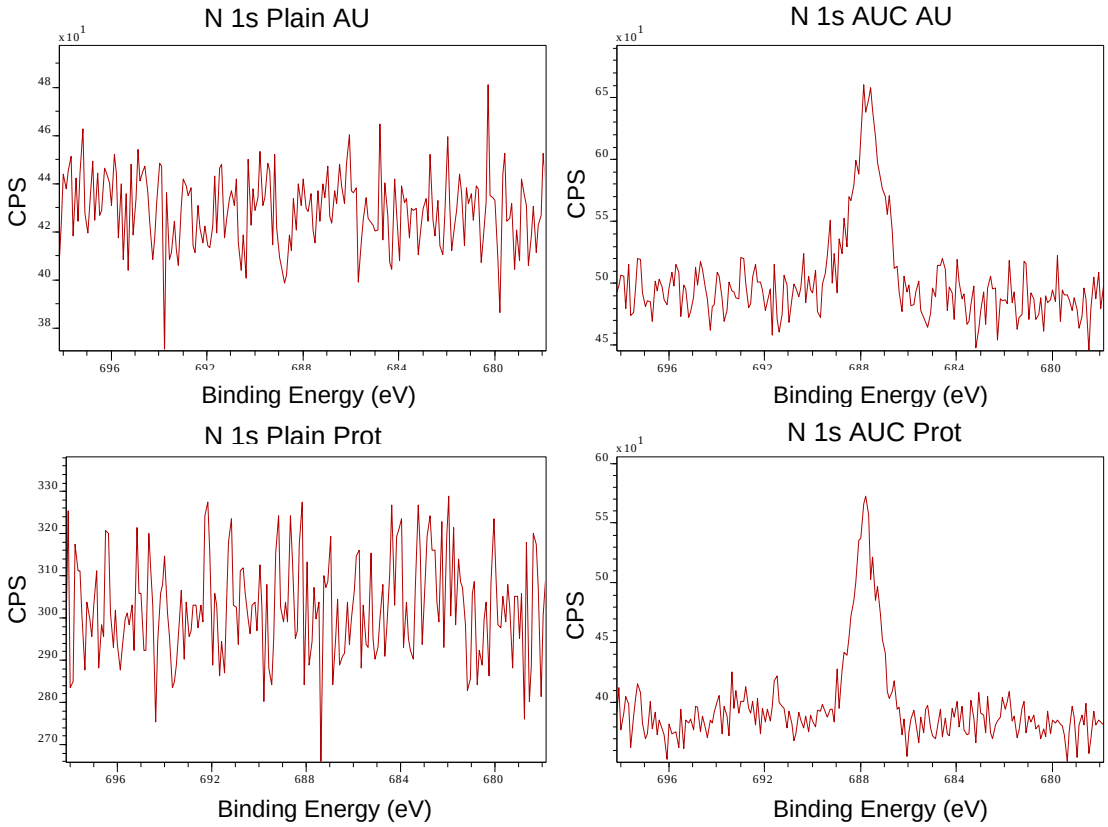
There was no significant difference (p=0.3695, one-way ANOVA) of the average N between the four groups. However, the appearance of the N peak differs between the four experimental groups. As seen in Figure 3-30 the shape of the N peak from the all-silicone catheters both with and without *P. mirabilis* is a single sharp peak, whereas the AUCs with and without *P. mirabilis* have a peak with multiple peaks within it. This suggests that the N deposited from urine, such as crystals, is different from the antibiotic N resulting in a rougher peak due to their different binding energies of the sources of N.

Figure 3-30 N 1s (nitrogen) peaks from an all-silicone (Plain) and an antimicrobial-impregnated (AUC) catheter segment perfused with artificial urine only (AU), and an all-silicone (Plain) and an AUC segment perfused with AU inoculated with *P. mirabilis* (prot)



In support of the detection of the antimicrobials on the surface of the catheter being detected by XPS is that fluorine (F), which is present in sparfloxacin, is detected only in the AUC segments (Figure 3-31).

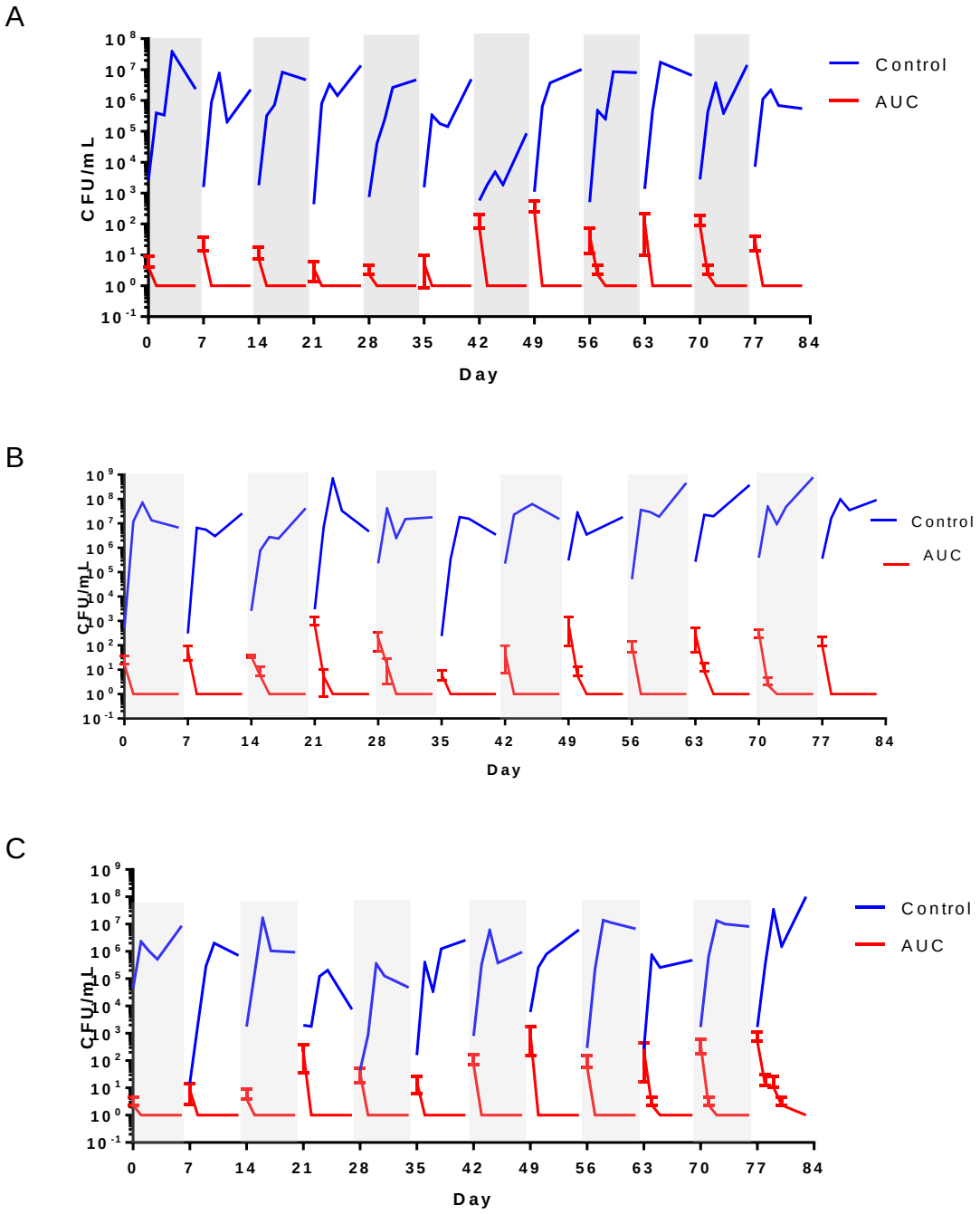
Figure 3-31 F 1s (Flourine) peaks from an all-silicone (Plain) and an antimicrobial-impregnated (AI/AUC) catheter segment perfused with artificial urine only (AU), and an all-silicone (Plain) and an AUC segment perfused with AU inoculated with *P. mirabilis* (prot)



3.3.9 **In vitro flow challenge model – efficacy experiments**

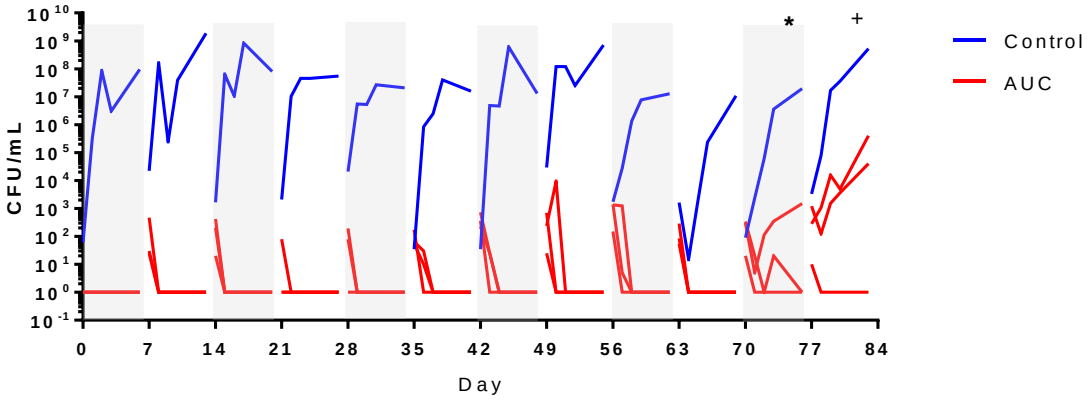
The AUC was able to prevent colonisation *by S. saprophyticus*, ESBL *E. coli*, and NDM-1 *E. coli* for 12 weeks. The all-silicone controls colonised successfully each week (Figure 3-32).

Figure 3-32 In vitro flow challenge model results of all-silicone (control) and antimicrobial-impregnated catheters (AUC) challenged with A.) F2637 *S. saprophyticus*. B.) F3986 extended-spectrum beta-lactamase producing *E. coli*, C.) F3802 New Delhi metallo-beta-lactamase producing *E. coli*. All control and AUCs were challenged weekly.



The AUC was able to prevent colonisation by MRSA for approximately 84 days (Figure 3-33). At 70 days one catheter failed, another failed at 77 days weeks, and the third catheter successfully prevented colonisation for the entire 84 day experimental period. The control catheter successfully colonised each week.

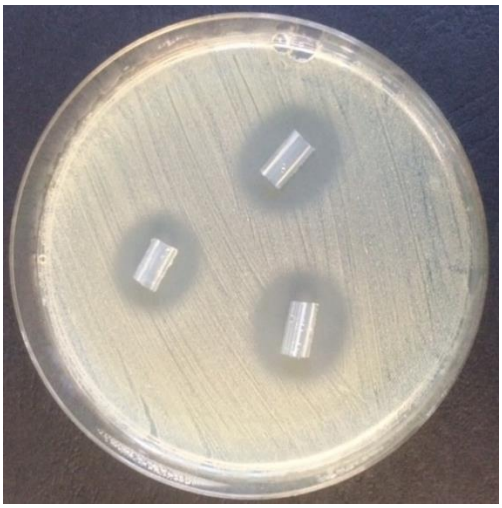
Figure 3-33 In vitro flow challenge model results of all-silicone (control) and antimicrobial-impregnated catheters (AUC) challenged with F4142 MRSA. All control and AUCs were challenged weekly. *: point of failure of MRSA AUC 2, +: point of failure of MRSA AUC 3



Throughout days 70-84, the control catheters of the MRSE and MSSA groups failed to colonise. The MRSE and MSSA controls would attach at 0 hours but die or fall off by 24 hours. Several strategies were employed to solve the problem. The first concern was that the strains had lost their ability to attach to silicone so fresh bacteria were re-subcultured from the freezer and inoculated into the model. At day 70, there was a change in weather and the laboratory was colder than it had been over the summer, so the perfusion medium was kept in an incubator at 37°C. Neither intervention was successful. It was then suspected that the all-silicone control catheters had incorporated something antimicrobial so that they were now exerting antimicrobial activity.

The control catheters were removed from the model and 1.0 cm segments were removed and placed onto a lawn of either F4001 MRSE or F3991 MSSA. As seen in Figure 3-34 zones of inhibition are clearly visible around the segments suggesting the control catheters acquired antimicrobial activity.

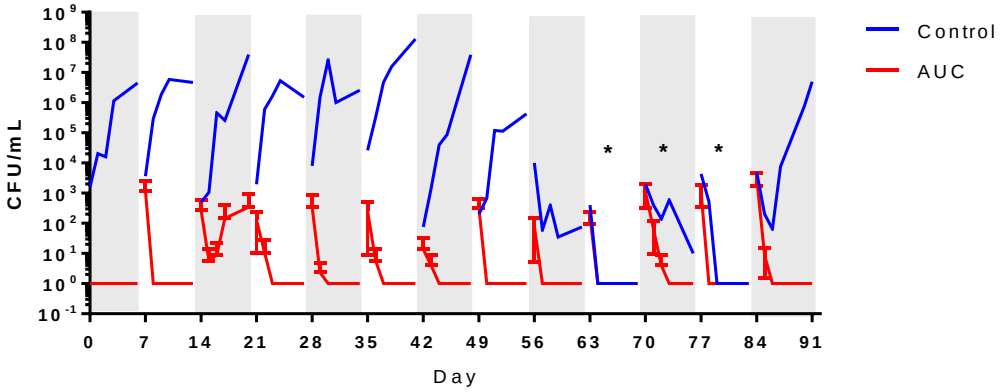
Figure 3-34 1.0 cm segments of the control catheter for the in vitro flow challenge model at 84 days placed onto a lawn of F3991 MSSA.



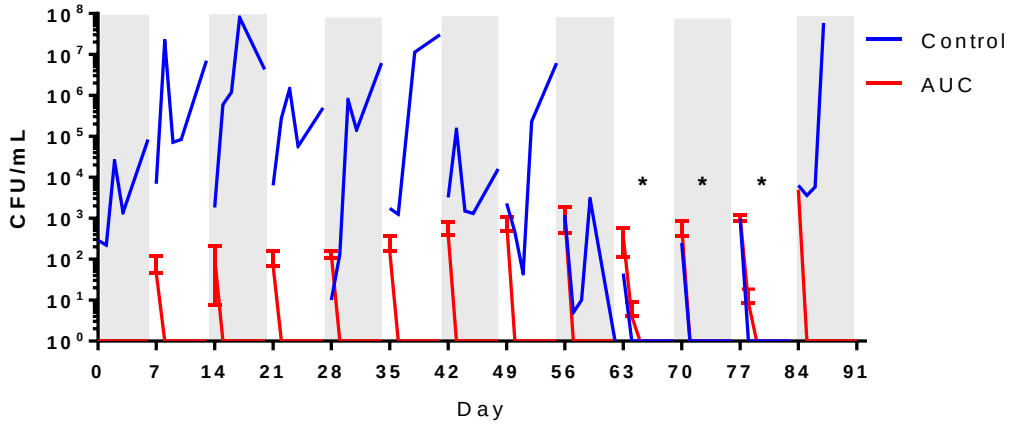
It was suspected that when the control catheters for this week were autoclaved for sterilisation, residual antimicrobial molecules were present in the water in the reservoir of the autoclave. These molecules may have been dispersed during the autoclave cycle. After a thorough cleaning of the autoclave and replacement of the water in the reservoir, a new set of control catheters were autoclaved and the in vitro flow challenge was run for a 13th week. During these investigations, the antimicrobial catheters inoculated with MRSE and MSSA did not colonise and were constantly perfused with medium as normal so that the surface of the catheters was still subjected to flow during this time. At 91 days the control catheters colonised exponentially over the course of the week and the antimicrobial catheters failed to be colonised. Therefore, despite a lack of proper controls during weeks 10-12 the success of the antimicrobial catheters in preventing colonisation at 91 days suggests the same is true of the previous weeks. Therefore, the antimicrobial catheters failed to prevent colonisation by MRSE and MSSA for 91 days weeks (Figure 3-35).

Figure 3-35 In vitro flow challenge results over 91 days of all-silicone (control) and antimicrobial catheters (AI) inoculated with A.) methicillin-resistant *Staphylococcus epidermidis* and B.) methicillin-susceptible *Staphylococcus aureus.* * indicates timepoints where the control catheters failed to colonise. During this time the antimicrobial catheters were continually perfused with media. All control and AUCs were challenged weekly.

A

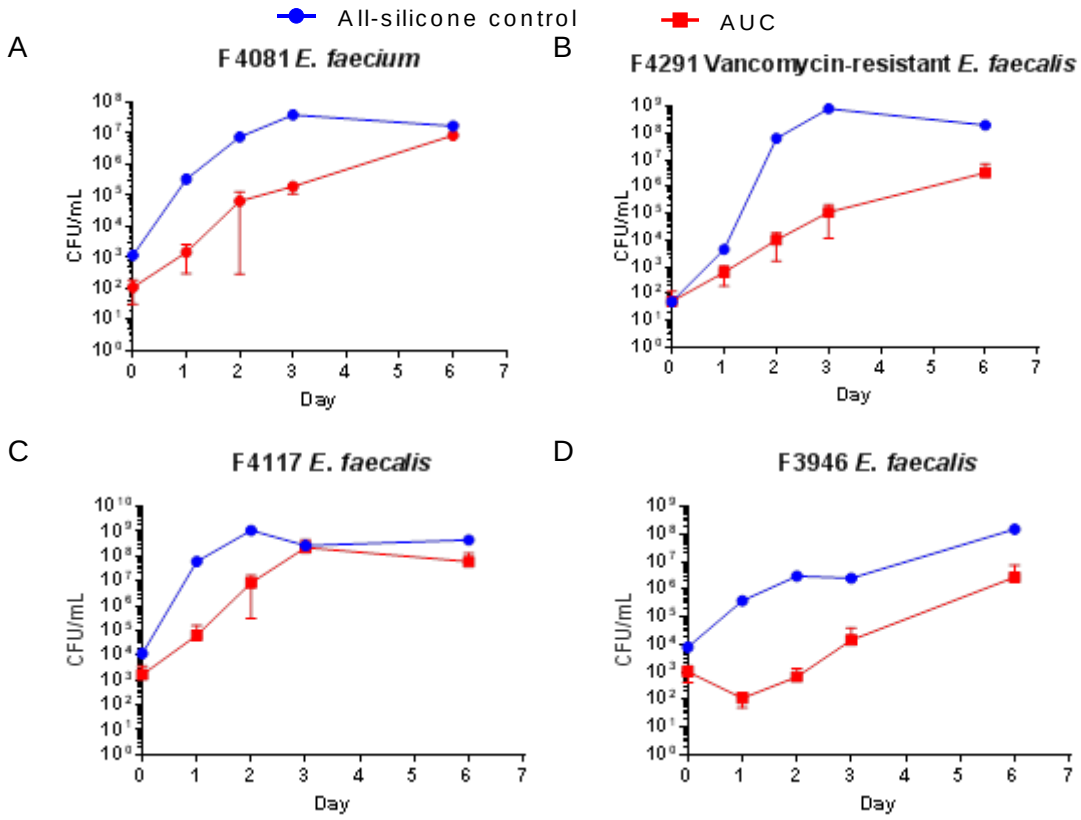


B



The AUC was unable to prevent colonisation by *E. faecalis* or *E. faecium* after the initial bacterial challenge in the in vitro flow challenge model (Figure 3-36). The Tk100 assay showed that the AUC was able to kill 100% of F4117 within 72 hours but was unable to do so under flow conditions. However, at 72 hours, there were statistically significantly fewer bacteria attached to the AUC than the all-silicone control for F4117 ($p<0.0001$), F4291 VRE ($p<0.0001$), and F3946 ($p<0.0001$), but not for F4081 ($p=0.1330$) (multiple t-tests, Holm-Sidak correction).

Figure 3-36 In vitro flow challenge results of all-silicone (control) and antimicrobial catheters (AUC) inoculated with A.) F4081 *E.* faecium, B.) F4291 vancomycin-resistant *E. faecalis*, C.) F4117 *E. faecalis*, D.) F3946 *E. faecalis.* All control and AUCs were challenged weekly.



3.3.9.1 **Susceptibility testing of surviving isolates**

MRSA isolates from the AUCs that failed at 70 days (MRSA AUC 2) and 77 days (MRSA AUC 3) were tested for their MICs and MBCs to the antimicrobials in the catheter. The original MICs for the MRSA were 0.008, >6.4, and 1.0 mg/L for rifampicin, sparfloxacin, and triclosan respectively. The MICs and MBCs for each isolate were similar to the original values, with the MIC for rifampicin raised by one dilution (Table 3-15). Both isolates remained susceptible to rifampicin.

Table 3-15 Minimum inhibitory concentration (MIC) (mg/L) and minimum bactericidal concentration (MBC) of MRSA isolates from the antimicrobial urinary catheters (AUC) in the in vitro flow challenge model that failed at 11 weeks (MRSA AUC 2) and 12 weeks (MRSA AUC 3).

	Rifampicin		Sparfloxacin		Triclosan	
	MIC	MBC	MIC	MBC	MIC	MBC
MRSA AUC 2	0.015	0.5	6.4	>6.4	0.5	8.0
MRSA AUC 3	0.015	0.5	6.4	>6.4	1.0	16.0

None of the AUCs challenged with the enterococci was able to prevent colonisation after the initial challenge. The surviving enterococci from each AUC had their MICs and MBCs determined (Table 3-16). The MICs to triclosan and to sparfloxacin remained unchanged, although the MIC of F3946 to sparfloxacin was raised by one dilution factor it still remained susceptible. However, all four isolates had raised MICs to rifampicin from the original, most notably being F4291 VRE which was susceptible to rifampicin with a MIC of 0.25 mg/L and had increased to approximately 2.0 mg/L (intermediate susceptibility) to rifampicin. As with F4117 its MIC to rifampicin increased by two-fold changing from intermediate sensitivity to resistant.

Table 3-16 Minimum inhibitory concentration (MIC) (mg/L) of *Enterococcus faecalis* (F4291, F3946, and F4117) and *Enterococcus faecium* (F4081) before and after inoculation into the in vitro flow model

	Rifampicin MIC		Sparfloxacin MIC		Triclosan MIC	
	Before	After	Before	After	Before	After
F4081 AUC 1	8.0	16.0	>6.4	6.4	32.0	32.0
F4081 AUC 2	8.0	16.0	>6.4	>6.4	32.0	32.0
F4081 AUC 3	8.0	16.0	>6.4	>6.4	32.0	32.0
F4291 AUC 1	0.25	1.0	>6.4	>6.4	32.0	32.0
F4291 AUC 2	0.25	2.0	>6.4	>6.4	32.0	32.0
F4291 AUC 3	0.25	2.0	>6.4	>6.4	32.0	32.0
F3946 AUC 1	4.0	8.0	0.05	0.1	32.0	32.0
F3946 AUC 2	4.0	8.0	0.05	0.1	32.0	32.0
F3946 AUC 3	4.0	8.0	0.05	0.2	32.0	32.0
F4117 AUC 1	2.0	8.0	0.4	0.8	32.0	32.0
F4117 AUC 2	2.0	8.0	0.4	0.4	32.0	32.0
F4117 AUC 3	2.0	8.0	0.4	0.4	32.0	32.0

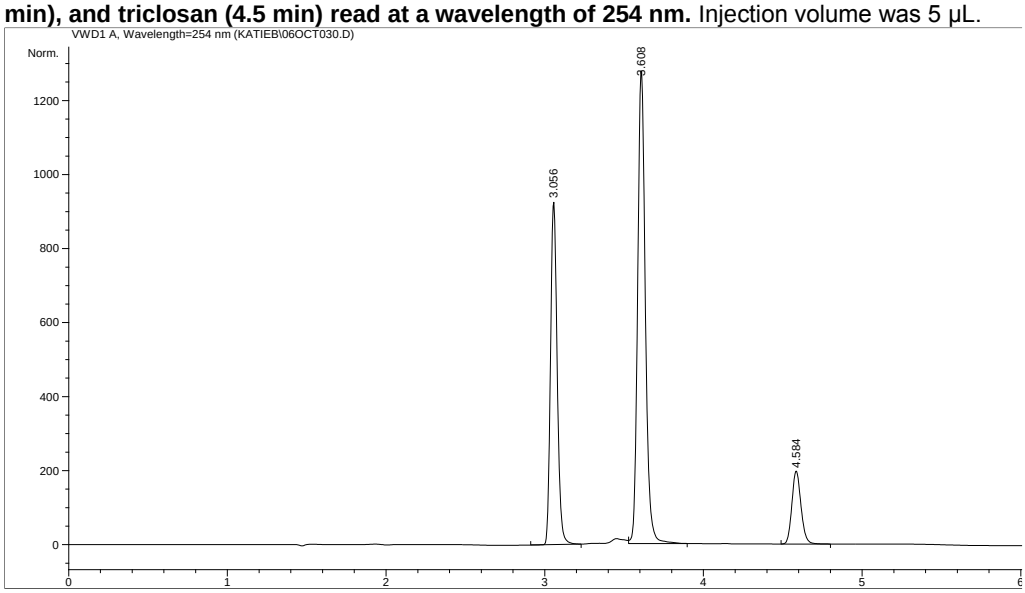
In summary, the AUC was able to prevent colonisation of MRSA, MSSA, MRSE, *S. saprophyticus*, ESBL-producing *E. coli*, and NDM-1-producing *E. coli* for its likely clinical lifetime as determined by the clinically predictive in vitro flow model. The AUC did not eradicate enterococci

including VRE in the same model but may have reduced attachment compared to the control catheters.

3.3.10 HPLC of catheters after perfusion and removal from the in vitro flow challenge model

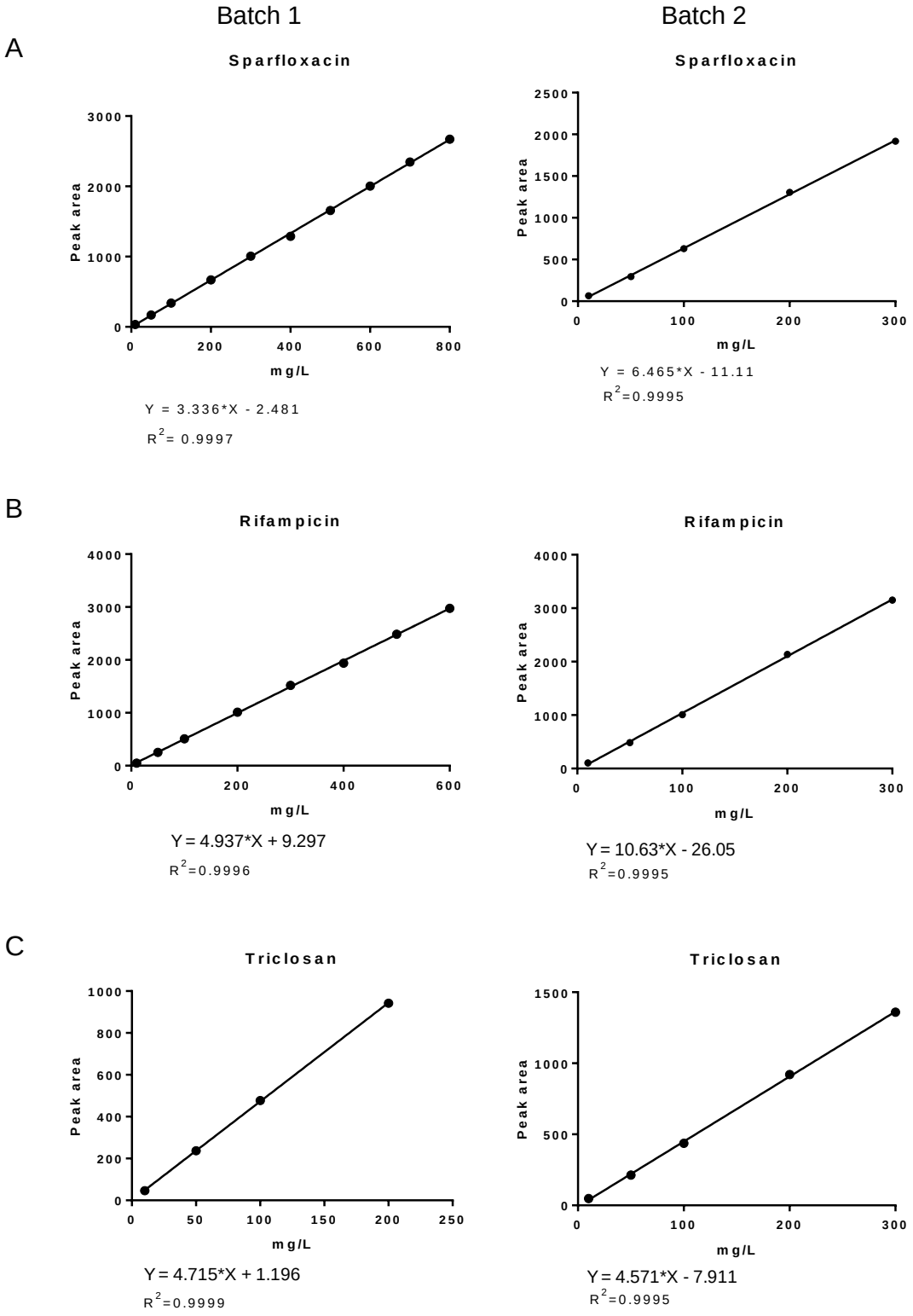
A typical chromatogram of the peaks of the three antimicrobials is displayed in Figure 3-37. The peak for sparfloxacin was at 3.07 minutes, for rifampicin at 3.6 minutes, and for triclosan at 4.5 minutes.

Figure 3-37 Chromatogram of the 800 mg/L standard of sparfloxacin (3.0 min), rifampicin (3.6 min), and triclosan (4.5 min) read at a wavelength of 254 nm. Injection volume was 5 µL.



The values from the standards were used to create calibration curves and interpolate the concentrations of the samples. The samples were analysed by HPLC in two batches due to the number of samples and therefore each batch had fresh standards prepared and the sample values were interpolated from the standard curves for its batch (Figure 3-38). For Batch 1 standards, concentrations were prepared up to 800 mg/L with concentrations of 700 mg/L and 800 mg/L rifampicin had peaks greater than a height of 1000 mAU, which is the arbitrary cut-off for the maximum height. The values for these figures were excluded from the standard curve. Due to smaller concentrations being detected in these samples, standard concentrations up 300 mg/L were sufficient and the standard curve was adjusted for Batch 2.

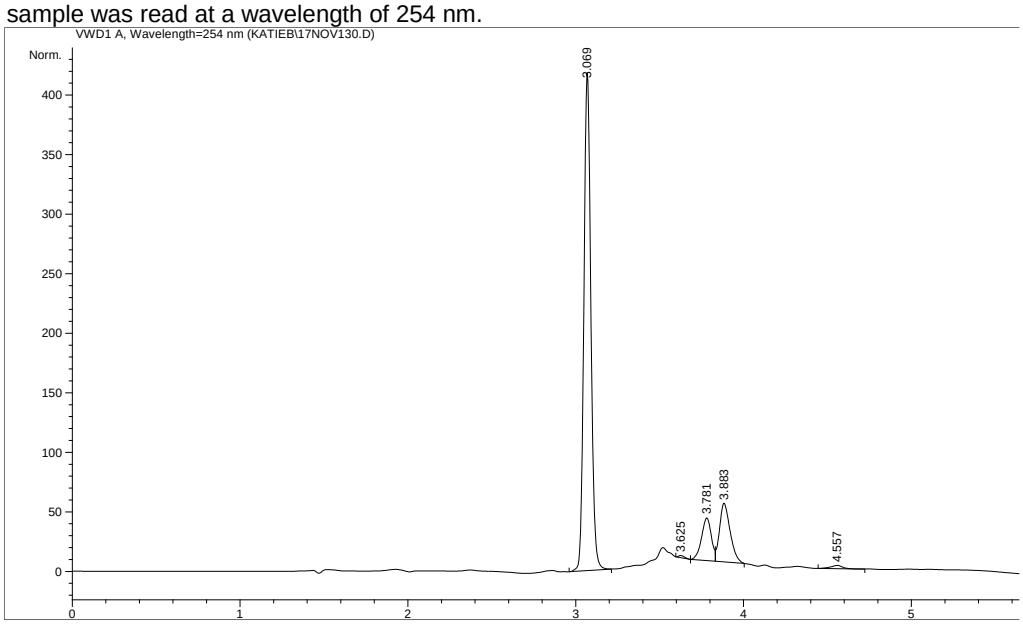
Figure 3-38 Standard curves of concentrations of A.) Sparfloxacin (read at 254 nm), B.) Rifampicin (read at 254 nm), and C.) Triclosan (read at 279nm) plotted against the peak area as determined by HPLC. Each value represents the average of three values and standard deviation. The slope of the line and goodness of fit (R^2) of the line are represented below each graph. Two batches of standards were prepared for the two rounds of HPLC required for the number of samples.



A typical chromatogram of the drug extract from a catheter that had been subjected to the in vitro flow challenge model for 13 weeks can be seen

in Figure 3-39. Of note is that the chromatograms of the samples had two peaks additional to the three for the antimicrobials of interest. These were present at approximately 3.7 and 3.9 minutes and represent rifampicin degradation products. They were not included in the quantification of rifampicin.

Figure 3-39 Chromatogram of sample MRSA 3.1 in which sparfloxacin (3.0 min), rifampicin (3.6 min), and triclosan (4.5 min) are present but also two peaks at approximately 3.7 and 3.9 minutes. These peaks represent rifampicin degradation products. The injection volume was 5µL and the sample was read at a wavelength of 254 nm.



As described in the methods section, additional modifications to the HPLC method were introduced to increase the sensitivity of detecting triclosan. Increasing the injection volume and changing to a more sensitive wavelength was not sufficient to increase the noise:signal over 10.0, which would indicate that the peaks could be reliably quantified without fear of quantifying noise. Changing the noise:signal baseline region away from the unresolved peaks of the rifampicin degradation products at 3.7 and 3.9 minutes increased the noise:signal ratio above 10.0 for each triclosan peak of every sample.

Sparfloxacin was clearly present after 13 weeks in all catheters at a median proportional drug content of 0.2020% with an interquartile range (IQR) of 0.101% or a total drug content of 22.41 mg (IQR=11.21 mg). The proportional drug content of sparfloxacin per catheter ranged from 0.096% (10.65 mg) - 0.264% (29.29 mg).

Triclosan was detectable and quantifiable after modifications to the HPLC method and was present in the antimicrobial catheters after 12-13 weeks of perfusion with a median proportional drug content of 0.0210% with an IQR of 0.023% or which is equivalent to a total drug content of 2.329 mg (IQR=2.55 mg). The proportional drug content of triclosan per catheter ranged from 0.008% (0.0867 mg) - 0.049% (5.477 mg).

Rifampicin drug content was on the cusp of the limit of quantification and detection by HPLC so many were determined as ‘detectable, not-quantifiable’ as the peaks were detected by the analysis, but due to the height of the peaks, they could not reliably be separated from the ‘noise’ and therefore quantified for the majority of samples. Peaks not detected were reported as ‘not detected’. Rifampicin proportional drug content ranged from not detected to 0.0077% (0.853 mg) (Table 3-17). The median proportional drug content of samples that could be quantified was 0.0046% with an IQR of 0.00298% which is equivalent to 0.512 mg (IQR=0.331 mg). However, this median value may not be representative of all samples as the greater majority of samples could not be quantified or detected and therefore did not have an assigned value.

Table 3-17 HPLC outcomes for rifampicin including not detected, detected but not quantifiable drug peaks and the proportional drug content (% w/w) rifampicin after challenge in the in vitro flow model

HPLC outcome	Number of Samples (n=53)
Not detected	6
Detected, not quantifiable	25
≥0.0021% < 0.0045%	10
≥ 0.0045% ≤ 0.0077%	12

In summary, after 12-13 weeks of perfusion and bacterial challenge sparfloxacin and triclosan were still present in all catheters. Rifampicin was detectable in the majority of catheters, but the amount was often below the limit of quantification. A summary of the drug content of AUCs after 12-13 weeks of perfusion and bacterial challenge can be found in (Table 3-18).

Table 3-18 Summary of the proportional drug content (w/w%) and total drug content (mg) with interquartile range (IQR) of the antimicrobial urinary catheters challenged for at least 12 weeks in the in vitro flow challenge model

	Rifampicin	**Sparfloxacin**	**Triclosan**
Median proportional Drug Content (w/w)	0.0046% (IQR=0.00298%)	0.2020% (IQR=0.101%)	0.0210% (IQR=0.0230%)
Total drug content (mg)	0.512 (IQR=0.331)	22.41 (IQR=11.21)	2.329 (IQR=2.55)

Quality assurance of the AUCs made for the safety clinical study (Chapter 4) included analysis of the drug content after manufacture by HPLC using the same method described in this chapter. Using that information as the starting concentration of antimicrobials it is clear that the majority of antimicrobials were eluted over the 12-13 week period with 98.09% of triclosan eluted and 75.33% of sparfloxacin eluted (Table 3-19). Approximately 95.23% of rifampicin was eluted, although, as some samples after 12 weeks of perfusion could not be detected or quantified by HPLC, they could not be included in this calculation and therefore the proportion of drug eluted over 12-13 weeks could be slightly higher for rifampicin.

Table 3-19 Median drug eluted (mg) and proportion eluted of total amount impregnated from the AUCs over 12-13. * Rifampicin samples below the limit of detection and quantification were not included in calculation[273]

Antimicrobial	**Median drug eluted over 12-13 weeks (mg)**	**Proportion of drug eluted over 12-13 weeks**
Rifampicin	8.285*	94.09%*
Sparfloxacin	68.415	75.33%
Triclosan	119.66	98.09%

In summary, sparfloxacin and triclosan remain in the AUC in readily quantifiable amounts after 12-13 weeks of constant perfusion with the amount of rifampicin remaining being on the cusp of detection by HPLC.

3.4 Discussion

The studies presented in this chapter concern the effect of the AUC on preventing multi-drug resistant bacteria and mineral deposition, which is a major cause of blockage, bypassing of urine, and urinary retention for catheter users. The AUC should ideally resist bacterial colonisation and not predispose to antimicrobial resistance or to mineral encrustation.

3.4.1 Bacterial characterisation

3.4.1.1 Characterisation of isolates for encrustation experiments

P. mirabilis and its enzyme urease have been implicated as the driving force of mineral encrustation by the formation of struvite crystals, and also of kidney stones[243]. However, other gram-positive and gram-negative uropathogens also produce urease. The urease produced by *P. mirabilis* is more efficient than ureases produced by other species. For example, in a study of ureases produced by *P. mirabilis, Proteus vulgaris, P. stuartii*, and *Morganella morganii* the ureases produced by *P. mirabilis* had less affinity for urea than the other species. A high affinity to urea, in a urea - saturated environment such as urine, is not necessary and therefore not advantageous. *P. mirabilis* ureases are able to hydrolyse urea at a six – 30 fold higher rate than the other species and can do so efficiently with their low-affinity enzyme in urine[11].

S. saprophyticus is an uropathogen particularly affecting the female urinary tract, and also a known causative agent of prostatitis[274], and it also possesses a urease enzyme that has been associated with urinary stone formation[275, 276]. The urease produced by *P. mirabilis* contributes to its pathogenicity in that the alkaline environment it produces is nephrotoxic, and the urease has a role in cellular invasion of the epithelium of the kidneys [277], whereas the urease produced by *S. saprophyticus* is a virulence factor for invasion of the bladder tissue (cystopathogenic) [278]. Despite differences in the types of tissue, urease is important in tissue invasion by both species. *E. coli* does not possess the urease enzyme and therefore does not alkalinise the urine and is not capable of blocking the catheter due to mineral deposition[211]. Urease production was confirmed by API kit and was also determined by changes in the pH of artificial urine over 96 hours, in which there was a rapid increase in pH over 24 hours by *P. mirabilis,* a steady increase in pH by *S. saprophyticus* over 96 hours, and no pH change by *E. coli.*

3.4.1.2 Characterisation of isolates for efficacy experiments

The isolates collected for these experiments were largely isolated from the lumens of urinary catheters collected as part of the study in Chapter

2. MRSA was not isolated from any of the collected urinary catheters, which likely reflects the decrease in prevalence of MRSA isolates in the UK[37, 38], but was instead isolated from an MSU. As no carbapenemase - producing isolates were available for the collected urinary catheters, the NDM-1 producing isolate was also collected from an MSU. All isolates have urological origins and as clinical strains are likely to be more relevant than type-culture strains.

3.4.1.3 Determining antimicrobial susceptibilities

The susceptibilities of the bacteria to the drugs in the catheter were difficult to determine in that there are no clinical breakpoints for sparfloxacin or triclosan. EUCAST recommends that in the absence of clinical breakpoints to use data on pharmacokinetic/ pharmacodynamics (PK/PD) breakpoints and that if the MIC is less than or equal to the PK/PD it could be reported as clinically useful. PK/PD breakpoints were unavailable for sparfloxacin and triclosan and so in the absence of these breakpoints, EUCAST then recommends comparing the MIC to the wild-type MIC as determined by ECOFFs [279], which were available for triclosan by Morrissey et al[266]. Due to sparfloxacin's lack of current use, ECOFF values were mostly unavailable with the exception of *Enterococcus faecalis* for which EUCAST provides an ECOFF of ≤1.0 mg/L, which is in disagreement with the clinical breakpoint of ≤4.0 mg/L for ciprofloxacin based on the ciprofloxacin ECOFF of 4.0 mg/L according to EUCAST and CLSI. However, Fuchs et al. states that 'The achievable sparfloxacin levels are similar to those of ciprofloxacin when comparable doses are administered[280]. The MIC breakpoint for susceptibility to sparfloxacin is also ≤1.0 mg/L in reference to staphylococci. This is in agreement with the current EUCAST MIC breakpoint of ≤1.0 mg/L for staphylococci to ciprofloxacin[264]. Therefore, due to the similarity of drug concentrations in the body and MICs, the ciprofloxacin MIC breakpoint value provided by EUCAST could be used to interpret sparfloxacin susceptibility. The clinical breakpoint of 4.0 mg/L for enterococci to ciprofloxacin, as a marker of susceptibility to sparfloxacin, was used on the basis of similar drug concentrations in the body and that the breakpoint is more clinically predictive of resistance than an ECOFF despite the difference in ECOFFs.

Likewise, no EUCAST clinical breakpoints were available to enterococci. CLSI breakpoints were available for enterococci with resistance being defined as ≥4.0 mg/L[265] which is in agreement with the ECOFF provided by EUCAST of 4.0 mg/L for *E. faecalis* to rifampicin. This therefore increased confidence in the choice of these breakpoints for this assay, particularly as the CLSI method of antimicrobial susceptibility testing is in concordance with the EUCAST method including media (MHA) and incubation at 35°C in air for enterococci[265].

Antibiotic susceptibility screening was carried out according to EUCAST guidelines. The isolates were screened for ESBL production due to the increase in ESBL-producing *E. coli* in Europe, in which the mean population-weighted percentage of ESBL isolates in Europe increased from 11.9% in 2012 to 12.4% in 2016[36]. Confirmation involves comparing the MIC of the cephalosporin to the MIC of the cephalosporin + clavulanic acid, a beta-lactamase inhibitor. Both cefotaxime and ceftazidime +/- clavulanic acid strips are used, because the TEM and SHV ESBL types readily show resistance to ceftazidime, and CTX-M types show resistance to cefotaxime consistently [260]. One of the key features of ESBL enzymes is that their activity is inhibited by clavulanic acid, and therefore a reduced MIC in the presence of clavulanic acid indicates that the enzyme has been suppressed so that the cephalosporin is not hydrolysed and may then have greater activity on the bacteria.

For detection of carbapenemase enzymes, meropenem is recommended by EUCAST as the most specific and sensitive screening agent and confirmation then relies on the synergy between meropenem and enzyme inhibitors[260]. The synergy of meropenem with DPA and EDTA is characteristic of the Class B MBLs[260, 262]. β-lactamase classification systems separate MBLs from other β-lactamases according to the zinc ions at their active site. EDTA and DPA are metal chelators which sequester the zinc ion from the active site preventing binding of the active site rendering the bacteria susceptible to carbapenems[55, 281]. DPA is a useful addition to MBL detection as it has no intrinsic antimicrobial activity, unlike EDTA, and does not interfere with the beta-lactams[282]. Unlike other phenotypic susceptibility tests, carbapenemase detection is improved by use of MacConkey agar

instead of MHA most likely due to the presence of bile salts in MacConkey agar which may lyse the cells or increase their permeability to release β-lactamase enzymes[283]. The precise MBL had previously been verified by the Antimicrobial Resistance and Healthcare Associated Infections Reference Unit as a NDM-1 enzyme[62].

3.4.2 **TK100**

The AUC does not prevent bacterial adherence but works by killing the bacteria that attach as seen by the TK100 assay in which there was initial attachment at 0 hours (Figure 3-13), and then the ability of the catheter to kill the attached bacteria over 72 hours was assessed. An initial assay determined the percentage of TSB that maintained growth but did not allow growth to exponentially increase or decrease. It is apparent from the TK100 assays that the TSB percentages chosen were appropriate as the control all-silicone catheters maintained a constant viability over the experimental period.

3.4.2.1 **TK100 – encrustation studies**

The results of the soaking studies show that soaking in AU prior to the TK100 assay does not affect bacterial attachment. This is important because firstly, over the soaking period the antimicrobials were released into the soaking fluid and therefore there is less antimicrobial available at the catheter surface to prevent attachment. There are clearly enough antimicrobial molecules to exert protective activity even at two weeks despite the soaking period. Secondly, soaking in artificial urine may produce a conditioning film that facilitates bacterial attachment, but this does not appear to affect bacterial attachment in this model. Thirdly, the lack of an increase in bacterial attachment over the soaking period or with the catheters processed by the impregnation method without antimicrobials, suggest that even if the impregnation process of the catheters changes the surface roughness it is unlikely to be clinically significant.

Artificial urine was chosen as the medium for soaking as it a consistent, reproducible medium unlike urine from donors, but also replicates the protein content of urine, of which the conditioning film is largely

composed[239]. Tambe et al. found that catheters soaked in a proteinaceous medium for two weeks had greater bacterial adherence than those soaked for one week[284]. As previously mentioned the AUC does not prevent bacterial attachment, and the presence of a conditioning film will facilitate bacterial adhesion. The antimicrobial molecules must migrate through the film in order to exert their protective activity, and this has been shown to occur in the neurosurgical devices impregnated by the same method as the AUC[109].

3.4.2.2 **TK100 - efficacy studies**

The TK100 demonstrated that the AUC rapidly killed 100% of attached MRSA, MRSE, MSSA, and NDM-1 *E.coli*, but not ESBL-producing *E. coli*, even though numbers of attached bacteria were reduced. This is inconsistent with the results of the in vitro flow challenge model in which 100% of ESBL-producing *E. coli* was eradicated for 12 consecutive weekly challenges. It was considered that the TK100, although a useful screening tool, may not be predictive of bacterial attachment and survival of the AUC under flow conditions. Therefore, an extended TK100 was developed to determine if the survival of attached bacteria after multiple challenges would predict the length of time the AUC would survive in the in vitro flow model and was employed as an alternative method for TK100 for the enterococci.

The extended TK100 was extended only for F4117 *E. faecalis* and F3947 *E. faecium* as these were the only two isolates completely eradicated after the initial bacterial challenge. F3947 *E. faecium* was completely eradicated for four consecutive weeks by the AUC, most likely due to its susceptibility to rifampicin and sparfloxacin. F4117 *E. faecalis* was completely eradicated by the AUC in the TK100 first weekly challenge but was not eradicated from the AUC under flow conditions in the comparable first challenge. The predictive value of the TK100 is variable. Differences such as flow and build-up of antimicrobials in the surrounding solution between time points separate it from the in vitro flow challenge model. The variable predictive ability of the TK100 has been seen in other experiments in which silver neurosurgical catheters were challenged with *S. epidermidis* in the TK100 and in vitro flow challenge model described herein. All S.

epidermidis were eradicated at 72 hours by the silver catheter in the TK100 challenge, but when inoculated into the in vitro flow challenge model with the same bacterial inoculum density, *S. epidermidis* was not eradicated by the silver catheters by the end of seven days[285].

The TK100 assay may not be predictive of the protective lifetime of the in vitro flow challenge model but instead may serve as a useful screening tool for the ability of the bacteria of interest to attach and remain attached to the biomaterials being tested. All TK100 assays showed that the test bacteria were capable of attaching to all-silicone urinary catheters and remaining attached for the 72-hour duration. This is crucial for obtaining relevant results from the in vitro flow challenge model. The model would not be relevant if the bacteria were simply falling off of the catheter because of the inability to attach rather than due to killing activity of the AUC. For example, F3802 NDM-1 *E. coli,* F4142 MRSA, and some enterococci were not capable of producing a biofilm according to the crystal violet screening assay but were clearly able to colonise all-silicone catheter segments in the TK100 and then consecutive catheters in the in vitro flow challenge model.

Bacterial adherence in the crystal violet microtitre assay can be influenced by the material for attachment, the media used in biofilm growth, and reproducibility of the assay may be decreased for non-adherent and weakly adherent bacteria[268, 286]. The TK100 assay avoids these issues as it measures colony counts as this is the quantitative outcome of the in vitro flow challenge model so like for like is being measured. Secondly, the medium employed in the TK100 is less nutritious than 100% TSB supplemented with glucose, which is often used in crystal violet biofilm assays, to reflect the perfusion medium which is designed to mimic the nutritional content of urine. The TK100 also measures attachment to all-silicone urinary catheter segments, which are again used in the in vitro flow model as they replicate the clinical situation rather than the less clinically relevant tissue-culture polystyrene. Although the TK100 assays were not designed to assess reproducibility, all bacteria were able to attach and maintain growth on all-silicone control segments and this was then replicated for all test bacteria in the in vitro flow model.

3.4.3 **Surface roughness**

AFM is a useful tool for measuring surface roughness and displaying the surface topography of the catheter. Surface roughness is an important property of biomaterials as several studies have demonstrated that mineral encrustation increases as surface roughness increases[78, 79, 223] and the ease of catheter insertion and removal is affected by surface roughness [287]. Surface roughness was displayed by the R_q value, which is the root of the mean square and is a representation of the distribution of heights and roughness. The R_q is calculated by the equation $\sqrt{\frac{\sum(V_0-V_i)^2}{n-1}}$, where n is the number of data points scanned during microscopy, V_0 is the mean data point, and V_1 are all other data points.

However, due to the fact that it provides a high-resolution measurement of the catheter, the area measured is small, with the scan employed in this experiment measuring a square 2.0 µm x 2.0 µm. Scans were 'flattened' using the NanoScope Analysis software to take into account the curve of the catheter. To analyse a greater surface area of up to 5mm x 5mm a tactile measurement could be employed using a tip with a radius of 2 µm compared to the radius of the tip of 8nm used in these experiments [288]. Resolution is significantly lost, but a greater area could be examined. This may have been useful for samples where crystals were present on the surface to get a more comprehensive picture of the distribution of crystals. However, higher resolution was more desirable for these experiments as nucleation sites for crystal formation are influenced by nano-sized indentations[227].

AFM has been employed to measure surface roughness of silicone catheters in a study by Jones et al. (2004) in which silicone urethral catheters had R_q values (±SEM) of 43.11 ± 9.06 and 56.78 ± 13.46 [287]. In our study, all-silicone controls not soaked had a mean R_q value (± SEM, presented here for ease of comparison to the Jones et al. study) of 34.77 ± 3.435 and a mean R_q value of 36.27 ± 8.233 for the non-soaked silicone segments processed according to the impregnation method without the addition of antimicrobials. The values of silicone catheter surface roughness presented

in this study are similar to those in the Jones et al. study accounting for differences in equipment set-up and catheter manufacturer [287].

Previous studies of this AUC after soaking did demonstrate significant differences in surface roughness between the soaked AUC and the non-soaked control[77, 131], hence leading to further study. According to one-way ANOVA results of the R_q values between the groups, there was no significant difference in surface roughness between the experimental groups and the all-silicone control that was not soaked in artificial urine. The previous study noted that the all-silicone controls became smoother after the soaking period and the AI-catheters become rougher after soaking leading to further study here. In the previous study, PBS was used as the soaking medium instead of AU used here with AU being more similar to physiological urine. AU replicates a proteinaceous conditioning film as demonstrated from the XPS studies, and this conditioning film may modulate surface characteristics.

It is also important to note that surface roughness is not the only factor that may affect mineral encrustation and bacterial adhesion. Charges at the surface and the hydrophobicity of the surface may influence bacterial adhesion[289]. Silicone is a hydrophobic material which is more predisposed to bacterial and protein adhesion[290]. Previous work has shown that addition of the antimicrobials makes the silicone catheter more hydrophilic[77], and hydrophilic surfaces may be able to reduce protein and bacterial adhesion[291]. After soaking there is no significant difference in hydrophobicity between all-silicone and AUC segments[77]. The previous data showed that hydrophobicity, which may be initially advantageous, changed with the addition of antimicrobials, and the data on surface roughness as determined by AFM demonstrates that the surface properties of the silicone catheter are not negatively affected by the impregnation of antimicrobials.

3.4.4 Phosphate deposition

Methods such as spectrophotocolorimetric detection of attached phosphate and XPS quantified phosphate deposition on the catheter lumens and SEM provided qualitative supporting data.

3.4.4.1 **Spectrophotocolorimetry**

An existing spectrophotocolorimetric method[247] was adapted for these experiments. Spectrophotometry is advantageous in that it does not involve extraction of the phosphate and uses relatively simple and inexpensive equipment, so many samples can be analysed. The chemistry of the method relies on the formation of phosphomolybdate $(PO_4Mo_{12})^{3-}$, which creates a blue colour. The chemical reaction is as follows:

$$7(PO_4)^{3-} + 12(NH_4)_6Mo_7O_{24} + 36H_2O \rightarrow 7[(NH_4)_3PO_4Mo_{12}] + 51(NH_4)^+ + 72OH^-$$

Sodium sulphide is added as a reducing agent as phosphomolybdate is an oxidising agent. 0.25N sulphuric acid is added to create an acidic environment, as the acidity prevents the final complex from oxidising over time as phosphomolybdate could lose its negative charge and dissociate from the ammonium ion without this charge.

The intensity of the colour blue relates to the concentration of phosphate in the solution according to the Beer-Lambert law in which more of the transmitting light is absorbed as the concentration of the solution increases[292]. For Beer-Lambert's law to be true all samples need to be read at the same wavelength, the points of the calibration curve form a straight line that passes through the origin, and only one solute in the solution being measured is capable of absorbing the chosen wavelength[292]. The assumptions are true with the calibration curve and experimental samples in that all samples were read at 715 nm, the intercept of the calibration curve was 0.03609, and only the phosphomolybdate complex in the solution was blue. Two wavelengths were screened for optimal sensitivity to detect changes in the intensity of the blue colour, both of which, 630 and 715 nm, are red wavelengths and therefore ideally absorb red light but transmit blue-green light.

This method of using spectrophotocolorimetry is a novel approach for quantifying mineral encrustation on catheters. Typically the extent of mineral encrustation has been detected using SEM to visualise the encrustations and quantified by atomic absorption spectroscopy[78, 79, 223, 293]. Atomic absorption spectroscopy relies on the atoms of interest absorbing a certain wavelength

of light, which has enough energy to promote the electrons of the element of interest to a higher energy level. The light absorbed is quantified and the method is so sensitive that it can detect parts per billion of the element of interest [294]. However, an important disadvantage of this method is that phosphate interferes with detection of elements such as calcium[295], which is another common compound found in urinary crystals. Furthermore, phosphorus is very difficult to detect with atomic absorption spectroscopy as the wavelength needed to excite phosphorus electrons is within the ultraviolet light spectrum [296]. Therefore, with atomic absorption spectroscopy, only Mg and Ca can be measured and Ca detection may be reduced by the presence of phosphate. Phosphate was of particular interest as it is a specific component of struvite crystals, which are formed by urease-producing organisms, and of amorphous phosphate crystals, which are formed when the urinary pH is greater than neutral[184].

This method was validated for the purposes of these experiments by measuring phosphate precipitation in artificial urine when inoculated with the three test bacteria. As expected, more phosphate precipitated from AU in the presence of *P. mirabilis* than *S. saprophyticus* and *E. coli* over 96 hours, and phosphate precipitation appeared to be related to changes in pH over time. The sensitivity of the method allowed for significant differences of mineral deposition to be detected between all-silicone control and AUCs inoculated with *P. mirabilis* under both static and flow conditions. There were no significant differences between all-silicone controls and AUCs inoculated with *S. saprophyticus* and *E. coli* or AU adjusted to three different pHs. The minimum limit of detection by this method was 2.2 mg/L (also known as 2.2 ppm), which means 2.2 ppm differences between samples could be detected by this method. While this is not as sensitive as atomic absorption spectroscopy which can detect parts per billion, differences on such a small a scale as this are not likely to be clinically significant. The statistically significant difference in phosphate deposition between all-silicone control and AUCs inoculated with *P. mirabilis* correlates with the clinically relevant outcome of the control catheters blocking and the AUCs not blocking during the test period.

3.4.4.2 **XPS**

In this study, XPS was used to examine the differences in minerals as determined by the elemental composition at the surface of AUC and all-silicone catheters perfused with AU with or without *P. mirabilis*.

Previous XPS analysis of the AUC and all-silicone catheters after exposure to AU for one hour showed an increase of N and C on both catheter types after exposure due to the formation of a conditioning film[77]. XPS studies of ureteral stents also show N, C, Na, and Cl as markers of conditioning films[297]. Small amounts of Na and Cl were detected on most samples from AUC and all-silicone catheters both in the presence and absence of *P. mirabilis*, suggesting a deposition of minerals from the AU and not unique to the presence of *P. mirabilis*. All samples showed greater than 50% atomic concentration of C and 50% of this was most likely part of the silicone material; the additional C was likely to be from the conditioning proteins and bacterial cell deposition. There are three possible sources of N for the samples of these studies including proteins deposited from the artificial urine, bacterial cell adhesion, and antibiotics impregnated at the catheter surface. It is clear that antibiotics are being detected in the AUC as the shape of the N peak is different in the AUC samples compared to the all-silicone catheter samples. This suggests that regardless of cell and protein deposition, N is being detected from the antimicrobials in the AUC catheter, as well as F, which is present only on the AUCs and would not be found in any of the components of artificial urine. This indicates two possibilities; firstly that the conditioning film and deposited minerals are thin enough (10 nm or less) that the antimicrobials on the surface are detected by this method. The second possible explanation is that the antimicrobial molecules are capable of migrating through the conditioning film and mineral layer. From the encrustation TK100 results in which the segments were soaked in artificial urine to form a conditioning film, the AUC segments were still able to exert protective activity, confirming that the antimicrobial molecules can migrate through the protein film. The SEM images of AUC and all-silicone catheters show uneven crystallisation of the minerals across the surface, suggesting that many of the areas sampled by XPS may have had crystals absent from that location. It is clear from sample Plain Proteus 2 from the

SEM images that despite three-dimensional crystal structures, there are still gaps between the crystals. However, for this sample, it seems likely that the XPS irradiated the surface covered by mineral as only 8.1% atomic concentration of silicon was detected in this sample compared to 15.7 – 24.1% atomic concentration in all other samples.

An alternative to XPS which would allow certain sections of the material to be analysed, is environmental scanning electron microscopy (ESEM) with energy x-ray microanalysis. This method was employed by Stickler and Morgan in which segments from catheters perfused with artificial urine inoculated with *P. mirabilis* were imaged by ESEM and areas with crystal deposition and areas from the naked catheter surface were identified and then an elemental composition spectrum using x-ray microanalysis was generated from these areas. They found that the crystal deposit area was rich in Ca and P, whereas the spectrum of the bare catheter surface was composed mainly of Si with some C and O[123]. This is consistent with the XPS results here where Si is present in all samples and the Ca and P content is greater in Plain Proteus 2. In this sample the Si content is less indicating that crystal deposition was detected. For the other samples, the effect is not as noticeable by XPS. Therefore, XPS was useful at demonstrating the presence of a conditioning film on all catheter types, and confirmed noticeable crystallisation on an all-silicone catheter inoculated with *P. mirabilis*, but this was easily seen with SEM, and spectrophotocolorimetric quantification of phosphate of the lumen of the entire catheter lumen gave a more well-rounded analysis of differences in mineral deposition between the control and experimental groups.

3.4.5 **The efficacy of the AUC**

The extension of the activity of the AUC to prevent colonisation by MSSA, MRSA, MRSE, *S. saprophyticus*, ESBL *E. coli* and NDM-1 *E. coli* for the lifetime of a long-term urinary catheter is a unique characteristic that is unavailable with any other commercial or experimental catheters to the author's knowledge. Previous in vitro flow challenge studies did include one strain of MRSA, and ESBL *E. coli*[77, 131], but due to their importance in healthcare-associated infections it was important to verify that the activity of

the AUC was not limited to one strain. The results of the in vitro challenge model showing the protective activity of the AUC for 12 weeks against F4142 MRSA and F3986 ESBL *E. coli* is in agreement with the MRSA and ESBL *E. coli* used by Fisher et al[131].

The three AUCs inoculated with ESBL *E. coli* and NDM-1 *E. coli* had the lowest amount of sparfloxacin remaining in the catheter at the end of the in vitro challenge test period according to HPLC, which is of interest as these organisms had intermediate susceptibility to sparfloxacin according to the MIC values. These were the only organisms with decreased sensitivity that were tested and were still killed by the AUC even after 12 weeks. On the other hand, two of the three AUCs challenged with MRSA were not able to resist colonisation entirely even though MRSA was susceptible to sparfloxacin and had more sparfloxacin remaining in those catheters than those challenged by the two *E. coli* isolates. However, both *E. coli* isolates had wild-type sensitivity to triclosan with a MIC of 0.5 mg/l, according to the ECOFF values, whereas the MRSA isolate had a greater MIC (MIC 1.0 mg/l). Triclosan may be more important agent in determining the protective lifetime of the AUC than the other antimicrobials.

Rifampicin could possibly also contribute to the protective activity against gram-negative organisms. Despite gram-negative organisms being intrinsically resistant rifampicin, this drug has been used as an adjunct to colistin to treat MDR *A. baumanii* infections based on a synergistic relationship between colistin disrupting the cell wall allowing access of rifampicin into the cell[298]. A possible synergistic mechanism may exist between rifampicin and triclosan in that the disruption of fatty acid synthesis by triclosan disrupts the cell membrane possibly increasing the cell's permeability to rifampicin, although this has yet to be investigated.

The in vitro flow challenge model was challenged with four enterococci that had differing susceptibilities to the antimicrobials in the catheter. Despite susceptibility to rifampicin or sparfloxacin, but none to triclosan, by the *E. faecalis* isolates none were completely eradicated by the AUC after the initial challenge. As suggested above, this may implicate triclosan as the crucial antimicrobial that determines clinical success. This is in agreement with Williams and Stickler who found that in their bladder model in which they

inflated the balloon with a 0.3% triclosan solution it was incapable of reducing *E. faecalis* in the residual urine of the bladder model[92] after seven days as in the in vitro challenge model.

In the absence of triclosan exerting any selective pressure on the enterococci, the enterococci acquired increased MICs to rifampicin when faced with essentially, monotherapy. The rapid development of resistance to rifampicin in monotherapy is well documented[299]. The greatest MIC increases to rifampicin were seen by F4291 and F4117 E. *faecalis* which were sensitive and intermediate, respectively, compared to F4081 *E. faecium* and F3946 *E. faecalis*, which were resistant according to clinical breakpoints. The mutant selection window hypothesis by Drlica and Zhao proposes that bacteria with resistance mutations develop when exposed to antimicrobial concentrations that fall between the MIC of the population of bacteria and the mutant prevention concentration, which is the MIC of a subpopulation with less drug susceptibility[300]. Therefore, more susceptible organisms have a larger mutant selection window, which may explain the greater MIC increase of the more susceptible organisms. Resistance selection can be countered by increasing dosing or using two antimicrobials of two different classes and therefore targetting two different bacterial sites[132, 300]. The AUC is designed with three antimicrobials with three different mechanisms of action with the rationale that at least two of the three antimicrobials will target the major organisms colonising the catheter. However, of the eight enterococci used in these experiments, only F3947 *E. faecium* was susceptible to rifampicin and sparfloxacin and intrinsically resistant to triclosan, It appears that in this population of enterococci isolated from urinary catheters it is more common for isolates to be susceptible to one or none of the antimicrobials in the AUC.

20% TSB was employed as the perfusion medium in the in vitro flow challenge assays instead of AU used in the perfusion encrustation studies, as the minerals such as calcium and phosphate of AU were not required for these assays examining colonisation and not crystal formation. TSB is much easier to make in the large quantities needed, and is a nutritious medium that increases the chances of isolating the attached bacteria and may make the assay more susceptible than when AU is used.

3.4.6 **HPLC**

After 12 weeks of constant perfusion with 20% TSB in the in vitro flow challenge model, HPLC analyses showed triclosan and sparfloxacin clearly remaining in the AUCs. Small amounts of rifampicin remained in some samples, but for the majority of samples, it could not be quantified by the HPLC method. Less rifampicin was initially added to the impregnation solution and therefore, less was impregnated into the catheter material. 0.106% rifampicin was initially impregnated into the catheter, whereas 1.128% triclosan and 0.782% sparfloxacin were initially impregnated (Chapter 4). The limit of quantification of rifampicin was determined as 3.987 µg/mL, which is similar to the limit of quantification of 2.942 µg/mL as determined by Shah and Jasani although their samples were read at 282 nm instead of 254 nm[301]. The wavelength and mobile phase for these experiments chosen was in accordance with the International Pharmacopoeia HPLC method for determining rifampicin content in rifampicin capsules[302]. Rifampicin degradation products were present after 12 weeks of perfusion. Rifampicin can degrade into 3-formyl rifamycin, rifampicin N-oxide, rifampicin quinone, 25-desacetyl rifampicin[303]. An internal report from the company that commercially produce neurosurgical devices impregnated with rifampicin and clindamycin concluded that the rifampicin degradation products in silicone catheter are same as those produced during human drug metabolism and are not toxic[304].

As it was known that less rifampicin was added into the impregnation solution 254nm was chosen as triclosan and sparfloxacin could clearly be determined by this wavelength as well according to the standards. After 12 weeks of perfusion of the AUCs, the wavelength of 254 nm did not provide enough resolution to separate triclosan peaks from noise peaks. To improve the limit of quantification the samples were re-analysed at 279 nm with an injection volume of 10µL which improved the ability of the samples to be quantified. Triclosan could be quantified in only four samples at 254nm but at 279 nm all samples could be quantified.

12-13 weeks is likely to be the maximum length of time the AUC could be used due to ≥ 94.09% rifampicin and 98.09% triclosan being eluted over

the time period. Less sparfloxacin was eluted than the other two drugs. Previous studies demonstrated that 30% of triclosan and 20% of sparfloxacin were eluted after 28 days of perfusion. This demonstrates, firstly, a gradual drug release of the antimicrobials over the 12 weeks, and secondly, that the decreased release of sparfloxacin compared to triclosan is consistent and also apparent at an earlier timepoint. From Time-of-Flight Secondary Ion Mass Spectrometry studies it is apparent that triclosan and sparfloxacin are evenly distributed throughout the silicone matrix[77, 131]; it may be sparfloxacin's physiochemical properties that cause it to diffuse from the catheter at a lower rate. It should be mentioned that a slower or reduced diffusion rate from the catheter is advantageous if it means the drugs are retained at the catheter surface. Williams and Stickler examined the diffusion of various antimicrobial agents through all-silicone urinary catheter balloons and found that triclosan was capable of diffusing across the balloon surface but not rifampicin or the fluoroquinolones[305]. Rather than diffusing from the aqueous environment into silicone and then back into an aqueous environment, the AUC is governed by diffusion through silicone into an aqueous environment. Of the three antimicrobial agents in the AUC, triclosan has the lowest molecular weight, which is a key parameter affecting diffusion through silicone, which may increase its release. From the in vitro flow challenge model it is apparent that even though the majority of antimicrobials have been released by 12-13 weeks the antimicrobials are still present in quantities sufficient to prevent colonisation by various uropathogens.

3.4.7 Statistical analysis

Parametric or non-parametric tests were chosen according to histograms of the control data, in addition to the usual distribution of the data. Some data were log transformed so non-normally distributed data could be compared to normally distributed data. Non-parametric or parametric was chosen for each group of experiments. For example, non-parametric tests were determined to be most appropriate to analyse the data from the TK100 studies for several reasons. Firstly, a histogram of the data from the controls was not normally distributed. Secondly, there were several values of zero from the experimental data which would further skew the data and are difficult to

transform. Thirdly, taking into account the typical distribution of organisms (CFU/mL) they are inherently distributed according to Poisson distribution[306, 307]. Poisson distribution is appropriate for CFU/mL data generated from the TK100 data as it assumes the data is random and takes into account values of zero in the data. In liquid suspensions, distribution is often random due to the motility and clumping of bacteria[307], with bacteria in a biofilm forming microcolonies during initial stages of biofilm development and then redistributing with varying channels and voids of the mature biofilm[308]. Poisson distribution can be inferred by normal distribution if appropriate, but if there are few data points and they are skewed then a non-parametric approach is then more appropriate[309].

Non-parametric tests such as Kruskal-Wallis test of one-way ANOVA data have less power than ordinary one-way ANOVA analysis, meaning the p-value will be greater than if the complementary parametric test was used. This means it is less likely to obtain a significant result[310]. However, despite using this non-parametric comparison there was still a significant difference between the conditions tested by the TK100. Dunn's multiple comparison post-hoc test of these data compared the control to the experimental groups correcting for the multiple comparisons. Adjustments are made for multiple comparisons as the more multiple comparisons are made, the more likely a comparison will be significant due to chance rather than the actual difference (Type I error). This increases the p-value required to declare significance, and therefore increases the rate of type II errors, in which a significant difference is missed and not declared as such[311]. The comparisons between the control groups and all-silicone soaked experimental groups largely had p-values of $p>0.9999$, and it is unlikely that any values of significance were missed.

3.4.8 Summary

In summary, the interaction of a urinary catheter surface with uropathogens and urinary minerals is crucial in predicting the successful lifetime of an indwelling urinary catheter. Any newly developed biomaterials should have characteristics that do not enhance bacterial colonisation and mineral encrustation, and if possible, should reduce both. The TK100 assay

confirmed that the AUC did not predispose to increased bacterial attachment and was able to kill 100% of *two E. coli* isolates, two *S. saprophyticus* isolates, and one *P. mirabilis* isolate within 72 hours. A second *P. mirabilis* isolate had significantly reduced bacterial attachment to the AUC within 72 hours, but bacterial colonisation was not completely eradicated from catheter segments in triplicate.

AFM of AUC and all-silicone controls soaked in artificial urine over three time periods of one hour, one week, and two weeks demonstrated no significant changes in surface roughness compared to the all-silicone control that was not soaked. A spectrophotocolorimetric method of quantifying phosphate deposition was adapted for static and flow experiments of the ability of the AUC to resist encrustation. Under both conditions, the AUC had significantly less phosphate deposited on the catheter surface in the presence of AU inoculated with *P. mirabilis* compared to the all-silicone control. This was supported by SEM images of AUC and all-silicone catheter lumens inoculated with *P. mirabilis*, in which reduced mineral deposition was visible on the AUC segments. Unlike the all-silicone controls, none of the AUCs inoculated with *P. mirabilis* blocked under flow conditions within the 28 day test period. There was no difference in phosphate deposition between the AUC and all-silicone control in the presence of *S. saprophyticus*, *E. coli* or AU adjusted to pH 6.1, 7.1, and 8.3 indicating that phosphate deposition is not increased on the AUC surface. XPS was able to detect the presence of antimicrobials and a conditioning film on the catheter surface but was less reliable in determining the extent of mineral encrustation.

The second round of TK100 of MDR organisms demonstrated the AUC's ability to eradicate MRSA, MRSE, MSSA, and NDM-1 producing *E. coli* but not ESBL-producing *E. coli*. This is in contrast to the results of the in vitro flow challenge in which all isolates successfully eradicated by the TK100 were also eradicated consistently by the in vitro flow challenge model for 12 weeks, as well as the ESBL-producing *E. coli*. The utility of the TK100 as a screening tool for success in the in vitro challenge model was then assessed by a newly developed extended TK100. The extended TK100 of enterococci demonstrated that it was possible to extend the TK100 assay, but its ability to predict the protective period of activity of the AUC was variable.

The in vitro flow model also demonstrated the protective activity of the AUC against significant MDR uropathogens including MRSE, MRSA, MSSA, ESBL-producing *E. coli*, and NDM-1-producing *E. coli* for 12 weeks. The activity did not extend to *E. faecalis* and *E. faecium* and this is most likely due to their intrinsic resistance to triclosan. HPLC of catheters that had been perfused constantly for 12 weeks showed that the three antimicrobials remained in the AUC although at reduced levels. Sparfloxacin was retained in the catheter more than triclosan and rifampicin.

Overall, the AUC does not predispose to mineral deposition under various conditions including after soaking and exposure to AU inoculated with common uropathogens and AU at varying pH levels. Furthermore, the AUC has reduced mineral deposition on its surface in the presence of *P. mirabilis*, a common cause of catheter blockage. The AUC is likely to be able to prevent colonisation by a range of uropathogens including difficult to treat MDR organisms in clinical use.

Chapter 4. A novel antimicrobial urinary catheter for long-term catheter users: a study of its safety

4.1 Introduction

The safety and patient acceptability of the AUC in the urological environment have not yet been determined in patients. Based on the promising in vitro studies, the next step towards commercialisation of the AUC and its use by patients is to understand its safety and patient acceptability.

4.1.1 Previous urinary catheter safety studies

The CATHETER Trial was a multicentre, randomised controlled trial to investigate the effectiveness of the silver alloy-coated latex catheter (Bardex IC®) and the nitrofurazone-impregnated silicone catheter (Release NF®) at reducing the incidence of CAUTI compared to PTFE catheters. Patients were randomly allocated to a catheter type for up to 14 days. Neither treatment catheter significantly reduced CAUTI according to the trial, and of interest, patients receiving the nitrofurazone catheter reported increased discomfort during use of the catheter and at removal[101].

A single-centre randomised controlled safety and efficacy study in the USA trialled a urinary catheter that released ionic silver from the catheter and also included an ‘accessory device’ that contained a copper and silver matrix. They noted adverse events (AEs) such as decreased urine output and urinary retention were ‘possibly’ related to the trial catheter and to the control catheter[312]. Another randomised controlled trial of the silver-alloy catheter and a standard catheter in SCI patients noted that 4.9% of patients had an AE related to the catheterisation procedure in the experimental group and 1.6% in the control group. AEs included haematuria, urinary leakage around the catheter, itching, catheter obstruction, suprapubic pain, rash, and urinary sepsis[313].

The European Commission defines an AE as ‘Any untoward medical occurrence, unintended disease or injury or any untoward clinical signs…in

subjects, users, or other persons.'[314] It is important to investigate whether impregnation of catheters with antimicrobials may produce unanticipated AEs or discomfort in patients in the urological environment even though the safety of the AUCs has a foundation in previous animals studies and clinical use in other applications[130, 315]. Animal studies of CAPD catheters impregnated with rifampicin, triclosan, and trimethoprim using the same method of impregnation as urinary catheters demonstrated no difference in peritoneal cavity inflammation between control and impregnated catheters. Throughout the 30 day period of implantation, no animals showed signs of distress or disease[130]. Furthermore, shunts impregnated with rifampicin and clindamycin were implanted into rat brains and the brains were stimulated to the threshold of seizures. The study found that the impregnated shunts reduced seizure susceptibility compared to the control group possibly due to the anti-inflammatory effect of clindamycin[315]. As the impregnated devices have not been tested in the urethral and bladder mucosa, but have a foundation of safety, this study will investigated AEs specific to urological use attributable to impregnation of silicone urinary catheters with antimicrobials.

4.1.2 Medical device or medicinal product?

The initial consideration for the design of this clinical trial of the AUC was underpinned by whether the AUC was classified as a medical device or a medicinal product as it is a medical device impregnated with pharmacological products. The UK Medicines and Healthcare Products Regulatory Agency (MHRA) defines a medical device according to Article 1 of the European Medical Devices Directive (MDD). The definition of a medical device in the MDD is as follows:

> ' 'Medical device' means any instrument, apparatus, appliance, software, material, or other article, whether used alone or in combination…intended by the manufacturer to be used for human beings for the purpose of:
>
> - diagnosis, prevention, monitoring, treatment or alleviation or disease,
> - diagnosis, monitoring, treatment, alleviation of or compensation for an injury or handicap,
> - investigation, replacement or modification of the anatomy or of a physiological process,
> - control of contraception,

> and which does not achieve its principal intended action in or on the human body by pharmacological, immunological, or metabolic means, but which may be assisted in its function by such means…' [316].

Alternatively, Article 1 of Directive 2001/83/EC defines a 'medicinal product' as 'Any substance or combination of substances presented as having properties for treating or preventing disease in human beings…' [317]. Both medical devices and medicinal products can be used for prevention, which is the intended purpose of the addition of the three antimicrobials. According to the MHRA guidance document '*Borderlines between medical devices and medicinal products'* the decision as to whether the product is a medical device or medicinal product is based on two considerations: the intended purpose of the product and the method by which the primary intended action is achieved[318]. The AUC conforms to this definition as a medical device as the intended purpose of the AUC remains primarily to drain urine from the bladder and does this by physical means, and the addition of the antimicrobials impregnated into the catheter protect the catheter, not the patient, from bacterial colonisation. The antimicrobials are not essential for the urinary catheter to function as its intended purpose as a urine drainage device. The MHRA guidance provides further examples of borderline products and their classification; example 21b deems devices impregnated with bacteriological coatings such as chlorhexidine, silver, and/or antibiotics to be a medical device unless the primary intended purpose is to treat [318]. It was decided that as the AUC would likely to be regulated as a medical device, the clinical trial would be of a medical device.

4.1.3 Medical devices regulations

The requirements of the UK regulations, the MDD and the new Medical Devices Regulations (MDevR) specify that the medical device must meet Essential Requirements to CE mark the device. CE marking is a mark that must be displayed on the device before being placed on the European market. The full Essential Requirements can be found in the MDD or in Annex I of the MDevR, but can be distilled into four basic requirements:

1. Device does not compromise patient safety or the safety of the user

2. Device achieves its primary intended purpose
3. Any risks associated with the use of the device are outweighed by the benefits to the patient
4. The design and manufacture of the device supports the safety and performance requirements 1-3 [316, 319, 320]

There are further essential requirements for medical devices which incorporate a medicinal product, in that the medicinal products, if it acts on the body '…ancillary to that of the device…' then its safety, usefulness, and risks versus benefits must also be investigated [316]. The ancillary action of the antimicrobials will influence the medical device classification of Class IIb or Class III, which determines the regulatory approvals needed for CE marking. Implantable devices that remain in place for 30 days or greater fall under Rule 8 of the classification of medical devices by the European Commission[321]. The majority of implantable devices are Class IIb devices. However, Rule 13 covers devices which incorporate a medicinal product stating that:

> 'All devices incorporating, as an integral part, a substance which, if used separately, can be considered to be a medicinal product as defined in Article 1 of the Directive 2001/83/EC, and which is liable to act on the human body with action ancillary to that of the devices, are in Class III' [321].

Previous studies of the drug release of the three antimicrobials on a daily basis from the AUC showed that the amount of rifampicin was undetectable by HPLC, the amount of sparfloxacin released per catheter per day was less than the serum concentration of sparfloxacin after a single therapeutic dose, and the amount of triclosan released per catheter per day was less than 400 µg day, which is less than triclosan concentrations in urine after exposure to consumer products containing triclosan[77]. It could be argued that the antimicrobials do not have any ancillary action. After consultation with a regulatory affairs manager from MHRA, it was advised that if the data supports the claim that the antimicrobials do not act on the body and there is no ancillary action, then Class IIb may be acceptable (M Carlos, personal communication 23 March 2016).

However, in May 2017 the new MDevR was published, which replaces the MDD, requires that all medical devices need to be compliant with the MDevR within three years. If the AUC was brought to market under the new MDevR it is likely that it would now be classified as a Class III device. There is a slight change of wording from Rule 13 of the MDD to the wording in Rule 14 (Annex VIII) of the MDevR:

> 'All devices incorporating, as an integral part, a substance which, if used separately, can be considered to be a medicinal product, as defined in point 2 of Article 1 of Directive 2001/83/EC, including a medicinal product derived from human blood or human plasma, as defined in point 10 of Article 1 of that Directive, and that has an action ancillary to that of the devices, are classified as class III'[320].

In the new MDevR, the liability of the medicinal product, antimicrobials, to act on the human body is irrelevant for its classification. If they are incorporated it appears that this classifies the device as a Class III device. Class III devices require more pre-market assessments but the Essential Requirements remain the same. Therefore, to achieve CE marking for commercialisation and before progressing further to studies of efficacy, the following clinical trial was designed to assess the safety of the addition of the antimicrobials to all-silicone urinary catheters in its intended environment of the human bladder. Furthermore, the trial will assess the risks associated with the use of the AUC and the severity of any risks. Whether the device achieves its intended primary purpose of urine drainage will be assessed by patient acceptability. Assuming that the AUC will be deemed safe, as is expected, the data from this trial will underpin a definitive clinical trial of efficacy and this trial can also be used to gain insight into the feasibility of a larger such trial.

4.1.4 Quality analysis of trial catheters

The new MDevR states that the design and manufacture of medical devices need to ensure that it supports the performance and safety of the device. Part of this includes removing the risks associated with processing residues. Importantly, this must include confirmation that any residual chloroform has been removed from the AUCs before their use. Additionally,

as the AUCs for the trial were sterilised by ethylene oxide, the removal of the ethylene oxide gas also must be verified.

4.1.4.1 Bioburden testing

Bioburden refers to a population of viable microorganisms present on a medical device or packaging. Understanding the bioburden before sterilisation is important for validating the sterilisation process. The success of sterilisation by killing all viable organisms is governed by the bioburden. Statistically, the greater the bioburden, the greater the probability that an organism may survive the sterilisation process. Therefore, the bioburden is useful for determining the extent of the sterilisation process. ISO 11737-1:2018 provides guidance for how to carry-out bioburden testing[322]. There are three methods of bioburden testing including direct plating, most probable number dilution method or membrane filtration, which is the method recommended for antimicrobial substances. The method must verify that if any antimicrobials are present, they are neutralised either by dilution or a neutralizing agent, which has been verified as not being toxic to the microorganisms[323].

4.1.4.2 Ethylene oxide sterilisation and validation

Ethylene oxide is a gas with broad-spectrum bactericidal, virucidal, and sporicidal activity. It is an alkylating agent in that it adds alkyl groups to nucleic acids and proteins so that normal cellular metabolism and reproduction is interrupted. However, due to its mechanism of action, it is also toxic to humans and its residuals on medical devices are a concern. If chloride ions are present on the device, secondary residual ethylene chlorohydrin can form. If water is present ethylene glycol can form as an additional secondary residual, and these residuals are also toxic[324]. Therefore, ethylene oxide sterilisation and validation of medical devices are regulated by ISO 11135:2014. ISO 10993-7:2008 regulates the removal of ethylene oxide sterilisation residuals such as ethylene oxide and ethylene chlorohydrin. Ethylene glycol residuals are not covered by this standard as if the ethylene oxide residuals are controlled it is unlikely that clinically relevant residuals of ethylene glycol would remain. Despite its toxicity, it is compatible

with many materials including those that are temperature or moisture sensitive or sensitive to degradation[324].

4.1.4.3 Chloroform removal validation

Chloroform is a probable human carcinogen according to the Environmental Protection Agency and International Agency for Research on Cancer and may cause cumulative kidney and liver damage. Therefore, it is important to ensure that it has been removed from the AUCs before use. As the process of impregnating silicone medical devices using antimicrobials dissolved in chloroform is novel and patent-protected there are no standards for quantifying chloroform content of medical devices. However, the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use provides guidelines for residual solvent levels and which solvents are acceptable for use in producing drugs. Chloroform is considered a Class 2 solvent, which has less toxicity than Class 1, and can be used in drug production, unlike Class 1, but must be limited to protect patients from toxicity. Chloroform's permitted daily exposure limit is 0.6 mg/day or a concentration of 60 ppm[325].

Gas chromatography-mass spectrometry combines two techniques to separate gases by chromatography and to identify the components by mass spectrometry. The sample is injected into a heated port and a carrier gas transfers the sample to a column containing the stationary phase. As with HPLC, separation is achieved by the distribution of the analytes of the sample between the carrier gas (mobile phase) and the column (stationary phase). The analytes are then transferred to the mass spectrometer, which measures the mass/charge ratio (m/z) of the separated gas ions. The m/z can be compared to a library of known m/z values to identify the component and then the ions of that m/z value can be quantified[326]. GC-MS has been used to quantify chloroform in counterfeit drugs[327] and chlorinated drinking water[328].

4.1.5 Patient and public involvement

The merits and importance of patient and public involvement (PPI) in the delivery of health services and research are becoming increasingly well-

recognised, and have been written into national policy in Part 14 of the Local Government and Public Involvement in Health Act 2007[329] and Part 12 of the National Health Service Act 2006[330]. To facilitate patient and public involvement in research, the Department of Health set-up INVOLVE as part of NIHR. INVOLVE's mission is to support public involvement in healthcare research, and they define PPI as research 'with' and 'by' members of the public [331]. Professor Dame Sally Davies defines involvement as '…an active partnership…rather than using people as the 'subjects' of research'[332].

The benefits PPI can bring to research include setting up and prioritising research at the initial stages, developing the research protocol and patient materials during the research stages, analysing the research, and disseminating the findings[333]. Specifically, a report from INVOLVE assessing the impact of PPI on research showed PPI particularly benefitted clinical trials by improving trial design and advocating for relevant outcome measures[331]. Patient and public involvement when designing the clinical trial also assist in the ethical review process by making the research more relevant to patient groups, improving the experience of the participants, and improving patient information documents to facilitate approval by the Research Ethics Committee (REC)[334]. Furthermore, recruitment to clinical trials and other research studies was increased when members of the public were involved[332].

There are possible challenges associated with PPI such as the time and cost of planning meetings and building relationships with users in the community. It may also be difficult to recruit and build relationships with older people and minority ethnic groups[333, 335]. Not only does the research benefit from PPI, so do the research team and the lay members themselves. A systematic review of the impact of being involved in research on lay members and communities showed that lay members who were engaged in research felt valued and empowered, and felt as if they were giving back to their community. In some cases, participants felt their involvement increased their self-worth and confidence and they felt part of the team. Likewise, the researchers also gained new insights into a community and their health and social priorities[335].

4.1.6 Chapter aims

This clinical trial aimed to assess the safety and patient acceptability of the AUC in long-term catheter users throughout the duration of their normal catheterisation length. This study also aimed to assess the feasibility of recruiting long-term catheter users to a clinical trial of the AUC to plan for a future RCT of efficacy. The study design was informed by patient and public involvement.

4.2 Materials and Methods

4.2.1 Patient and public involvement

At the initial stages of this project, an advertisement was placed on the People in Research website (http://www.peopleinresearch.org/), which is a website supported by INVOLVE to support members of the public looking to get involved in research, and researchers looking to find members of the public for their studies. The advertisement (Appendix 1) asks for long-term catheter users or carers of long-term catheter users in the Nottingham area, who have an interest in research, but not necessarily any experience with it to get involved as a member of the Research Management Committee (RMC). The aims of the RMC were to meet several times a year over the three years of the project to review the progress of the laboratory studies, plan for future work, review patient information for the clinical study, and shape the clinical study protocol. All travel expenses were covered and for each meeting of two to three hours, the lay members could receive a payment of £45. Tea, coffee, and refreshments were provided.

The aims, duties, and remuneration policy for RMC members can all be found in the Role Description of Lay Panel Member (Appendix 2), which was distributed to those interested in getting involved so they could make an informed decision about their involvement. The RMC also consisted of Professor Roger Bayston, Mr. Richard Parkinson, and Dr. Gillian Shuttleworth, a licensing executive for the University of Nottingham. Therefore, the RMC was a collaboration between catheter users and carers of those with catheters, as well as academic staff, clinical staff, and commercialisation experts.

At the introductory meeting on 29 June 2015, all three lay members were asked to sign a confidentiality agreement (Appendix 3) to protect any sensitive personal information they shared and to protect intellectual property that was discussed. They were also asked to sign a conflict of interest statement (Appendix 4) for future intellectual property and publication purposes. All meetings were recorded using a voice recorder (Sony IC Recorder) so that an accurate reflection of the minutes could be distributed to the RMC members. Payments for attending the meetings and reimbursement for travel were recorded on the University of Nottingham Non-Staff Expenses Claim Form and were reimbursed by bank transfer. The payment for attendance could be donated to a charity of their choice if they chose not to receive the payment themselves. A fourth member joined us at the second RMC and signed the confidentiality agreement and conflict of interest statement.

After each RMC meeting, minutes were produced and sent to the lay members by email and post to prevent the member from incurring printing costs or if they had difficulty using technology.

4.2.2 Study approvals

The University of Nottingham offered a statement of sponsorship for the study on 13 July 2016 (Appendix 5), which accompanied the IRAS application (IRAS Ref: 206184) submitted to the Research Ethics Committee and the Health Research Authority on 27 July 2016. Favourable ethical opinion was granted by the West Midlands - Edgbaston Research Ethics Committee on 12 September 2016 (REC Ref: 16/WM/0353), after meeting with the Committee on 17 August 2016 (Appendix 6). Approval from the Health Research Authority (HRA) was given on 11 October 2016 (Appendix 7). Nottinghamshire Healthcare NHS Foundation Trust granted approval for Nottingham CityCare to act as a Participant Identification Centre on 14 October 2017 (Appendix 8). Nottingham University Hospitals NHS Trust confirmed they had the capacity and capability to deliver the trial on 02 December 2016 (Ref: 16UR001) (Appendix 9) and on 06 December 2016 provided approval for the use of the trial antimicrobial urinary catheter under

article 4.3 of the Managing Loan Medical Devices Procedure v1.0 at NUHT (Appendix 10).

The study was also adopted by the NIHR Clinical Research Network Portfolio (Ref 33365) on 19 January 2017, which supported the registration of the study to the ISRCTN registry with the trial ID of ISRCTN12606737. The trial was funded by the National Institute of Health Research Innovation for Innovation grant award II-LA-0214-20007.

4.2.2.1 Amendment approvals

Throughout the course of the study, several non-substantial and substantial amendments to the clinical trial were submitted to the appropriate regulatory authorities depending on the type of amendment (Table 4-1).

Table 4-1 Amendments to the clinical trial after receiving the initial study approvals. NS: non-substantial amendment, SA: substantial amendment

Trial amendment code	Changes to original	Documents changed	Date of HRA approval	Date of REC Approval
NS 1	New start and end dates: 21 November 2016 – 20 November 2017	Protocol (v3.0 20/10/2016)	21/10/16	
NS 2	Covering letter to accompany PIS posted to participants	Covering letter for PIS (v1.0 10/01/2017)	17/01/17	
SA03	Collecting the original urinary catheter from participant	Covering letter for PIS (v1.5), GP letter (v1.5), PIS (v2.5), Protocol (v3.5), SOP v(2.0)	22/02/17	21/02/17
NS 3	Extension of the trial end date by 3 months	Amendment to research contract between UoN and NUHT, Protocol (v5.0)	11/09/17	
NS 5	Changes to letter sent to patients via NIHR CRN GPs	Recruitment letter v2.0 05/09/2017	21/09/17	
SA05	Inclusion of suprapubic urinary catheter users	Protocol (v5.5 11/09/17), Consent form (v3.5 11/09/17), and PIS (v3.5 11/09/17)	3/10/17	6/10/17
SA06	Modification of recruitment target to 31 patients	Protocol (6.0)	20/12/17	20/12/17

Amendments to add NIHR Clinical Research Network (CRN) East Midlands Primary Care and district nursing teams from Nottinghamshire Healthcare NHS Foundation Trust as participant identification centres (PIC) were submitted to the HRA as non-substantial amendments. However, correspondence with HRA confirmed that as PIC activity was already approved in the original application for clinical trial, the addition of PIC sites was not considered an amendment. Therefore, the addition of the PIC sites was reflected in the updated protocol (v4.5 date 19/07/2017).

4.2.3 Study design and clinical intervention

The study was a single-centre, non-randomised feasibility trial with the aim of evaluating the safety of CE-marked medical device with modifications. CE-marked indwelling silicone urinary catheters were impregnated with antimicrobials to produce the indwelling antimicrobial urinary catheter long-term urinary catheters. Long-term urinary catheter users were catheterised with the AUC after providing written consent (see Appendix 11 for consent form) at their next catheterisation date and interviewed about adverse events and acceptability 24, 48, and 72 hours post-catheterisation and then once weekly for rest of the trial duration (see Appendix 12 for telephone interview form). The per participant trial duration varied according to the participant's normal catheterisation schedule. To keep things as close to normal as possible, if a patient regularly has their catheter changed every six weeks, for example, the trial catheter would only be in place for six weeks. Patients were also given a questionnaire to ask about the feasibility of a RCT of efficacy of the AUC. The original urinary catheter and the trial catheter (AUC) were collected from the participant and analysed in the laboratory according to SOP entitled 'SOP of collection, transport, and analysis of trial devices when removed from participants enrolled in the Antimicrobial Urinary Catheter Safety Study' (v2.0, date 01/02.2017). Further detail about study design can be found on the ISRCTN website at http://www.isrctn.com/ISRCTN12606737.

Informed written consent was provided from each participant before collecting any data and catheterisation with the trial catheter. All members of

the research team had up-to-date Good Clinical Practice training. Informed consent was provided no less than 24 hours after the participant had the participant information sheet (PIS)(Appendix 13), so they had time to consider their participation.

4.2.3.1 Participants and setting

Adults (age 16 years or greater) who currently had an indwelling urethral urinary catheter in place for 28 days or greater and required another urinary catheter for 28 days or greater were initially considered for inclusion. Participants were recruited from the community and hospital settings as long as they were able to travel to NUHT for the fitting of the trial catheter and satisfied the inclusion and exclusion criteria (Table 4-2).

Table 4-2 Inclusion and exclusion criteria

Inclusion Criteria
Age: 16 years old or greater
Currently fitted with a urinary catheter for at least 28 days and will require another urinary catheter for 28 days or greater
Able to understand written English and speak English fluently
Able to verbally respond and to speak on the telephone
Exclusion Criteria
Pregnant or likely to become pregnant
Adults lacking the ability to consent for themselves
Allergy to: - Rifampicin - Sparfloxacin or any other fluoroquinolone antibiotics - Triclosan - Silicone
History of uncontrolled/unmanageable autonomic dysreflexia
Significantly impaired sensation of the bladder and/or urethra

The setting of catheterisation was an acute teaching NHS Trust, Nottingham University Hospitals NHS Trust, Nottingham, UK, specifically the Urology Centre at City Hospital campus. If the participant was in the community, the participant needed to attend two trial visits at NUHT for the insertion and removal of the trial catheter. Travel expenses were reimbursed.

4.2.3.2 Recruitment

Participants were recruited in several different settings including patients on wards at either the City Hospital or Queen's Medical Centre campuses at

NUHT. Patients were also recruited from attendance at the Trial Without Catheter (TWOC) clinics on Harvey 2 ward, which is a male urology ward.

Potential participants in the community were recruited by several strategies. Firstly, Nottingham CityCare which provides continence supplies to patients in the community, posted a letter of expression of interest (Appendix 14) to those on their database of long-term catheter users. The letter was accompanied by a pre-paid and self-addressed envelope for posting their reply of permission for the research team to contact them about the study. The replies were collected in the first instance by Nottingham CityCare and those giving permission to be contacted were passed to the research team.

This same strategy was adopted by NIHR CRN East Midlands Primary Care team, in which the NIHR Clinical Research Network (CRN) contacted GP practices in Nottinghamshire and Derbyshire with information about the trial. GP practices then expressed interest to the CRN and undertook a database search of their patients who fitted the inclusion and exclusion criteria for the trial. The identified patients were sent a modified version of the same letter of expression of interest on the GP practice letterhead by the GP practice with a pre-paid and addressed envelope for the patient reply. Patients who then replied giving consent to be contacted were contacted with more information by the research team.

District nursing practices under the jurisdiction of Nottinghamshire Healthcare NHS Foundation Trust identified patients whom they believed would be eligible for the trial and spoke with the patients by telephone. Those patients who consented to receive more information about this trial were then contacted by the research team.

4.2.3.3 **Addressing bias in patient recruitment**

This trial aimed to include a representative patient population, while at the same time being mindful of the primary outcome of assessing the safety of this novel device. Despite the fact that the safety has been assessed of the CE-marked silicone catheters, which were then modified by antimicrobial impregnation, the effect of the antimicrobial impregnation in the lower urinary tract has never been assessed. Therefore, there were some elements of

selection bias resulting from the eligibility criteria. For example, participants were excluded if they not did have sensation in the urethra and/or bladder as they would be unable to self-report some symptoms and also for their safety. If the AUC was to cause irritation, allergy or discomfort both the participant and research team would be unaware. This by extension excluded patients with spinal cord injury or cauda equine syndrome, a large population of indwelling urinary catheter users. Likewise, many participants with dementia may require a urinary catheter to manage incontinence but were not eligible to participate due to the possibility that they may not have been able to accurately report new symptoms or adhered to the telephone interview schedule. These exclusion criteria were put in place to protect patients and to preserve the accuracy of the data collected.

Due to the nature of the trial, a modified medical device manufactured in-house, it has to be fitted at the site that is responsible for its manufacture as it is then not regulated by Medical Devices Regulations[319]. Therefore, an element of selection bias may have been introduced as the majority of long-term catheter users are managed in the community and would need to be motivated to come to hospital for their catheter change. To ensure that cost was not a prohibitive factor that prevented people from travelling to hospital, transport was reimbursed or a taxi was pre-booked and pre-paid. Similarly, participants were always telephoned by the research team so that they did not incur any charges to their phone bill for their participation in the telephone interviews.

Furthermore, to encourage participation of participants who required a wheelchair, wheelchair accessible taxis could be arranged and hoist facilities were available at the Nottingham Urology Centre so mobility was not a prohibitive factor. Due to the nature of conditions that predispose to the long-term urinary catheterisation such as stroke, enlarged prostate, and other age-related conditions, long-term urinary catheter users are generally an older population. Frailty of a potential participant could be prohibitive to them wanting to leave their home or to leave a frail partner that they care for, especially if their catheter is routinely changed at their home by the district nurse. Funds were available to pay for an alternative carer for their trial visits, and also for child-minding arrangements for those participants with children.

Finally, the participant's decision to enrol in the trial may have introduced an element of selection bias. As participation is entirely voluntary for this trial, participants may have varying motivations for engaging in the trial, such as current problems with their urinary catheter. It may be that patients without any catheter issues are less likely to participate as they are content with their current catheter care. To investigate this the feasibility questionnaire asked questions about reasons for interest in the trial to combat future selection bias issues if there were any that arose.

4.2.3.4 **Outcome measures**

Outcomes were largely assessed by patient self-reporting, which is why the inclusion criteria required the ability to respond verbally, ability to speak on the phone, and ability to speak and understand English. Furthermore, patients without capacity to consent were excluded due to the likelihood that they might be unaware or unable to verbalise any problems or concerns with the AUC. Likewise, patients unable to consent may be unaware of irritation or discomfort that is relevant to the outcome measures of the clinical trial.

4.2.3.4.1 Primary outcome measure

The primary outcome measure of this study was the rate of adverse events attributable to the antimicrobials or the impregnation process. All adverse events were recorded in the case report form and a score of severity and a score of relatedness to the AUC was given to the adverse event by the research nurse and adjudicated by the principal investigator. The definition of AEs was that agreed by the ICH Guidelines on Clinical Safety Data Management, Definitions, and Standards for Expedited Reporting[336].

Relationship of the adverse event to the antimicrobial impregnation of the urinary catheters was assessed according to the WHO-UMC system as it is a standardized case causality measure[337]. An adjudication system was in place so that all AEs were classified by the research nurse and adjudicated by both the principal investigator and chief investigator so that the AE causality was appropriately classified. Having a standardized case causality system is beneficial as it is a study-wide classification system and having the

causality measures pre-defined prevented disagreement between assessors as to the interpretation of an AE. However, a classification does not prove the connection between the antimicrobial catheter and the event, but it does provide a large degree of agreement about the cause. Telephone interviews were used to capture initial reports of AEs, the details, and validity of which were then confirmed with electronic patient medical records. Particularly for the reporting of SAEs, patient medical records were accessed to capture full, and accurate details of events. Therefore, there was an element of cross-checking of reporting of AEs. Adverse events were detected by patient self-reporting via the weekly telephone interview and, if necessary, followed up by a review of the patient's notes.

4.2.3.4.2 Secondary outcome measures

Secondary outcome measures included patient acceptability, whether the trial device needed removal before the planned end date for the trial, and microorganism colonisation of trial catheters. Withdrawal from the trial and reason for withdrawal wert also recorded in the case report form to capture information about early removal of trial catheters. Patient acceptability was measured by the telephone interviews including multiple-choice questions and free text answers. The free text answers were grouped into sub-themes and themes according to the principals of thematic analysis[338] to organise these data.

The participant's original urinary catheter was collected when removed at the appointment to have the AUC fitted according to the protocol and then the trial catheter was collected at the end of the trial. The contents of the balloons and lumens of original and trial catheters were investigated according to the SOP entitled 'SOP of collection, transport, and analysis of trial devices when removed from participants enrolled in the Antimicrobial Urinary Catheter Safety Study', which is based on the method of catheter collection and analysis in Chapter 2.

Information about the feasibility of an RCT was collected by the questionnaire entitled 'A new antimicrobial urinary catheter for long-term use: a feasibility study for planning a future randomised controlled trial' (Appendix 15). This questionnaire was assessed for clarity and utility by four lay

members of the RMC. They provided written feedback and were reimbursed for their time.

4.2.4 Sample size determination

One of the aims of this feasibility study was to use data generated from this study to power the definitive trial of efficacy. Therefore, the sample size was based on the number of catheters collected from this same patient population within one year at NUHT for the Catheter Collection Study in Chapter 2. Indwelling urethral urinary catheters were collected from patients at NUHT over one year and this helped to determine the sample size of 60 participants.

4.2.5 Analysis

The populations analysed included the safety set, which included all participants who received the trial catheter. The Full Analysis Set included all participants who received the trial catheter and for whom at least one telephone interview assessment was available. The per protocol set included all participants in the Full Analysis Set who were deemed to have no major protocol violations.

Adverse events were classified by intensity (mild, moderate, or severe) and causal relationship to impregnation of the antimicrobial urinary catheter. Relationship causality was classified as unrelated, unlikely, possible, probable or definite according to the algorithm given by the World Health Organisation[337] and as explained in the trial protocol. Further classification grouped AEs as non-serious or serious. An AE was classified as serious (SAEs) if it was fatal, life threatening, resulted in hospitalisation or prolonged hospitalisation or resulted in persistent or significant disability or incapacity. Adverse event classification was adjudicated by the Principal Investigator and Chief Investigator.

Although CAUTI was not an outcome measure, but instead an AE, a pragmatic diagnostic criterion was still used. The definition of CAUTI was symptomatic presentation with prescription of antibiotics for treatment of suspected CAUTI. CSU samples were not considered as they are useful to only exclude an infection.

4.2.6 **Thematic analysis**

As alluded to in Section 4.3.3.4, the patient acceptability free text answers were analysed according to the principals of thematic analysis. Thematic analysis is a qualitative research method for interpreting patterns in text to identify and organise the text into themes[339]. The six phases of thematic analysis are familiarisation with the data, initial coding of the text, searching for themes, reviewing the themes, definition and naming of themes, and production of a final report[338]. Free text responses were collated into a table and were given codes according to topics that had been identified. If there was more than one codable section in the free text responses, the response was divided into multiple coded sections. For example, the following free text response was coded into three sections:

> 'draining much more urine than usual; not bypassed and not expelled' Daughter stated that her mother seemed 'much more upbeat than usual as she has depression due to catheter problems'

A comment about drainage was separated from text about bypassing and expulsion, which was also separated from free text about the participant's mood.

Similarly coded items were grouped together to create themes. The items in each theme were reviewed and moved if necessary. It was clear from the original themes created, that several themes could also be grouped together and therefore became sub-themes. The themes and sub-themes were reviewed at the final stage before being assembled into a report. A trail of changes to the groupings was kept for audit and traceability purposes.

4.2.7 **Manufacturing the trial devices**

Two hundred and five all-silicone, two-way, catheters were received from Yushin Medical Co., Ltd in five lumen sizes and two lengths (Table 4-3) with 50 of the catheters to be used for validation analysis carried out by Yushin Medical Co., Ltd, five of the catheters to be used for in-house batch analysis, and the 145 remaining catheters to be available for use in the trial. The quantity decided was based on the lumen sizes collected as part of the collection of catheters from NUHT. Only standard length (400mm) catheters

are used at NUHT due to safety concerns about men being catheterised inadvertently with female length (280 mm) catheters. In the community both standard and female catheters are used as the catheter is issued for the individual patient so this minimises the risk of selecting the wrong length (A. Cartwright, personal communication, 27 June 2016). However, both hospital and community patients could enrol in the trial if eligible so it was important that patients receive the catheter that they were used to. Since the trial was being carried out in specialist urology setting with a specialist trial nurse performing the catheterisation it was decided that it was acceptable to use female and male length catheters for the trial.

Table 4-3 Quantities of urinary catheters impregnated for the clinical trial

Lumen size	Catheter length	Balloon fill volume (mL)	Quantity impregnated	Quantity available for use in the trial
12 Ch	Standard	5	74	22
12 Ch	Female	5	30	30
14 Ch	Standard	5-10	34	31
14 Ch	Female	5-10	24	23
16 Ch	Standard	5-10	14	14
16 Ch	Female	5-10	10	8
18 Ch	Standard	5-10	10	9
20 Ch	Standard	5-10	9	8
			205	**145**

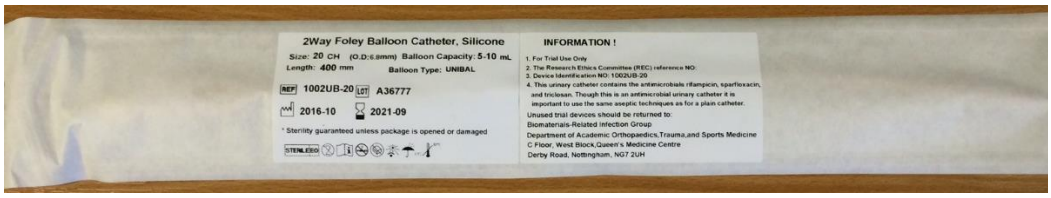
The plastic rings, which are fitted around the balloon port to assist in balloon inflation, were removed prior to the impregnation process. Plastics are not compatible with the chloroform impregnation solution. Catheters were impregnated according to Standard Operating Procedure of antimicrobial impregnation of silicone urinary catheters (v2.0, date 26/08/2016) submitted for REC and HRA approval (Appendix 16) which includes a processing checklist and record form for traceability purposes. The record forms were counter-signed by an additional person supervising the impregnation process. The catheters were impregnated in three batches and assigned three batch numbers and all chemicals, reagents, and materials for each batch were recorded in the processing record form and kept in the trial master file.

Five catheters, one of each length, were randomly chosen and removed for batch analysis of the impregnation process. The remaining antimicrobial impregnated catheters and the removed plastic rings were returned to Yushin Medical Co., Ltd to have the plastic rings replaced onto the balloon ports, sterilisation by ethylene oxide, bioburden testing and for sterilisation validation analysis. Before sterilisation, they were individually packaged in plastic sleeves and labelled with the batch number, lumen size, catheter length, a trial identification number, and additional trial information (Figure 4-1).

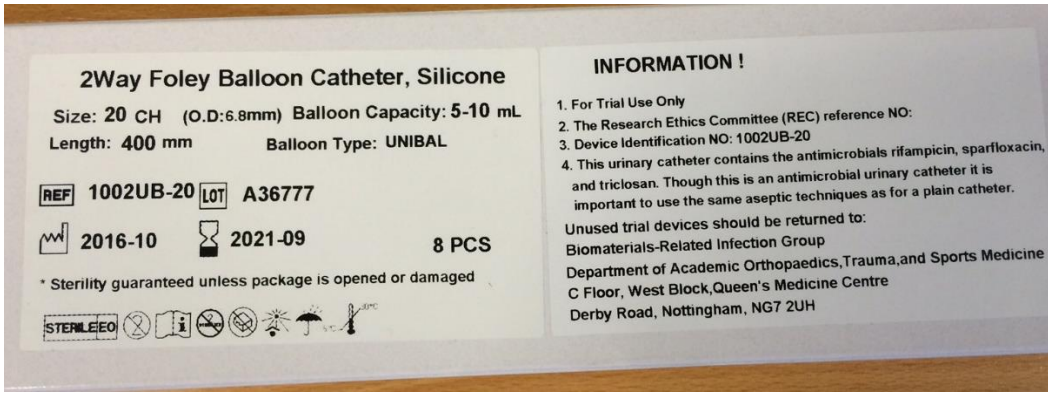
Figure 4-1 Packaging of antimicrobial impregnated trial catheters. A) Clear Tyvek front of the packaging, B) Opaque Tyvek packaging with the catheter identification information, C) Close up of packaging label on the box containing trial catheters

A

B



C



Five catheters were randomly selected for Bioburden testing and it was carried out according to ISO 11737-1 by the Yushin Medical Co., Ltd laboratory. An additional five catheters were randomly selected 72 hours after ethylene oxide sterilisation for analysis by gas chromatography of ethylene oxide and ethylene chlorohydrin residuals. This too was carried out by Yushin Medical Co., Ltd. Briefly, the samples were analysed by a Varian gas chromatograph, using a 10 volt detector. The injection volume was 1µl.

4.2.7.1 Batch analysis of trial devices

One catheter of each lumen size was randomly removed for analysis of drug content by HPLC and verification of removal of chloroform by mass spectrometry - gas chromatography (MS-GC) (Table 4-4). One catheter of each of the lumen sizes were chosen to verify the proportional drug content remained consistent across increasing lumen diameters and increasing silicone material. These five catheters also represent all three antimicrobial impregnation batches to also verify consistency across batches.

Table 4-4 Trial catheters removed for batch analysis

Trial Identification Number	Lumen size	Catheter length
12S01	12 Ch	standard
14S07	14 Ch	standard
16F08	16 Ch	female
18S04	18 Ch	standard
20S09	20 Ch	standard

4.2.7.1.1 High performance liquid chromatography

After manufacture, the balloon inflation and drainage ports were removed by scalpel from the catheters for batch analysis. Three 1.0cm segments per catheter were cut from the tubing section. To extract the drugs from the catheter, each segment was placed in 2 – 4 mL of chloroform so that the chloroform covered all surfaces of each segment. The drugs were extracted for one hour, then the segments were removed allowed to dry for one hour at room temperature under air flow, and then the process was repeated two more times at which point the chloroform remained clear. The chloroform and drug content from each extraction was pooled together and the chloroform was left to evaporate off over 24 hours at room temperature. The remaining drug residue was then stored in the freezer for five days until being reconstituted in 10.0 mL of HPLC grade methanol (Fisher Scientific) for subsequent analysis.

Standards of rifampicin, sparfloxacin, and triclosan were prepared in concentrations of 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100, 50 µg/mL. They were prepared in one solution as all were detected at the same wavelength.

The aqueous phase mixture was composed of 15 mM sodium dihydrogen phosphate (pH 2.5, Sigma-Aldrich, adjusted with phosphoric acid (Fluka) if

necessary) and 10% acetonitrile (HPLC grade, Fisher Scientific). The organic phase was composed of 100% methanol (HPLC grade). The flow rate was set at 1.0 mL/min with an injection volume of 5.0 µL.

Please refer to Section 3.2.8.6 for information on the calculations to determine the proportional drug content and total drug content.

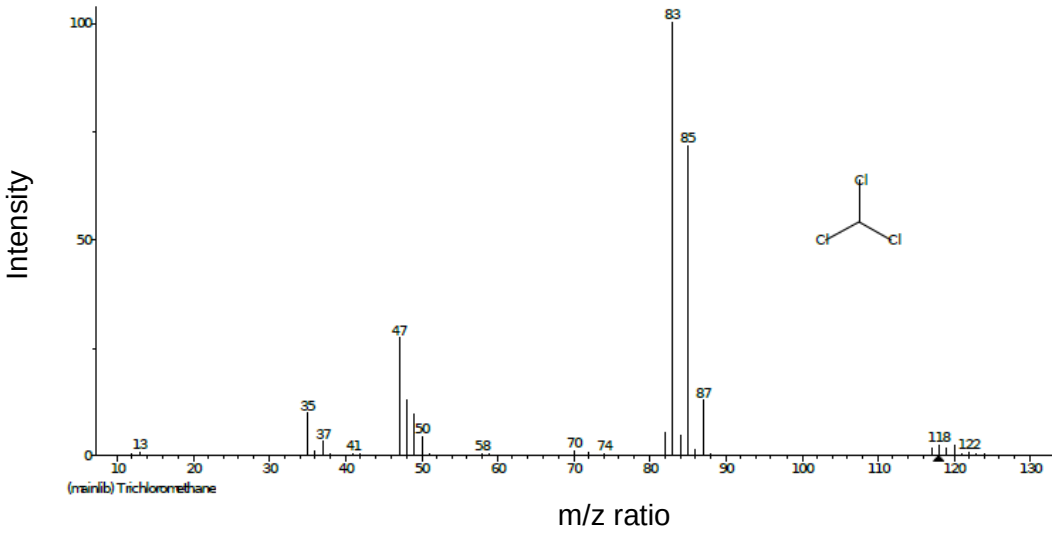
4.2.7.1.2 Gas chromatography – mass spectrometry method development

After manufacture, including importantly a minimum of 12 hours drying time post-soaking and rinsing with ethanol, three additional 1.0 cm long segments were removed from the same catheters used for drug content analysis by HPLC for GC-MS. They were immediately placed in a 2.0 mL amber vial (ChomatographyDirect.com) with a PTFE cap with PTFE septa. The caps were tightened and then wrapped in parafilm and placed in sealable plastic bags to prevent chloroform from escaping. The samples were then placed in the freezer until analysis.

Analysis was carried out using a JEOL AccuTOF GCX (JEOL Ltd., Tokyo, Japan) mass spectrometer and an Agilent 7890B (Agilent Technologies Inc., Wilmington, USA) gas chromatograph. The acquisition mass range was between 40 -130 Da. Perfluorotributylamine was used to calibrate mass for the mass spectrophotometer. The ion source for MS was electron ionisation at an energy of 70eV and a temperature of 150°C. The column for GC was a J&W DB-5MS 30m x 0.25mm x 25 µm (Agilent Technologies Inc.) column and was maintained at 50°C. The carrier gas was helium, inlet temperature was 120°C and the transfer line temperature was 180°C.

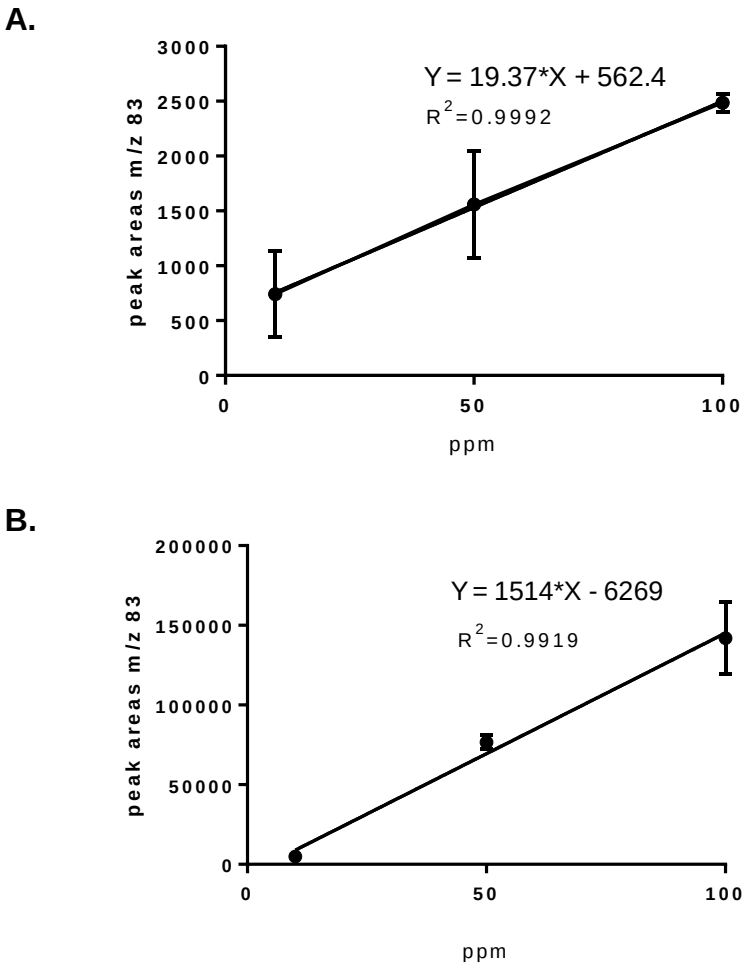
Masses of 82.94 and 84.94 can be used to detect chloroform due to the isotopes of chlorine (Figure 4-2).

Figure 4-2 Gas chromatography – mass spectrometry reference spectrum of chloroform (trichloromethane). Peaks represent ion detection at select mass to charge (m/z) ratios.



Several different strategies were tested for developing the most appropriate standards for quantifying chloroform content. Originally strips of catheter material 1.0 cm x 3.0 mm (matching the size of the inlet of the gas chromatograph) were soaked in chloroform (Fisher Scientific) concentrations of 100 ppm, 50 ppm, and 10 ppm diluted in acetonitrile (Fisher Scientific).The catheter strips were directly placed in the GC inlet as this replicates placing strips of the trial catheters into the GC inlet. This was compared to the standard solutions of 100, 50, and 10 ppm that the catheter segments were soaked in (Figure 4-3). However, a chloroform liquid standard was being used as a standard for the chloroform vapour being extracted from the catheter in the inlet tubing. Therefore, it was not ideal to compare a liquid standard to a vapour sample.

Figure 4-3 Standard curves of A.) solutions of 10, 50, and 100 ppm chloroform and its goodness of fit value and B.) catheter strips 1.0 cm x 3.0mm soaked in 10, 50, and 100 ppm chloroform solutions



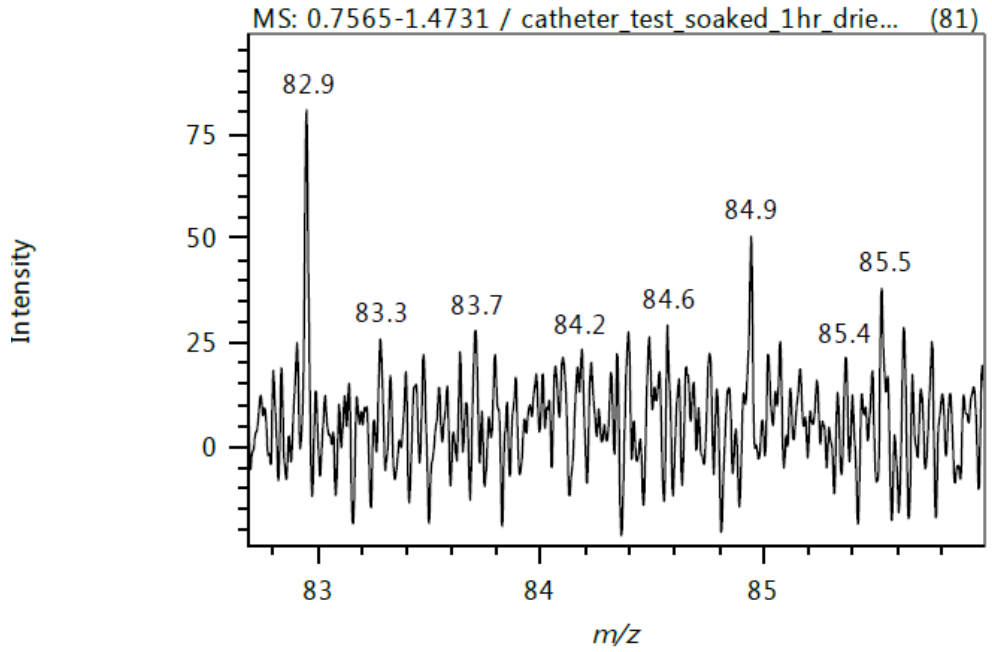
As seen from Figure 4-3 the peak areas of the standards in a vapour state are roughly 100 times greater than those standards in solution highlighting the disparity between the two types of standards. Furthermore, there is some variability within the standards, which is possibly expected from the catheter segments as the amount of chloroform present in the standard is dependent on the chloroform being impregnated into the silicone of the catheter and then being released during injection into the gas chromatograph.

Therefore, a new method of developing standards and harvesting the remaining chloroform from the catheters was determined. In the same principles of drug extraction for HPLC, chloroform was extracted from the catheter segments by three rounds of soaking in acetone. The chloroform

standards were then prepared in acetone. For both the standards and the experimental samples all chloroform was measured dissolved in acetone.

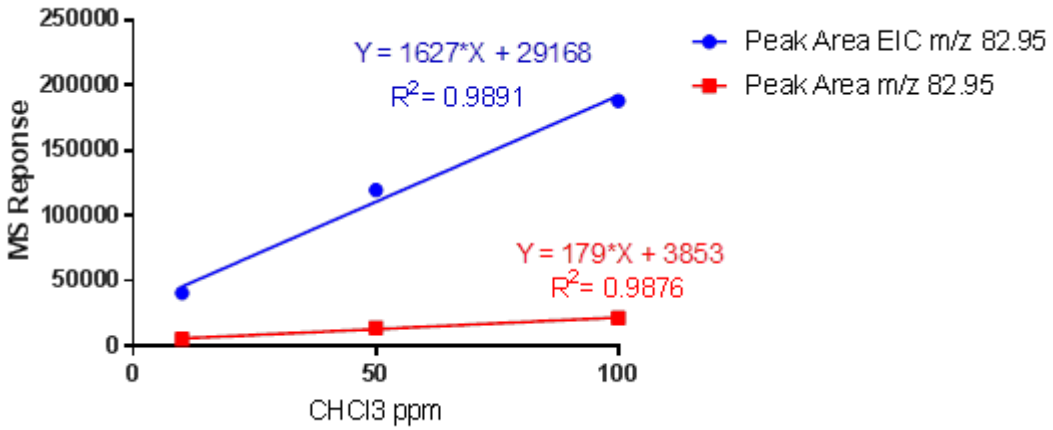
An initial analysis of a test catheter segment showed that the chlorine isotopes were detected at 82.9 m/z and 84.9 m/z in the mass spectrum (Figure 4-4). This is a total ion current trace in which all peaks of the range of masses being detected are represented on the spectrum.

Figure 4-4 Mass spectrum of a test catheter segment showing chlorine isotopes being detected at 82.9 m/z and 84.9 m/z



The signal at 82.9 m/z was stronger than that at 84.9 m/z, therefore to focus on this peak of interest the mass spectrum was also processed as an extracted ion current trace (EIC) for m/z 82.95. An EIC monitors the mass of interest. Furthermore, it is a more sensitive method of detection compared to calculating the peak ion area from the total mass spectrum (Figure 4-5). It was determined that EIC for 82.95 m/z was a sensitive method of detecting chloroform residues as demonstrated by the increased slope of the calibration curve, and was employed for the catheter samples.

Figure 4-5 Chloroform ($CHCl_3$) parts per million (ppm) as detected by GC-MS and peak area quantified by extracted ion current trace (EIC) or by calculating the peak ion area from the mass spectrum. Both peak areas were calculated at 82.95 m/z.



4.2.8 Statistical analysis

Standard curves and graphs were prepared using GraphPad Prism. Data were analysed for normality by production of histograms. Data that were not normally distributed were log transformed for subsequent analyses so all data sets could be analysed by parametric tests for conformity of statistical testing.

4.3 Results

4.3.1 Patient and public involvement

Four lay members were recruited to the RMC. Please see Table 4-5 for the lay members of the RMC and their relevant experience to the studies.

Table 4-5 Lay members of the Research Management Committee

Lay member	Life experience
1.	Tetrapelegic after a spinal cord injury – uses an indwelling urinary catheter to manage his/her bladder on a long-term basis
2.	Stroke survivor – was an indwelling urinary catheter user during his/her rehabilitation and was hospitalised from urosepsis
3.	Carer of patients with dementia and frail, older people who suffer from urinary incontinence
4.	Breast cancer survivor – was an indwelling urinary catheter user during treatment and is currently prescribed long-term antibiotics to prevent UTIs

Nine Research Management Committee meetings were held over the course of the clinical trial process from planning stages to data dissemination. Duties fulfilled by the lay members included commenting on trial design and trial recruitment strategies at the meetings. One unexpected benefit of integrating PPI was the development of a commercial business plan for the antimicrobial urinary catheter. Lay member 2 was an entrepreneur in his/her working life and had significant experience in developing business plans and understanding the values of technologies. This was developed to aid the future commercialisation process and process of licensing the technology to develop the AUC.

The lay members outside of the meetings reviewed patient information sheets, advertising posters, and consent forms before submission to REC. Lay member 3 attended the REC review meeting of the trial at West Midlands - Edgbaston Research Ethics Committee and he/she spoke on behalf of the lay members in support of the study at the meeting. The PPI contribution was discussed in the letter of provisional opinion from the Edgbaston REC following the meeting in which it was remarked that ‘The committee voiced to the applicants it being clear a positive amount of PPI has been undertaken in respect of this study and agreed this as commendable’. The success of this PPI partnership was highlighted in a blog for NIHR[340] after an award was presented by the NIHR Clinical Research Network East Midlands 2016-2017 (Research Awards to the author for Patient/Carer/Member of the Public Nominated Researcher). All lay members gave written approval for their names to be published in the above NIHR blog.

4.3.2 **Production of trial catheters**

4.3.2.1 **Sterilisation quality assurance**

All catheters passed bioburden testing and had levels of ethylene oxide and ethylene chlorohydrin residual levels below the acceptable standards and passed. Please see Appendix 17 for the sterilisation certificate, bioburden testing certification, and ethylene oxide residual gas test reports.

4.3.2.2 **Drug content as determined by HPLC**

Initially HPLC was performed on three trial catheter segments after they had only undergone one round of drug extraction. This was carried out to assess if there were any remaining drug residues on the surfaces that may be responsible for variability (Table 4-6).

Table 4-6 Drug determination of proportional drug content (w/w) of three samples per trial catheter, media proportional drug content per catheter and total mean proportional drug content of all catheters and standard deviation after only one round of drug extraction

Sample	Rifampicin		Triclosan		Sparfloxacin	
	Drug content (w/w)	Median drug content (w/w)	Drug content (w/w)	Median drug content (w/w)	Drug content (w/w)	Mean drug content (w/w)
12s1	0.210%	0.137%	1.780%	1.299%	1.426%	0.982%
12s2	0.137%		1.299%		0.982%	
12s3	0.133%		1.258%		0.955%	
14s1	0.081%	0.081%	0.886%	0.886%	0.590%	0.590%
14s2	0.086%		0.977%		0.672%	
14s3	0.076%		0.868%		0.590%	
16f1	0.067%	0.067%	0.743%	0.743%	0.493%	0.493%
16f2	0.082%		0.872%		0.624%	
16f3	0.058%		0.673%		0.458%	
18s1	0.079%	0.079%	0.836%	0.836%	0.585%	0.585%
18s2	0.060%		0.728%		0.490%	
18s3	0.100%		1.037%		0.736%	
20s1	0.067%	0.081%	0.765%	0.823%	0.527%	0.602%
20s2	0.081%		0.849%		0.603%	
20s3	0.086%		0.823%		0.602%	
Total Median and IQR		0.081% (0.021%)		0.868% (0.213%)		0.602% (0.148%)

Catheter 12s, particularly sample 12s1, had a much greater drug content compared to the two other 12s segments and the other trial catheters. It is likely this was due to a remaining drug accretion. This catheter was then flushed with ethanol to remove any excess residues and the full drug extraction (three rounds) was performed again, and the extracts were analysed by HPLC (Table 4-7). As seen in Table 4-7, sample 12s1 was now more consistent with the other 12s values and the other catheters.

Table 4-7 Proportional drug content (w/w) as determined after flushing with ethanol to remove excess drug residues, according to each 1.0 cm segment removed from the five catheters for quality assurance testing from the batches of catheters made for the clinical trial, and the mean proportional drug content per catheter.

Sample	Rifampicin		Triclosan		Sparfloxacin	
	Drug content (w/w)	Median drug content (w/w)	Drug content (w/w)	Median drug content (w/w)	Drug content (w/w)	Median drug content (w/w)
12s1	0.090%	0.090%	1.278%	1.273%	0.948%	0.948%
12s2	0.090%		1.273%		0.961%	
12s3	0.078%		1.242%		0.891%	
14s1	0.078%	0.075%	1.084%	1.086%	0.704%	0.704%
14s2	0.075%		1.088%		0.698%	
14s3	0.073%		1.086%		0.721%	
16f1	0.080%	0.080%	1.054%	1.041%	0.704%	0.692%
16f2	0.073%		1.024%		0.691%	
16f3	0.081%		1.041%		0.692%	
18s1	0.113%	0.113%	1.118%	1.118%	0.785%	0.785%
18s2	0.126%		1.163%		0.850%	
18s3	0.091%		1.062%		0.697%	
20s1	0.079%	0.079%	0.983%	0.985%	0.689%	0.695%
20s2	0.078%		1.007%		0.698%	
20s3	0.083%		0.985%		0.695%	
Proportional Drug Content (w/w) ± IQR		0.080% (0.013%)		1.084% (0.138%)		0.704% (0.155%)
Median mg/g ± IQR		0.799 (0.129)		10.81 (1.39)		7.035 (1.55)
Total drug content (mg) (IQR)		8.805 (5.081)		121.992 (40.9)		90.825 (34.88)

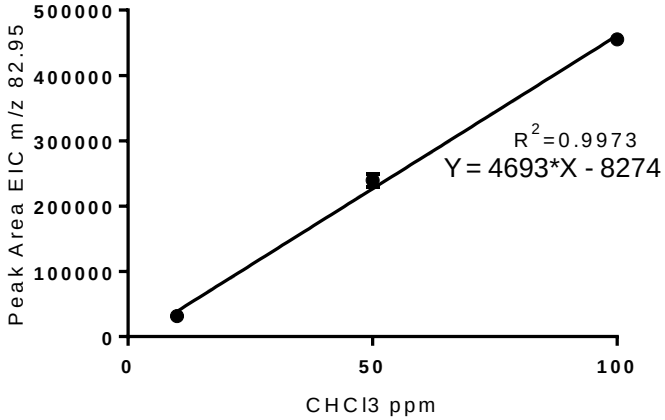
Three rounds of drug extraction, as expected, recovered more drug from the catheters than only one round, and an additional rinse with ethanol aided the removal of microscope drug accretions. There was less rifampicin present in the catheter as less is added to the impregnation solution. Of interest, the same proportion (1.0% w/v) of sparfloxacin and triclosan are added to the impregnation solution, but there was a difference in the amount impregnated into the catheters. The amount impregnated may be affected by the size and properties of the drug or its solubility in chloroform. The molar mass of triclosan is 289.54 g/mol and it is 392.41 g/mol for sparfloxacin.

4.3.2.3 **Chloroform residuals as determined by GC-MS**

A standard curve was created using the method described. Unknown chloroform concentrations from the catheter segments taken from the

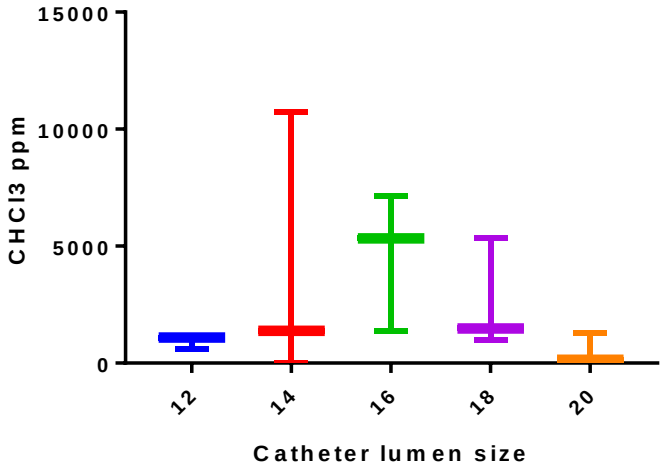
impregnated catheters before sterilisation by ethylene oxide were interpolated from the standard curve in Figure 4-6.

Figure 4-6 Calibration curve of chloroform ($CHCl_3$) standards in parts per million (ppm) for pre-sterilisation catheter segments



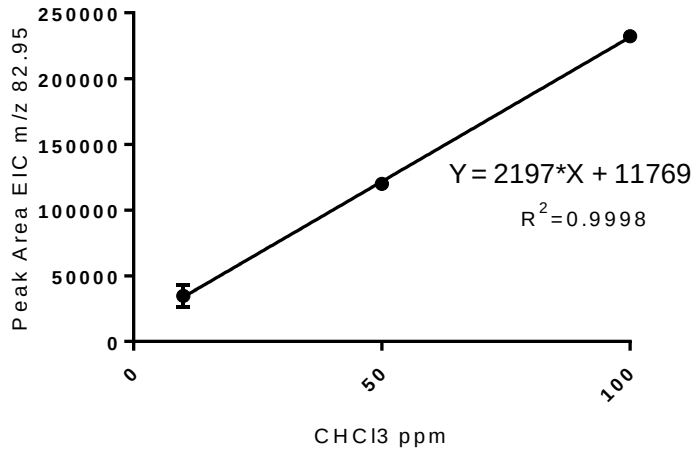
All three segments of each of the five catheters tested pre-sterilisation by GC-MS for chloroform residues had chloroform residues ranging from 23.4 ppm – 10,714.4 ppm. There was variation between segments from the same catheter and between catheters as seen from the box and whisker plots (Figure 4-7). These residual chloroform amounts were unacceptable for patient contact.

Figure 4-7 Tukey's box and whisker plot of chloroform ($CHCl_3$) parts per million remaining on 3 x 1.0 cm segments removed from five catheters before sterilisation



An additional standard curve was created for the catheter segments analysed after sterilisation. Unknown chloroform concentrations from the catheter segments taken from the impregnated catheters after sterilisation by ethylene oxide were interpolated from this standard curve (Figure 4-8).

Figure 4-8 Calibration curve of chloroform ($CHCl_3$) standards in parts per million (ppm) for post-sterilisation catheter segments



Two of the three catheters had no chloroform residues detected by the established method for all three catheter segments tested. Two of the three segments of the third catheter had no chloroform residuals detected. The third segment of this third catheter did have chloroform detected but when interpolated from the standard curve the value was -11.839 ppm, meaning the chloroform detected was virtually indistinguishable from zero. These residual levels of chloroform were deemed acceptable for patient contact.

4.3.3 **Clinical trial results**

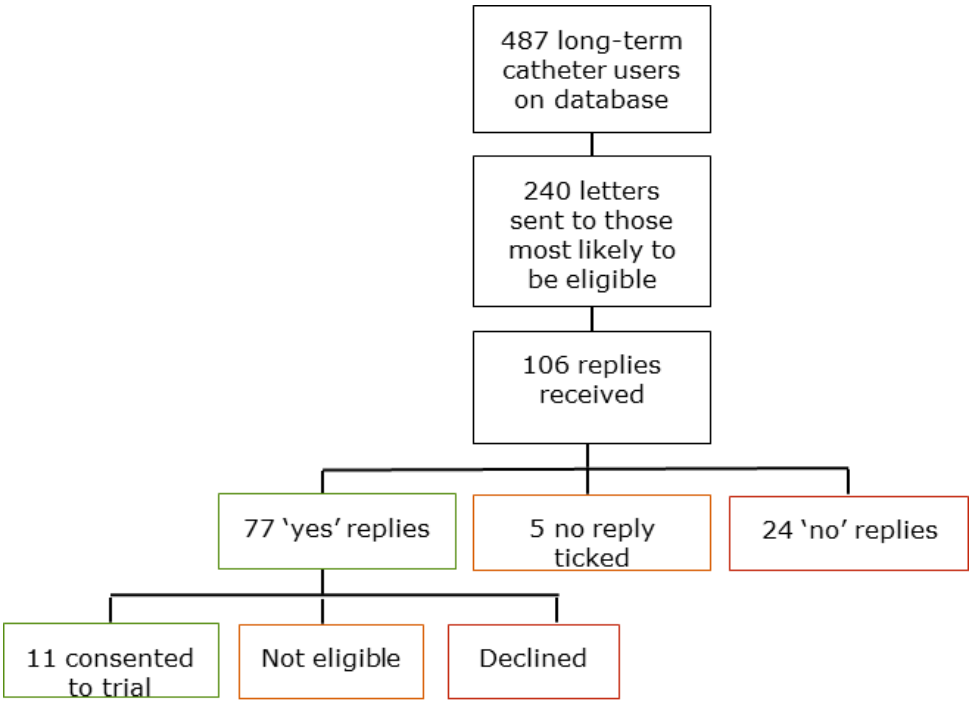
The trial and recruitment began on 1 December 2016 and the trial ended on 28 February 2018. 30 participants were recruited to the study.

4.3.3.1 **Recruitment**

Several recruitment strategies were employed, the first of which included sending a letter of interest to long-term catheter users who ordered their catheters supplies through Nottingham CityCare. 487 long-term catheter users were on the database and letters were initially sent to 240 who were most likely to be eligible and likely to respond to the patient letter, such as being in their own home and not in a nursing facility. The response rate to the mail out was 44.2% and of those who responded 77 patients were interested in receiving more information about the trial (Figure 4-9). Several patients did not indicate ‘yes’ they would like more information or ‘no’ they do not wish to be contacted. Where there was no response selected, a second

letter was sent as they couldn't be contacted about the trial without giving explicit consent. There were still several who did not tick either reply.

Figure 4-9 Replies to patient mailout of long-term catheter users on the Nottingham CityCare database



Common reasons for not being eligible for the trial included recently passing the TWOC clinic and a catheter was no longer needed, catheter had been successfully removed, and not medically suitable. People declined for reasons including feeling overwhelmed by other health problems and their appointments, too much travel to the hospital or difficulty travelling.

15 GP practices in Nottinghamshire and Derbyshire completed a search of their database for patients fulfilling the trial inclusion criteria. In total 156 patients were identified and contacted and 60 replies were received (38.5% response rate). Fifty seven of the 60 replies received were from patients who wanted to receive more information about the trial. As with the CityCare mail-out, there were many patients who were not eligible for reasons such as currently doing intermittent self-catheterisation, passed TWOC, or did not currently have a catheter in. Those who declined, did so due to inability to travel or did not want to travel. Two patients from this mail-out were recruited to the trial.

Other participants recruited to the trial were identified by consultant urologists who discussed the trial briefly with the potential participants, and these patients were then followed up by the research team. Likewise, other suitable patients were identified by ward staff at NUHT. The Harvey 2 TWOC diary, which kept a record of patients coming in for the TWOC clinic, was prospectively reviewed for patients who would have had their catheter in for a minimum of 28 days by the time of the TWOC clinic. They were contacted before the clinic and given the PIS in the post if interested. If they failed the TWOC clinic, meaning they required another indwelling urinary catheter, they were consented to the trial at that point. However, it was not possible to predict who would pass or fail, so those that passed would not need a catheter and were not recruited to the trial. This strategy recruited 11 patients to the trial.

The original recruitment target was 60 based on how many catheters had been collected from a similar group of patients within the year for the catheter collection study. Throughout the trial, it was clear thar this was an over-ambitious target within the time frame as the catheter collection study did not take into account patient sensation, ability to travel, or mental capacity. A reduction in the recruitment target was discussed with REC and a substantial amendment was approved to consent 31 patients instead based on projected recruitment.

4.3.3.2 **Participant demographics**

Thirty participants were recruited to the trial and received the antimicrobial urinary catheter (Table 4-8). The mean length of catheterisation with the trial catheter was 56.03 days with a range of 1-84 days. There were a total of 1681 days of participants catheterised with the AUC.

Table 4-8 Participant demographics for the antimicrobial urinary catheter safety study

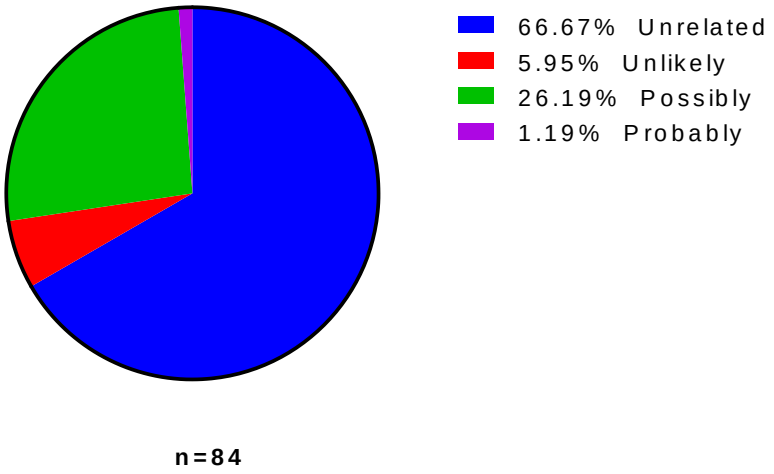
Trial Participants (n=30)		
Age (years)		
	Mean	71.4
	Range	43 - 92
Gender		
	Male	26/30 (86.7%)
	Female	4/30 (13.3%)
Catheterisation Route		
	Urethral	29/30 (96.7%)
	Suprapubic	1/30 (3.3%)
Reason for catheterisation		
	Acute retention	12/30 (40.0%)
	Chronic retention	11/30 (36.7%)
	Chronic retention with incontinence	2/30 (6.6%)
	Incontinence	2/30 (6.6%)
	Neurogenic bladder	1/30 (3.3%)
	Urethral stricture	1/30 (3.3%)
	Immobility	1/30 (3.3%)
Catheter lumen size		
	12 Ch	7/30 (23.3%)
	14 Ch	18/30 (60.0%)
	16 Ch	5/30 (16.7%)

28 participants were included in the full analysis set, meaning they had at least one telephone interview after catheterisation. Regarding the 2 participants who were not included in the full analysis set, when called at 24 hours post catheterisation for the first interview, one catheter had been expelled. For this participant, the balloon had burst as confirmed by the research nurse and then back in the laboratory. It was later confirmed that this participant had bladder stones, which are a common cause of burst catheter balloons. The second participant, who has a history of self-expelling catheters, self-expelled the trial catheter when sitting up in bed on the morning after catheterisation.

4.3.3.3 **Primary outcome: adverse events attributable to the AUC**

A total of 84 AEs were recorded for the 30 participants in the safety set, and on a per participant basis, the number of AEs ranged from 0-11. The majority of AEs reported were unrelated or unlikely to be related (72.62%) to the AUC (Figure 4-10).

Figure 4-10 Relatedness of adverse events to antimicrobial impregnation of the trial catheters



The AEs considered to be 'possibly' related to AUC (26.19%) included blockage of the catheter, CAUTI episodes, and stinging after catheterisation as these are AEs associated with any urinary catheterisation. The exception to this was one participant who experienced severe stinging following catheterisation that the participant did not experience with previous catheters (Table 4-9). This AE was classified as 'probably' related to antimicrobial impregnation of the catheters due to the noticeable difference between the AUC and the normal catheters. The stinging subsided within 48 hours and the participant then went on to consider the AUC no different from their normal catheter. None of these AEs were considered serious or severe.

Table 4-9 Description of adverse events (AEs) 'unlikely', 'possibly' or 'probably' related to antimicrobial impregnation of trial catheters.

AE relationship to antimicrobial impregnation of the trial catheters	Number of AEs			
	Mild	Moderate	Severe	Total
AE 'Unrelated'	**37**	**14**	**5**	**56 (66.67%)**
AE 'Unlikely'	**4**	**1**	**0**	**5 (5.95%)**
Pelvic infection	1			1
Dizzy after catheterisation	1			1
Catheter fell out spontaneously	1			1
Dizziness/generally unwell		1		1
Catheter displaced due to bag being full and falling off catheter strap	1			1
AE 'Possibly'	**19**	**3**	**0**	**22 (26.19%)**
CAUTI	2	2		4
Blockage	4	1		5
Burning at the beginning and end of passing urine via the flip flow valve	2			2
Early catheter change due to shorter length	2			2
Catheter expulsion	1			1
Bypassing catheter	1			1
Sensation of needing to void	1			1
Catheter bag/valve pushing off connection	1			1
Haematuria	1			1
Stinging	1			1
Testicular ache	1			1
Difficulty connecting the catheter bag to catheter	1			1
Small sore on foreskin	1			1
AE 'Probably'	**1**	**0**	**0**	**1 (1.19%)**
Heightened stinging following catheterisation	1			1

Four SAEs were reported during the trial, all of which were classified as such due to hospitalisation of the participant. All four were classified and agreed as unrelated to the study intervention. Details of the SAEs can be found in Table 4-10.

Table 4-10 Description of serious adverse events (SAEs) of trial participants

Patient Identifier	Description of SAE
160454	Presented to Accident and Emergency (A&E) at Queen's Medical Centre with acute shortness of breath, cough, and intermittent chest pain. Diagnosed with community acquired lobar pneumonia and admitted to Nottingham City Hospital
020170	Experienced a tonic clonic seizure lasting two minutes in Resus, A&E, Queens' Medical Centre. Seizure self-terminated. Participant has a long-standing history of complex partial seizures.
261132	Presented to GP with severe headache, and was then admitted to Queen's Medical Centre via GP for further assessment. CT scan showed no acute findings and lumbar puncture results were normal.
140448	Presented to A&E at Queen's Medical Centre after taking an accidental overdose of 20 paracetamol resulting in a fall and dizziness. Patient was transferred to ward for further head injury and paracetamol level monitoring

No participants reported a rash in the genitourinary region. One participant had a rash around a nephrostomy tube 13 days after its insertion. Another participant developed a painful rash around his 'waistband' eight days after catheterisation with the AUC. He was seen by the principal investigator that day, was diagnosed with shingles and the participant was prescribed acyclovir. Neither rash was judged to be associated with allergy or hypersensitivity to the AUC.

4.3.3.4 **Secondary outcomes**

Patient acceptability was a key secondary outcome and was assessed by the telephone interviews over the duration of catheterisation.

4.3.3.4.1 Catheter blockage

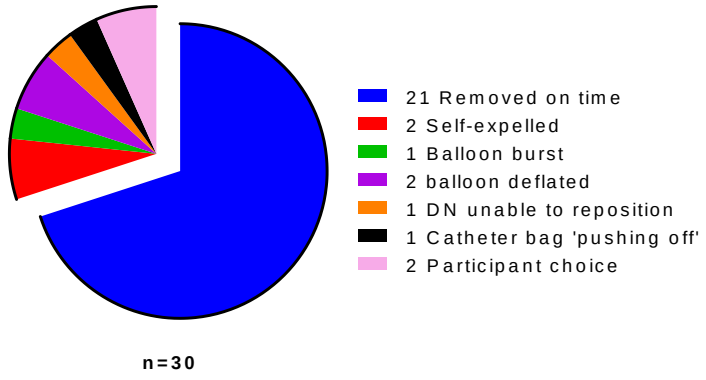
The telephone interviews asked about blockage of the catheter which is an important issue for patient dignity and catheter management. Five participants experienced catheter blockages which required a bladder washout. Two of the blockages were caused by blood clots. For one of the patients 500 mL of bloodstained urine was drained by a nurse practitioner in A&E and the haematuria was believed to have been caused by aspirin use. For a second participant who experienced a blockage caused by a blood clot, it was dislodged by the washout performed by the district nurse. The

catheter then did not leak for the rest of the trial duration. From analysis of the two other catheters that blocked one contained *C. albicans* extensively producing hyphae and the cause of the other blockage was unclear.

4.3.3.4.2 Participant withdrawal

Nine of the 30 participants ended the trial earlier than expected (Figure 4-11). Seven of the nine participants ended the trial earlier than expected not due to choice, but for reasons such as the catheter falling out due to self-expulsion, a burst balloon, and balloon deflation. One catheter was pulled out of position slightly and it was not within the district nurse's remit to deflate the balloon, reposition the catheter, and re-inflate the balloon. One participant had not had any problems at the 42 days post-catheterisation interview, but at day 47 the participant reported bladder spasms leading to increased emptying of the catheter using the catheter valve. The participant switched to a leg-bag instead, and on day 48 reported that the catheter connector kept disconnecting from the leg bag and he could watch it 'pushing off' from the catheter. High bladder pressure was suspected. Two of the nine participants ended the trial early voluntarily, both due to what they felt, was a shorter catheter length. Both received standard length catheters, which were 400mm in length. No catheters were removed over safety concerns.

Figure 4-11 Reasons for early catheter removal during the AUC safety trial

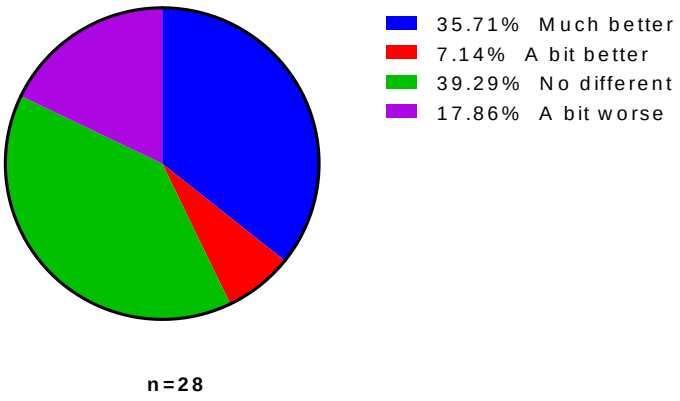


4.3.3.4.3 Patient acceptability

One question of particular relevance in the telephone interview was 'How would you rate this catheter compared to your usual catheter?', which was followed by the options of 'much better', 'a bit better', 'no different', 'a bit

worse', or 'much worse' and then a section of free text for the reason for rating the catheter. 82.14% of participants rated the AUC as no different or better than their previous catheters. Five participants (17.86%) rated the catheter as 'a bit worse' than their usual catheter (Figure 4-12).

Figure 4-12 Participant responses to the telephone interview question 'How would you rate this catheter compared to your usual catheter?'



Thematic analysis of the free text responses to this question from the first and last recorded interviews can be found in Table 4-11 and Table 4-12, respectively. All free text responses were included in the tables as recorded. However, for some participants there was no free text response included alongside the catheter rating. This only occurred when the catheter was rated as 'No different' and were included in the theme representing this.

Four themes were identified from the first interview that summarised participants' opinions on how the AUC compared to their usual catheter. The first of these was that there was no noticeable difference to previous catheters. The participants felt it was too soon to detect any differences or that they were confident that there was no difference between the AUC or usual catheters. The second theme was catheter maintenance issues which included leakage, bypassing, and urine flow. The comments were mainly positive in that there was improved flow and less bypassing, however, one participant commented that there was increased leakage and another commented that there was a more frequent need to empty the catheter. The third theme was 'catheter comfort' in which the comments were divided into sub-themes of 'improved comfort', which were positive comments, and 'stinging and soreness', which were negative comments. The responses were contrasting with two participants saying the AUC was so comfortable

that they did not know the catheter was in place, while several other participants said there was stinging and soreness after catheterisation. Stinging is common after catheterisation, but for one participant the stinging was greater than normal and recorded as an AE as discussed previously. The fourth theme was mental health in which one participant reported her positive mood to her friends on Facebook, and the daughter of one participant commented that the participant was feeling much more upbeat than usual, which was significant as the participant suffered from depression either partly or wholly due to catheter issues.

Table 4-11 Thematic analysis of free text responses to the question 'How would you rate this catheter compared to your usual?' from the first telephone interview

Main Themes	Sub-Themes	Free text responses
No noticeable difference to previous catheters	Too soon to make a judgement	- 'can't tell any difference so far' - 'hard to judge because of the camera (flexible cystoscopy)' - 'too early to tell the difference as it takes a few days to settle down normally' - 'too early to tell any difference' - 'too early to tell if it is going to be much better at the moment' - 'bit too soon to say'
	Cannot tell a difference	- 'business as usual' - 'the catheter is the same' - 'feels no different at the moment' - 'about the same' - 'can't tell any difference to the to other one' - 3X 'no different ratings' but no free text
Catheter maintenance	Urine flow	- 'flow is good' - 'draining beautifully clear urine – as good as the usual catheter' - 'draining much more urine than usual' - '…more frequent need to empty it compared to the usual catheter'
	Leakage and bypassing	- 'bypassing is less than other catheter' - '…not bypassed and not expelled' - A bit worse 'due to …leakage plus the Statlock keeps unclipping itself'

Catheter comfort	Stinging and soreness	- A bit worse ‘due to the soreness and stinging’ - ‘experiencing stinging and soreness but this is present with the usual catheter when there is an infection present’ - ‘uch worse due to the intense stinging which wasn’t happening with the other catheter’ - ‘a bit sore initially but that’s normal after a catheter change’ - A bit worse ‘due to the stinging sensation which has resolved now’ - A bit worse ‘due to the pain’
	Improved sensation	- ‘not felt a thing. Would think it had been removed. Been to the shops on my scooter and had a walk around’ - ‘It feels a lot more comfy. Usually when I’m walking around I can feel it in, with this one I don’t even know it’s there and keep forgetting’ - ‘feels more comfortable’
Mental Health	Impact on mental health	- ‘quite happy at the moment, put it on Facebook yesterday’ - Daughter stated that her mother seemed ‘much more upbeat than usual as she has depression due to catheter problems’

The four main themes from the first interview carried over to the last recorded interview with the addition of the theme ‘Participant satisfaction’ (Table 4-12). Again many participants commented that the AUC was not noticeably different from their usual catheters. The sub-themes within the ‘Catheter maintenance’ main theme changed with ‘infection’ and ‘catheter valve issues’ replacing ‘urine flow’. Any novelty in a change of flow was replaced with three positive comments about the participants being free of infection throughout the trial and problems with flip-flow valves. It is unlikely the flip-flow valves are related to antimicrobial impregnation of the trial catheters as they were not involved in the impregnation processing. One of the main themes of ‘Catheter comfort’ was divided into two contrasting sub-themes of ‘improved comfort’ where participants reported less pain and more comfort, and ‘discomfort’ in which two participants reported the AUC felt shorter and one felt the need to pass urine more often. There were no reports of soreness or stinging. There were two comments related to improved mental health including improved quality of life of a participant who needed to empty his bladder less at night with the AUC and was able to get a better quality of sleep. The fifth, and additional main theme, from the last interview, was ‘Participant satisfaction’ in which five participants or their relatives asked if they could have another AUC or that they didn’t want to have the AUC removed.

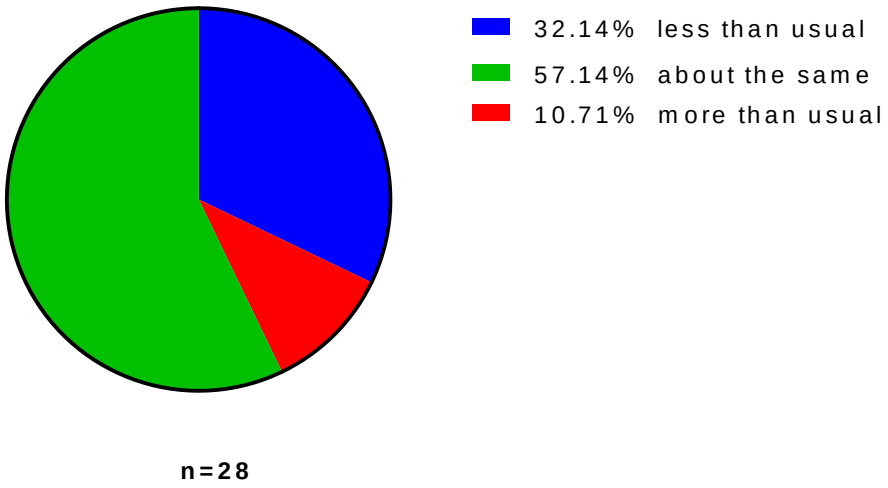
Table 4-12 Thematic analysis of free text responses to the question 'How would you rate this catheter compared to your usual?' from the last telephone interview

Main Themes	Sub-Themes	Free text responses
No noticeable difference to previous catheters	Cannot tell a difference	- 'much the same' - 'no different to other catheter' - 'equal to the best of these I have had' - 'no real difference to the usual catheter' - 'no problems with it' - 'It's the same as the other catheter' - 3X no free text about it being no different
Catheter maintenance	Infection	- 'better because I definitely would have had an infection by now' - 'no infections and normally suffers from several' - 'better because currently free from infection'
	Leakage and bypassing	- 'although it's leaked, it hasn't leaked as much as usual.' - '…leaking slightly more as its at the end of the catheter life-span- it's happened with the usual catheter as well.'
	Catheter valve issues	- 'changed from flip-flow to leg bag and the leg bag keeps popping off. Had to secure it with micropore overnight' - 'experiencing burning at the beginning and end of passing urine via the flip-flow valve, which is more than usual'

Catheter comfort	Improved comfort	- ‘less pain’ - ‘more comfortable. At times I don’t know it is there. Been very happy with it.’ - ‘have had no pain with this catheter’ - ‘more comfortable- no sliding in the urinary tract. Others tend to move in and out’ - ‘its comfortable’ - ‘it's better because I've had less problems than normal'
	Discomfort	- ‘its difficult to judge as due to the shorter length it provides lack of comfort’ - ‘discomfort due to the feeling of needing to pass urine’ - ‘due to the shorter length of the catheter, the catheter can get caught or pulled. Using a net leg hold but no G-strap. Ache in testicles is not there with usual catheter’
	Catheter material	- ‘the catheter material is stiffer, not as flexible’
Mental Health	Impact on mental health	- ‘better at night-only emptying it twice which is a massive improvement. To get 3-4 hours sleep in one go has improved the quality of my life’ - ‘feel really happy with this catheter in’
Participant satisfaction	Desire to keep the AUC	- ‘Wish I could keep it in. Don’t think I will give it back’ - ‘can’t believe I’ve only got one more week left- dreading having it removed’ - Participant doesn’t want to give it back
	Desire to have a second AUC	- Participant asked if he could have another trial catheter - Son asked if the participant could have another trial catheter

A summary of the participant's opinion on pain caused by catheterisation during the trial was captured by the telephone interview question of 'Have you had any pain from the catheter?' with 89.28% of participants having the same amount of pain or less pain with the AUC as compared to their normal catheter at their last interview (Figure 4-13). For many who responded that the pain was 'about the same' this was because they did not have pain with previous catheters and did not have pain with the current catheter. The three participants who had more pain than usual, also rated the catheter as 'a bit worse' due to the shorter length, pain when passing urine through the flip flow, and feeling the need to pass urine more often.

Figure 4-13 Participant responses to the telephone interview question 'Have you had any pain from the catheter'



4.3.3.5 **Microbiological contents of original and trial catheters**

29 of the 30 original catheters from each trial participant were processed and analysed for their microbiological contents. One original catheter could not be analysed as it had fallen out and was lost before the patient had arrived at the clinic for consenting and catheterisation with the trial catheter. No catheter lumens or balloons from the original catheters were culture negative. 20/29 lumens and balloons had the same microorganisms isolated. The most common organisms isolated were *E. faecalis* and *E. coli* in both the lumen and balloons (Table 4-13). Two MDR bacteria were isolated and included MRSE and vancomycin-resistant *E. faecalis*.

Table 4-13 Microbiological contents of 29 original catheters from participants in the trial

Original catheter contents (n=29)				
Organisms isolated from catheter lumens			**Organisms isolated from catheter balloons**	
Enterobacteriaceae	**25**		**Enterobacteriaceae**	**25**
E. coli	8		*E. coli*	8
Serratia marcescens	5		*Proteus mirabilis*	5
Proteus mirabilis	4		*Serratia marcescens*	4
Klebsiella oxytoca	3		*Klebsiella oxytoca*	3
Enterobacter cloacae	2		*Enterobacter cloacae*	3
Enterobacter aerogenes	1		*Klebsiella pneumoniae*	1
Klebsiella pneumoniae	1		*Citrobacter freundii*	1
Citrobacter freundii	1			
Enterococci			**Enterococci**	
Enterococcus faecalis	14		*Enterococcus faecalis*	15
***Pseudomonas* spp.**	**4**		***Pseudomonas* spp.**	**3**
Staphylococci	**6**		**Staphylococci**	**6**
Staphylococcus aureus	4		*Staphylococcus aureus*	4
Staphylococcus epidermidis	1		*Staphylococcus epidermidis*	1
Staphylococcus haemolyticus	1		*Staphylococcus haemolyticus*	1
Yeasts	**3**		**Yeasts**	**5**
Candida albicans	2		*Candida albicans*	4
Candida parapsilosis	1		*Candida parapsilosis*	1
Others	**7**		**Others**	**7**
Streptococcus acidominimus	2		*Corynebacterium spp.*	2
Chryseobacterium meningosepticum	1		*Streptococcus acidominimus*	2
Stenotrophomonas maltophilia	1		*Stenotrophomonas maltophilia*	1
Alcaligenes xylosoxidans	1		*Alcaligenes xylosoxidans*	1
Streptococcus constellatus	1		*Streptococcus constellatus*	1
Moraxella osloensis	1			
Total:	**59**		**Total:**	**61**

28 of the 30 trial catheters were processed and analysed in the laboratory. Two catheters were lost as they were self-expelled by participants in their own homes and could not be recovered. Two lumens were culture negative. 16/28 trial catheters had the same organisms isolated from the lumen and balloon. The most commonly isolated organisms from the lumen were *E.*

faecalis and *E. coli*, and *E. faecalis* and *Pseudomonas* spp. in the balloons (Table 4-14). Two MDR bacteria were isolated and were both ESBL-producing *E. cloacae.*

Table 4-14 Microbiological contents of 28 trial (AUC) catheters from participants in the trial

Trial catheters (n=28)			
Organisms isolated from catheter lumens		**Organisms isolated from catheter balloons**	
Enterobacteriaceae	**24**	**Enterobacteriaceae**	**19**
E. coli	7	*E. coli*	5
Serratia marcescens	6	*Serratia marcescens*	4
Enterobacter cloacae	3	*Enterobacter cloacae*	3
Klebsiella oxytoca	2	*Proteus mirabilis*	2
Proteus mirabilis	2	*Klebsiella oxytoca*	2
Klebsiella pneumoniae	2	*Klebsiella pneumoniae*	2
Serratia liquefaciens	1	*Citrobacter freundii*	1
Citrobacter freundii	1		
Enterococci	**12**	**Enterococci**	**10**
Enterococcus faecalis	12	*Enterococcus faecalis*	10
Pseudomonas spp.	**6**	**Pseudomonas spp.**	**6**
Staphylococci	**0**	**Staphylococci**	**3**
		Staphylococcus epidermidis	3
Yeasts	**2**	**Yeasts**	**4**
Candida albicans	1	*Candida albicans*	3
Candida tropicalis	1	*Candida tropicalis*	1
Others	**4**	**Others**	**5**
Corynebacterium striatum/amycolatum	1	*Corynebacterium striatum/amycolatum*	1
Stenotrophomonas maltophilia	1	*Peptostreptococcus prevotii*	1
Streptococcus acidominimus	1	*Stenotrophomonas maltophilia*	1
Alcaligenes xylosoxidans	1	*Streptococcus acidominimus*	1
		Brevibacterium spp.	1
Total:	**48**	**Total:**	**47**

A paired analysis of the contents of the original catheter and the corresponding trial catheter (n=27) suggested a trend towards a lower burden of microorganisms attached to the lumen and the balloon, although neither was statistically significant in this small population. There were significantly fewer (p=0.0088) species of micoorganisms attached to the trial

catheter balloons compared to the matched original catheter balloons and a trend towards fewer species in the catheter lumens (Table 4-15). This is reflected in that only 16/28 trial catheter lumens and balloons contained the same organisms, as there were fewer organisms attached to the trial catheter balloons. There was no difference in the number of MDR organisms isolated from original or trial catheters.

Table 4-15 Paired summary of characteristics of original catheters and trial catheters (AUCs). SD: Standard deviation, MDR: multi-drug resistant

Variable	Original Catheters	AUCs	Paired t-test
Length of catheterisation (mean ± SD)	60.74 ± 25.81 days	61.63 ± 22.52 days	p=0.8428
pH of lumen sonicates (mean ± SD)	6.926 ± 0.661	6.796 ± 0.524	p=0.1992
Number of species in lumen (mean ± SD)	2.074 ± 0.829	1.778 ± 1.121	p=0.0881
Number of species in balloon (mean ± SD)	2.185 ± 0.834	1.778 ± 0.801	p=0.0088
Burden of species in lumen (log mean ± SD)	6.908 ± 1.371	6.276 ± 2.304	p=0.1259
Burden of species in balloon (log mean ± SD)	5.808 ± 1.247	5.190 ± 1.571	p=0.0873
Number of MDR isolates in lumens	2	2	
Number of MDR isolates in balloons	2	2	

While this is a quantitative analysis of the microbiological contents of the two catheter types, as demonstrated in Chapter 2, catheter colonisation may not necessarily correlate with presentation of CAUTI symptoms, and therefore this is not a reflection of CAUTI rates.

4.3.3.6 **Anecdotal case studies of the impact of the AUC**

This trial was not designed to assess the efficacy of the AUC in reducing the incidence of CAUTI. However, there were a few case studies with interesting supporting evidence of the CAUTI preventative activity of the AUC. These cases may also provide interesting considerations for future trials of efficacy.

4.3.3.6.1 Participant 030338

Participant 030338 is a 79 year old female whose medical history includes hypertension, cancer of the bladder, angina, and frequent urinary tract infections. She uses an indwelling urethral urinary catheter for convenience due to immobility following a fractured right ankle (the author appreciates that this is not a recommended reason for indwelling catheterisation). The participant was catheterised with a 12Ch standard length AUC. At 72 hours, her son enquired if the AUC prevented infection would his mother be able to have another. It was discussed with him that as explained in the PIS, another AUC would not be available to his mother. At the telephone interview at 28 days, the participant stated that she 'would normally have had at least one bladder infection by now'. This was repeated at the telephone interviews until the final interview at 63 days in which she rated the AUC as 'Better because definitely would have had an infection by now'. Again, at this interview, her son asked if she could have another AUC and it was discussed that it was not possible.

A few weeks later, after the trial catheter had been removed, Participant 0303338 returned to the Nottingham Urology Centre for an unrelated appointment and spoke to the trial Research Nurse in passing. The participant stated that she had one CAUTI episode since the trial, and this was greatly reduced for her. A carry-over effect of the AUC bears consideration in future clinical trial designs.

4.3.3.6.2 Participant 300473

Participant 300473 is a 43 year old female whose previous medical history includes asthma, polycystic liver and kidney disease, fibromyalgia, pelvic venous embolism and frequent CAUTI episodes. She uses an indwelling urethral urinary catheter to manage chronic retention due to nerve damage from surgery following childbirth. She was catheterised with a size 12Ch female length AUC and reported mild stinging for the first 10 hours following catheterisation. Throughout the trial, she rated the AUC as better compared to her usual catheter, predominantly for comfort reasons. She suffered one blockage and received antibiotics for one episode of CAUTI during the trial period, though, according to her comments, this is greatly

improved for her (Table 4-16). She also kept her trial catheter despite CAUTI.

Table 4-16 Timeline of Participant 300473 free response comments at each telephone interview over the trial period.

Interview Number	Free response comments
24 hours	'Quite happy at the moment. Put it on Facebook yesterday'
48 hours	'Not sore down there, not swollen, and not painful when I sit down. Usually, have to sit on my side but can sit down normally now. I've put it on Facebook again'
72 hours	'tempted not to bring it back. Can sit down without any pain, so pleased'
7 days	'don't even know I've got it in half the time. Don't have to keep phoning my district nurse of GP because I've got an infection. My district nurse will have forgotten me as I haven't phoned her in so long'
14 days	'two or three times during the week, used to call the district nurse and haven't had to call since having the new catheter'
21 days	Catheter was blocked and district nurse did a bladder washout and urinalysis which was positive, but the participant did not want antibiotics. 'Urine is now clear and pain has resolved. Happy with the catheter again.'
28 days	'I keep putting it on Facebook. Disappointed that the weeks are flying by too quickly'
35 days	'Still don't want to part with it'
42 days	'Dreading having the other one back in'
49 days	'Dreading having the other catheter back in'
56 days	Daughter pulled the catheter in error, husband pushed the catheter back in and did a bladder washout. A CSU was taken by the practice nurse due to burning sensation in urethra. 'Want to hang on to it for as long as possible'
63 days	Was prescribed trimethoprim during the previous week. Recorded CAUTI AE. 'Pain is much worse with usual catheter- can't sit upright normally, have to lie on my side. Can sit upright with this one, it's lovely'
70 days	'Much more comfortable- dreading having it taken out'
77 days	'Can't believe I've only got one more week left-dreading having it removed'

As seen from comments at seven and 14 days after catheterisation with the AUC, the participant mentioned accessing healthcare resources less often due to fewer catheter problems. Future clinical trials might consider a health economics analysis, which takes into account the impact of the AUC on contact with healthcare services. Although increased comfort was not universally reported during the trial for all participants, for several

participants, including Participant 300473, the improvement was noticeable and important to the participant. Possible mechanisms of improved comfort should be investigated.

4.3.3.6.3 Participant 300160

Participant 300160 is 57 year old male with a previous medical history which includes insulin dependent diabetes mellitus type 2, urethroplasty, urethrotomy, hypertension, lymphoedema, and gastric bypass. He requires an indwelling urethral urinary catheter due to stricture of the bulbar urethra. He was catheterised with a 16Ch standard length AUC. During the trial, the participant reported reduced leakage and improved comfort. At 49 days post-catheterisation the participant reported that he '[feels] more confident knowing the risk of infection is less. Feel more comfortable on the whole. Reassuring having the trial catheter in'. At 56 days post-catheterisation he reported 'Not had any infections, normally would have had several by now.' This comment was echoed at every subsequent telephone interview. At the final interview, he also stated that he didn't want to give the AUC back. This case is important because it adds to patient reports of reduced infection. This gentleman went 12 weeks CAUTI free, which was unusual for him. Participant 300160 commented that knowing he had a catheter which might prevent infection increased his confidence in his catheter. However, with knowing the catheter may prevent infection comes the risk of bias. Future clinical trials are unlikely to be blinded to the clinician or participant due to the red-orange colour of the catheter, and an element of bias of not reporting CAUTI due to the belief that it will resolve due to the protective activity could be introduced. Future studies will need to be carefully designed to minimise this.

4.3.3.6.4 Participant 120465

Participant 120465 is a 51 year old male whose medical history includes spinal injuries, ileostomy, cholecystectomy, insulin dependent diabetes mellitus type 2, peripheral neuropathy, asthma, hypertension, and frequent urinary tract infections. In the previous year, he suffered an episode of urosepsis. He requires an indwelling urethral urinary catheter due to chronic retention as a result of nerve damage. He was catheterised with a

12Ch standard length AUC. At catheterisation, he was currently taking 500 mg t.i.d cephalexin for an active CAUTI episode. Analysis of his catheter showed that the lumen contained 10^5 CFU/mL *Pseudomonas* spp., 10^6 CFU/mL *E. coli*, and 10^5 CFU/mL *Enterobacter aerogenes*. The balloon contained 10^6 CFU/mL *E. coli* and 10^5 CFU/mL *C. albicans*. At six days post-catheterisation, the participant called the research team to inform them that the district nurse had performed a bladder washout and taken a CSU due to malodourous urine. The CSU results 'came back clear' and the participant was not prescribed antibiotics. On Day 36 the participant rang the research team to say he had symptoms of CAUTI including cloudy and smelly urine. He attended a drop-in clinic and on the basis of urinalysis he was prescribed 200 mg b.d. nitrofurantoin. The following day he chose to have the AUC removed due to the catheter feeling too short and leakage. Analysis of the trial catheter showed 10^3 CFU/mL ESBL-producing *Enterobacter cloacae* and 10^4 CFU/mL *C. albicans* in the lumen and 40 CFU/mL ESBL *E. cloacae* and 10^3 CFU/mL *C. albicans* in the balloon. Microscopic examination of the lumen sonicate showed yeasts producing hyphae.

This was the only participant to have an active episode of CAUTI at catheterisation with the AUC. The AUC is designed to protect the catheter from colonisation and not to treat an active infection. Furthermore, the AUC does not have protective activity against yeasts and while the burden of yeasts on the balloon was reduced compared to the original catheter, the yeasts were able to colonise the lumen. The production of hyphae by yeasts is associated with invasive disease[341] and is likely responsible for symptoms when also considering that there were only 40 CFU/mL ESBL *E. cloacae* on the balloon. The presence of an ESBL-producing organism may have been predisposed by previous use of cephalexin, a first generation cephalosporin. When considering the inclusion criteria for future studies of efficacy, it is unlikely it will include patients with active CAUTI. The episode of CAUTI experienced during the trial may have been as a result of the previous infection that never cleared. Discomfort from the catheter feeling shorter could have been influenced by inflammation associated with an invasive infection.

4.3.3.6.5 Participant 160454

Participant 160454 is a 63 year old male with a longstanding medical history of chronic obstructive pulmonary disease, bronchiectasis, and atrial fibrillation. In 2014 he underwent a colostomy due to a perforated bowel and in 2015 an implantable cardioverter defibrillator was implanted. He requires a urethral urinary catheter for chronic retention. He was catheterised with a 14Ch standard length AUC. Throughout the trial period, he reported that the AUC was more comfortable and quite often he couldn't feel that it was in place. At the end of the trial period, the AUC was removed and another indwelling catheter was not replaced. Instead, he chose to perform intermittent self-catheterisation. He had previously done intermittent self-catheterisation and due to not feeling pain with the AUC his confidence increased and he felt able to once again perform intermittent self-catheterisation. This showcases and reinforces how the AUC may have the potential to influence mental health and quality of life, which should be investigated in larger studies.

4.3.3.7 Feasibility study questionnaire

Twenty-four feasibility questionnaires were returned. Several participants did not return theirs as they felt it was not relevant to them as after the trial period they no longer had a catheter due to successful TURP operations and would not be eligible for subsequent studies. The questionnaire asked questions regarding where the catheter user normally has their catheter changed, issues with their catheter, and reasons for getting involved in a clinical trial of an AUC (Table 4-17). The majority of catheter users enrolled in this trial have their catheters changed in their own home (70.83%), which was echoed during recruitment to this trial in which many were unable to leave their homes to participate. Of particular relevance to the patient acceptability studies, 54.1% of participants stated that they had some pain when catheterised, which suggests that stinging and soreness reported by participants initially was not atypical.

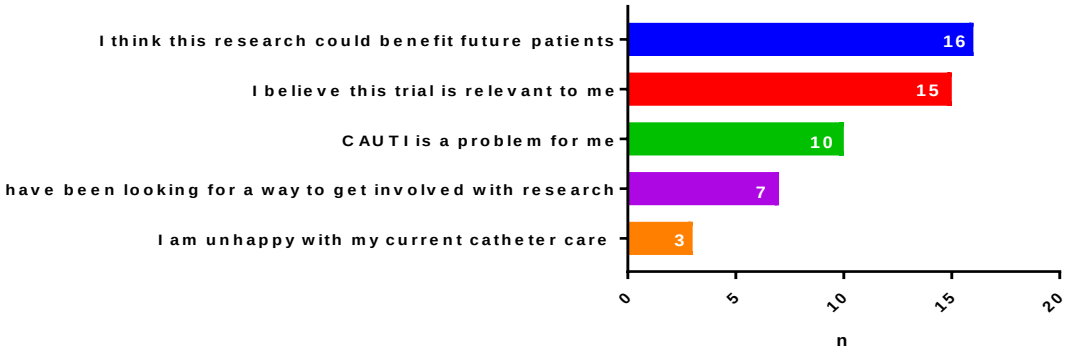
Table 4-17 Feasibility questionnaire responses (n=24)

Question	Responses (n)		
Where do you usually have your catheter changed?	**Hospital**	**Own Home**	**GP Practice**
	5 (20.83%)	17 (70.83%)	2 (8.3%)
In the past year, have you experienced a urinary tract infection when a catheter has been in place?	**Yes**	**No**	
	10 (41.7%)	14 (58.3%)	
If YES, have you had more than one urinary tract infection when the catheter has been in place?	**Yes**	**No**	
	8	2	
If YES, have you received antibiotics for a urinary tract infection in the past year?	**Yes**	**No**	
	9	1	
If YES, were you hospitalised as a result of a urinary tract infection when the catheter was in place?	**Yes**	**No**	
	3	7	
In the past year, have you sought medical attention for your catheter being blocked?	**Yes**	**No**	
	14 (58.3%)	10 (41.7%)	
If YES, has blockage of your catheter resulted in it being removed earlier than expected?	**Yes**	**No**	
	9	**5**	
In the past year has urine leaked around the outside of your urinary catheter?	**Yes, often**	**Yes, but not often**	**No**
	7 (29.2%)	11 (45.8%)	6 (25.0%)
In the past year have you been told you have bladder stones?	**Yes**	**No**	
	2 (8.3%)	22 (91.7%)	
Do you have pain when catheterised?	**Yes, often**	**Yes, but not often**	**No**
	5 (20.8%)	8 (33.3%)	11 (45.8)

Of particular consideration, several participants who joined the trial did not have a history of CAUTI and/or blockages. One concern when designing the trial was that there may be a bias towards people joining the trial who had issues with their urinary catheter, as participants without any issues might perceive that they would have less to gain by trying a new catheter. One of the questions in the feasibility questionnaire asked about reasons the participant might want to get involved in a randomised controlled trial of the AUC. The most common motivation for involvement was a sense of altruism

and a desire to be involved in further research that could potentially help future patients (Figure 4-14). 41.7% of participants would be motivated to take part in future studies of the AUC because they feel CAUTI is a problem, which supports the view that this current trial was an even mix of participants with frequent episodes of CAUTI and those without.

Figure 4-14 Responses (could choose several options) from 24 participantsto what would be their motivations for joining a trial of the AUC



The feasibility questionnaire helped to understand the characteristics of the long-term catheterised group of patients and identify reasons for patient involvement in future clinical trials of the AUC.

4.4 Discussion

4.4.1 Summary

AUCs were produced and validated for use in the antimicrobial urinary catheter safety study. A GC-MS protocol was developed to detect chloroform levels in the AUCs before and after sterilisation. The AUCs were validated as being sterile, free of ethylene oxide sterilisation residuals, free of chloroform, and contained the three antimicrobials in similar and expected quantities.

30 participants received AUCs in the safety trial after securing the appropriate trial approvals and input from the PPI group. This is the first clinical trial of these devices. The primary outcome measure was the rate of AEs attributable to antimicrobial impregnation of the trial catheters. 84 AEs in 30 participants across 1681 catheterisation days were recorded and of those only one was identified as being ‘probably’ related to the antimicrobial impregnation of the catheters. This event was stinging at catheterisation that

the participant had not experienced with other catheters. The AE resolved within 48 hours. The safety profile of the AUCs appears favourable.

Patient acceptability was also largely favourable with 82.14% of participants included in the full analysis set rating the AUC as ‘no different’ or better than their previous urinary catheters. Thematic analysis of participant free responses at the last interview showed that improved comfort and being free from infection spoke strongly about the positive impact of the AUC. Individual case studies provided anecdotal evidence about the efficacy of the AUC in preventing symptomatic infection. An unexpected finding was improvement in other aspects of the participant’s life, such as improved mental health and improved quality of sleep, which also had implications for mental health. Participants that thought the catheter was ‘a bit worse’ than previous catheters did so due to catheter valve issues and perception that the AUC was shorter in length than other catheters. The two participants who perceived the AUC to be shorter than other catheters were the only participants who chose to withdraw from the trial.

Microbiological analysis of the participant’s original and trial catheters demonstrated a significant reduction of the number of species attached to the balloon of the AUCs. There was a trend towards a reduced number of species isolated from the AUC lumens, and the burden of microorganisms in the AUCs but these were not statistically significant in this small population. Importantly, the use of the AUC did not increase the prevalence of MDR organisms or increase the prevalence of microorganisms that are non-susceptible to the activity of the AUC.

4.4.2 **Patient and public involvement**

PPI in this entire study has been extremely rewarding as it has added value to many aspects of the clinical trial, to the research team, and to the lay members of the group. An example of how the clinical study was shaped by PPI, was that originally the recruitment criteria excluded those with a history of autonomic dysreflexia. PPI member 1, who has a spinal cord injury, commented that in over 30 years of being tetraplegic he/she had only experienced one episode and thought it would be unfair to exclude those with rare episodes. Therefore the exclusion criteria were amended to reflect

those with a history of uncontrollable autonomic dysreflexia. The RMC felt this was a sensible modification as it would not outright exclude those with an episode of autonomic dysreflexia and therefore leave the decision up to the clinician.

Additional ideas for recruitment were also discussed at the RMC meetings. Lay member 3 and the author attended the NIHR Clinical Research Network Research Awards 2016-2017 evening after Lay member 3 had nominated the PPI of this project for recognition. At this event details on how NIHR CRN could assist with recruitment were provided and this information was delivered back to the RMC. It was decided that the research team would ask the NIHR CRN East Midlands Primary Care team for assistance identifying patients. The GP mail-out strategy was then discussed with the NIHR CRN East Midlands Primary Care team and implemented. Lay member 4, who has sat on Clinical Commissioning Groups suggested contacting local support groups through organisations such as the Stroke Association. One participant was recruited via information he/she had obtained through the Stroke Association.

The patient-facing materials and the clinical trial protocol were commented on by the lay members of the RMC both generally in a group setting at the meeting and individually when they were asked to review documents outside of the meeting. It was universally agreed that the original recruitment posters were not appealing to future participants. Group discussion created the recruitment posters that were then approved by REC and HRA and displayed in clinical settings. It was felt that the original posters were not designed in a way that grabbed the attention of our target population, and it was therefore agreed that the posters should read in large font ‘Are you a long-term urinary catheter user?’. Through these discussions, it was also decided not to include any pictures of people as there is no one person or group of persons who looks like a typical urinary catheter user.

Several challenges were identified when assembling the RMC. One of the challenges that was identified when planning lay member involvement was how to maintain confidentiality of the research developments, but also of the lay members own discussions about their treatment, care, and condition. A confidentiality agreement was created to protect the research and lay

members' personal details. This was signed by all members and to the author's knowledge, there were no instances of confidentiality being compromised. Another challenge of embedding PPI was recruiting lay members as the advertisement asked for a commitment of three years. Lay member 2 was unable to remain with the RMC for the whole three years due to other unforeseen commitments. However, his/her contribution during their involvement was astounding as they generated a business plan for the AUC. The third challenge of arranging the RMC meetings was finding an appropriate venue that was wheelchair accessible, not costly and welcoming. At the initial stages of recruitment, the lay members expressed that they did not like coming into the hospital for meetings as there was little parking and was often associated with bad memories. A venue was found at the University of Nottingham, University Park Campus that had parking, wheelchair access, served refreshments and was not associated with the hospital. Another consideration was to equally include 'professional' lay members who were involved with many instances of PPI and non-professional lay members who had little to no experience to retain a foundation in the everyday patient. Overall, the group dynamic in the RMC was extremely positive and supportive.

4.4.3 **HPLC**

HPLC of the trial catheters showed that they were impregnated with 0.08% w/w rifampicin, 0.704% w/w sparfloxacin, and 1.084% w/w triclosan. After impregnation, drying, and rinsing in ethanol the drug was extracted by one round of soaking in chloroform. This was initially carried out to determine if there were microscopic drug deposits on the surface that were not rinsed off with the ethanol wash. One sample had a concentration in which all three antimicrobials were at least double that of the median proportional drug content. After flushing with ethanol, the drug content of this segment was reduced and was similar to those of other segments from the same catheter. It appears that there may be some microscope accretions that remain in the lumens of catheters after rinsing by dipping in ethanol. Actively flushing the catheter lumens with ethanol via a syringe could be used in the future to minimise any drug residual accumulations.

The full drug extraction showed all three drugs were impregnated into the tested trial catheters. It was difficult to follow a standardised assessment protocol of determining the uniformity of drug content as current pharmacopoeial guidelines do not have a monograph for combination drug devices. ISO 12417-1:2015 is a standard on vascular device-drug combination products, and may be the most applicable standardisation protocol in the future as it is applicable to antimicrobial coated catheters or other medical devices where the drug has an ancillary action to the device[342]. However, it refers to the European Pharmacopeia and the US Pharmacopeia to determine drug content, again which do not have a monograph for combination drug devices, which specifies how to test for uniformity of drug dosage. The manufacturers of Bactiseal have determined an upper and lower manufacturing limit of rifampicin and clindamycin which are impregnated into the silicone neurosurgical devices. The manufacturing lower and upper range for rifampicin impregnated into Bactiseal devices is 0.25-0.7 mg/g (0.45 mg/g difference)[304]. The amount of rifampicin impregnated into Bactiseal is 0.054% ± 0.032% w/w compared to 0.080% ± 0.13% w/w of the AUCs, the small difference in median drug contents may be due to the presence of a balloon, thickness of silicone, size and number of eyelets, and impregnation with different antimicrobials (clindamycin vs triclosan and sparfloxacin) which may affect compatibility with the matrix. The range of rifampicin impregnated into the AUCs is 0.733 – 1.26 mg/g (difference 0.53 mg/g). The ranges of manufacture are broadly comparable for rifampicin in the AUCs compared to Bactiseal. However, there is no comparison for impregnation of sparfloxacin and triclosan into silicone. Acceptable drug levels could be informed by achievable, non-toxic serum concentrations. Future manufacturing of the AUCs will need a standardised assessment of drug content and an acceptable manufacturing range. For this small production of the AUCs, the presence of the three drugs in similar quantities was sufficient.

4.4.4 **GC-MS**

The method of determining the chloroform content of the AUCs is a novel method relying on the identification and quantification of chloroform by GC-

MS. The specific mass-to-charge ratio chosen for identification of chloroform was 82.9 m/z based on its greater sensitivity compared to a ratio of 84.9 m/z. The m/z ratio of 82.9 to detect chloroform has also been used by Deconinck et al. and provided a limit of detection of 0.589 ppm[327]. The greatest concern when developing this method was how to create standards that replicated the samples. For example, initially, to detect the chloroform in the catheter segments the segments were placed directly into the GC injection port. Standards were made by soaking plain silicone catheter segments in the chloroform standards. However, it could not be guaranteed that the entire amount of chloroform was impregnated into the catheter. To remove variability, standards were prepared in acetone. To compare like to like, the chloroform was extracted from the catheter segments into acetone.

Acetone was chosen as it is relatively safe compared to other solvents such as methanol and toluene, and due to its non-polar methyl groups, it is able to dissolve non-polar substances such as chloroform. Its m/z ratio of 43 or 58 was also sufficiently different from that of chloroform. From preparation of the standards, it appears chloroform in acetone was a stable solution which provided excellent goodness of fit values of the calibration curve of greater than 0.99.

Despite at least 12 hours of air flow at room temperature over the impregnated catheters, unacceptable levels of chloroform remained in the AUCs before sterilisation. In the plant that commercially produces the neurosurgical devices, chloroform is removed by vacuum over 10 hours, which could be employed to improve the removal of chloroform. However, after sterilisation by ethylene oxide chloroform was detected in only one sample and at a level virtually indistinguishable from zero. Although there are no available guidelines for chloroform content of medical devices the United States National Institute for Occupation Safety and Health recommends limiting exposure to 2ppm for 60 minutes[343] or a 60 ppm concentration in medical drugs and devices according to the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use[325]. Another concern was that any remaining chloride ions from the chloroform may have reacted with the ethylene oxide to form ethylene chlorohydrin, a toxic residual of ethylene

oxide sterilisation. The sterilisation reports did not detect any ethylene chlorohydrin in the five samples tested for remaining ethylene oxide post-sterilisation. The AUCs for the trial were free from chloroform and ethylene chlorohydrin.

4.4.5 **Trial design**

There were some limitations of the trial design that may have hindered participant recruitment. The AUC had to be fitted at NUHT as part of the agreement with REC. As a CE-marked device that had been modified, it could be used in the facility of manufacture without the need for MHRA involvement. This meant it could not be fitted in the community setting, which is where the majority of long-term catheter users have their catheter changed as demonstrated by the feasibility questionnaire. They are often seen by district nursing teams for catheter changes due to immobility. Therefore, many prospectively eligible patients were not interested in the trial due to the inability to travel.

For many long-term catheter users, they would come in contact with clinicians and the research team when having their catheter changed. For those who have their catheter changed every 12 weeks, this would mean they could not be consented to the trial until their next catheter change as they had not had 24 hours to read the PIS. Once they consented they would need another 12 weeks in the trial, so for some patients 6 months may have been necessary for recruitment and trial completion. In the future, the trial should be extended as recruitment ran out of time for some eligible and willing participants. The trial was extended by three months in order to capture additional participants for the above reason. The removal of the 24 hour window to read the PIS was discussed with REC and it was felt that the period of reflection was important for a trial of safety and especially for a non-emergency procedure.

Initially, only indwelling urethral urinary catheter users were recruited to the study as it was felt that it was important to capture any discomfort to the urethral mucosa, as all patients in the CATHETER trial underwent urethral catheterisation and reported that one in nine patients experienced urethral discomfort[101]. However, encouraging interim results of the safety and

patient acceptability were discussed with the RMC and it was decided that to increase recruitment and assess comfort in suprapubic catheter users, they should be recruited. The amendment to include suprapubic catheter users was approved by REC and HRA as a substantial amendment.

Patient acceptability was integrated as a secondary outcome measure to assess firstly, that the catheter performs as expected, in that it drains urine without increasing the risk of blockage or urine bypassing. Secondly, a Cochrane Systematic Review of indwelling urinary catheters by Lam et al. concluded that the silver-alloy and nitrofurazone coated urinary catheters were more likely to cause patient discomfort[122]. Patient comfort is paramount for clinical uptake. This was assessed by a structured telephone interview. The telephone interviews were conducted at 24, 48, and 72 hours then weekly. The heavy emphasis on discussion at the beginning of the catheterisation was because allergy and hypersensitivity would present early after catheterisation, and these were important outcomes to capture for the study. Feedback from participants was that they did not find the telephone interview onerous, and in fact, several participants commented that it was beneficial to have access to healthcare and research personnel invested in their treatment.

4.4.6 **Safety of the AUC**

A heightened sense of stinging at catheterisation was reported by one participant and this resolved within 48 hours. There were no sequelae to the event and it was a mild event. The increased stinging was the only AE to be ‘probably’ associated with antimicrobial impregnation of the trial catheters. The participant then went on to give the catheter a final rating of ‘no different’ to their normal catheter. It was the only associated event throughout the 1681 catheterisation days, which suggests the AUC has an equivalent safety profile.

The previous CATHETER trial of silver and nitrofurazone catheters demonstrated increased discomfort with the experimental catheters during catheterisation. Haematuria and septicaemia were the two recorded significant clinical events included in the CATHETER trial[103]. Haematuria and blockage of the catheter due to a blood clot were recorded during this trial,

but there were alternative reasons for the presence of blood such as taking aspirin and the presence of an enlarged prostate which is a recognised cause of haematuria[344]. During adjudication of AE causality, as haematuria was not present without other predisposing factors and was no worse than previous episodes, the episodes were determined as 'possibly' related as it is related to catheterisation but not necessarily catheterisation with the AUC.

The ESCALE trial, a trial of silver-alloy catheters in SCI patients, reported more adverse events possibly related to catheterisation with the experimental catheters compared to standard catheters, including itching which was not reported in the control group[313]. Itching was not reported by any participants at any time point during this trial. Other AEs captured by the ESCALE trial included haematuria, rash, blockage, and suprapubic pain[313]. Rash related to catheterisation and suprapubic pain were not reported by patients in this AUC safety trial. Blockage was reported during this trial.

Some AEs that were recorded during the AUC safety trial and not in the previous trials, included the sensation of needing to void, burning at the beginning and end of passing urine using a catheter valve, difficulty connecting the catheter bag to the catheter, and the catheter drainage system 'pushing off' the catheter connection. While these are mild events and mostly associated with the catheter drainage systems, they were still reported by participants as part of their catheter management. The base silicone urinary catheters that were impregnated with antimicrobials may have been from a different manufacturer than their normal catheter, which could have affected what the catheter user perceives as 'normal' from their catheter. Recording of these events aimed to increase the transparency of the study and it will help to inform the choice of the base silicone catheter for antimicrobial impregnation in future studies.

4.4.7 **Patient acceptability of the AUC**

For the majority of the patients catheterised with the AUC, the AUC was either no different, a bit better, or much better compared to their previous catheters at the end of the trial period. For some participants the reasons for it being better included being infection free, having less pain, and increased comfort. Five participants or their relatives voluntarily stated they

wanted to continue with the AUC even though they were not asked about using the AUC again. There were mental health improvement implications by improving catheterisation issues, which bears consideration for future studies. However, these will require careful investigation due to the risk of bias from increased patient interaction with healthcare personnel in a trial setting, which can affect participant satisfaction.

The themes derived from participant free responses to their rating of the AUC showcase that infection is important to participants, but also that managing the catheter requires a holistic approach and improving pain, leakage, comfort, and other aspects of life impacted by urinary catheterisation are just as important for many participants. When initially considering patient acceptability, no difference from the participant's usual catheter was considered a desirable outcome as the AUC did not predispose to discomfort. Reports of increased comfort and less pain were unexpected and beneficial.

Some participants reported stinging and soreness at the first interview post-catheterisation. A survey of male catheter users with prostate cancer or benign prostatic hyperplasia showed that 43.6% of those surveyed reported 'rather much'-'much' discomfort and 27.8% reported smarting at catheter placement[345]. Another questionnaire based study of long-term catheter users showed that 66% experienced discomfort at catheterisation and that it took between 1-7 days to subside[346]. The feasibility questionnaire administered during this AUC safety trial agreed with these previous studies in which 54.1% of participants reported that they sometimes or frequently experienced pain when catheterised. Six of the 28 (21.4%) participants who had a first interview reported stinging, soreness or pain, which was less than those values reported in the previous studies. The stinging and discomfort sensation subsided within 48 hours for the trial participants. One participant did report greater stinging than with previous catheterisation procedures and this was captured accordingly. The discomfort reported during the final interview was not related to stinging or soreness that could be associated with antimicrobial impregnation of the trial catheters, but instead with the length of the catheter and the feeling of needing to pass urine often.

The feeling of comfort and less pain associated with the AUC may be influenced by the anti-inflammatory effects of triclosan. Due to triclosan's long-standing role in oral care products, it has been suggested that triclosan may exert a direct anti-inflammatory effect which may enhance healing in chronic periodontitis patients and reduce inflammation of the gums[347, 348]. Investigations have shown that triclosan downregulates many acute and chronic inflammatory molecules, including interleukin-6, which is a determinant molecule for the transition between acute and chronic inflammation[347] and cyclooxygenase 2, a significant pro-inflammatory molecule[348]. The suppression of these molecules may be particularly relevant to those catheter users who suffer from recurrent episodes of CAUTI. Hannan et al elucidated that inhibition of cyclooxygenase-2 in mouse models of recurrent urinary tract infection prevented acute and chronic infection. The inhibition of cyclooxygenase-2 prevented mucosal damage by preventing neutrophil migration to the area, but did not disrupt beneficial immune responses such as shedding of infected urothelial cells and recruitment of other immune cells to the area[349]. Hannan et al then proceeded to show that raised levels of interleukin-6 were predictive of the development of chronic cystitis in the mouse bladder model[350]. Elwood et al investigated the effect of triclosan-eluting ureteral stents on inflammation and also found reduced expression of pro-inflammatory markers in human bladder cells when exposed to triclosan. They suggested that triclosan's mechanism of action may be two-fold by preventing inflammation from bacterial stress and non-bacterial mechanical stress of the presence of the stent, as well as exerting antimicrobial activity[351]. A reduced bacterial burden may also help to reduce inflammation and discomfort.

Additionally, for the one participant who reported an improved quality of life due to more sleep at night from not having to empty his bladder as often, this improvement in urgency may have been influenced by the presence of triclosan. In non-catheterised patients, increased urinary frequency is a sign of infection due to bladder inflammation. It is possible this participant's frequent emptying of the bladder was mediated by inflammation in the bladder. This is interesting in contrast to the participant who reported some discomfort due to feeling the need to pass urine more often. The

participant also suffered from constipation during the trial period, which can be a cause of an increased feeling of the need to pass urine more often. The role of triclosan and the AUC in mediating inflammation in the bladder, which could, in turn, have an effect on pain or recurrent infection, warrants further study.

4.4.8 **Thematic analysis**

Thematic analysis was used to organise the free text responses provided by the participants at the first and final telephone interviews. Thematic analysis is an advantageous method for interpreting patterns in free text responses as it is not restricted by the size of the sample population and can be used for large and small data sets, and therefore is used to analyse patterns across participants. Furthermore, due to its flexibility it is an easily accessible technique without extensive procedures for researchers unfamiliar with qualitative research[338, 339]. However, its flexibility and lack of rigorous defined procedures can lead to inconsistencies. To ensure the trustworthiness of the data it is essential that decisions and rationales used during the process are recorded so that analytic decisions can be audited and revisited if questioned[338].

Thematic analysis was useful in these studies as a simple grouping exercise to organise the data and identify commonality between participants. It is clear from the data that there were shared ideas about the patient acceptability of the AUC between participants. Changes to the names of themes or the groupings of coded texts was recorded for any required auditing purposes.

4.4.9 **Microbiological contents of catheters**

Twenty-nine original catheters and 28 trial catheters were analysed for their microbiological contents using the method developed in Chapter 2. One possibility was that eradication of organisms susceptible to the activity of the AUC would allow replacement by other organisms. However, this was not seen in the catheter analyses. For example, *E. faecalis* was not susceptible to the activity of the AUC as investigated in Chapter 3, and there was no overgrowth of *E. faecalis* in the AUCs, in fact five fewer AUC balloons and

two less lumens contained *E. faecalis* compared to the control catheters. In general the presence of all groups of organisms was reduced in the AUCs, with the exception of *Pseudmonas* spp*.* in which there were two more AUC lumens colonised with *Pseudomonas* spp*.* compared to the control catheters. The main limitation of this analysis is that the numbers are small and future studies will need to monitor the colonising microorganism populations.

From the in vitro flow studies (Chapter 3), the model demonstrated eradication of major uropathogens until the end of 12-13 weeks. Two AUC lumens in removed catheter were culture negative, but for many, although the number of attached micoorganisms was reduced, they were colonised by organisms that were susceptible to the activity of the AUC. There was a discrepancy between the in vitro flow model and the colonisation of the removed catheters. From the in vitro flow model studies, after inoculation of the catheters in the model, the killing effect of the AUC took 24-72 hours to eradicate the attached micoorganisms. During this time the model was not re-inoculated so that eradication of attached organisms could be monitored. It was also felt that because a common source of catheter contamination is changing of the drainage system[65], which is often changed weekly, weekly bacterial challenges might mimic weekly bacterial exposure that accompanies drainage system changes. However, if the residual urine in the bladder was serving as a constant bacterial reservoir the AUCs may have been continuously exposed to a bacterial inoculum. By the time the catheter was removed and analysed in the laboratory there may not have been sufficient time for the AUC to exert its complete antimicrobial effect. As seen in Chapter 3, as antimicrobials are eluted over time the killing effect of the AUC takes longer. For example, in the twelfth week of challenge of the AUCs with NDM-1 *E. coli*, it took six days for all bacteria to be eradicated.

However, as discussed previously and in Chapter 2, catheter colonisation does not equate with symptomatic infection. A clinically important reduction of infection can only be assessed by an appropriately powered clinical trial of efficacy. What these studies do show, importantly, is that even if the AUCs are not culture negative, the remaining attached microorganisms do not represent a population that is non-susceptible to the

catheter that is replacing the susceptible organisms that have been eradicated.

If organisms that are originally susceptible to the activity of the AUC are able to colonise the AUC, one concern is the development of resistance. There is no evidence to suggest that the antimicrobial impregnated neurosurgical devices, which have been in use for many years, cause or increase resistance[352, 353]. The EVDs have some similarities to urinary catheters in that both are implanted into the body but also with access to the environment for drainage. The rate of EVD infections can range from 3.9%[354] to 27.0%[355] with the reasons for the range of rates due to different diagnostic criteria or patient populations. The rate of CAUTI in control catheters during the CATHETER trial was 11%[101]. However, EVD infections are mainly caused by staphylococci and occassionally by enterococci, Enterobacteriaceae and *Pseudomonas* spp, which differs from CAUTI causative organisms which is mostly caused Enterobacteriaceae and enterococci with some staphylococci. On the basis of a similar risk of colonisation it might be reasonable to expect that the AUC might perform as the antimicrobial impregnated EVDs and will not cause or increase antimicrobial resistance. However, as seen in Chapter 3, the MICs to rifampicin by some enterococci were raised after exposure to the AUC. A combination of rifampicin and fluoroquinolone is well-documented for reducing, but not entirely preventing, the emergence of resistance to rifampicin by enterococci[356]. The number of colonising *E. faecalis* isolates was reduced on the trial catheter lumens and balloons suggesting some protective activity of the AUC. The susceptibility of catheter-colonising enterococci and development of resistance will require monitoring in future trials, although the rationale for the design and supporting evidence of the AUC suggests that it should not cause or increase resistance.

4.4.10 Conclusions

The AUC has an advantageous safety profile and was an acceptable alternative catheter to the majority of trial participants. Information gained from this trial will support future regulatory applications for commercialisation and larger studies of efficacy of the AUC.

Chapter 5. Discussion and conclusions

As an ageing population and reliance on the use of medical devices increase, device-associated infections have become a growing concern. Bacterial attachment on device surfaces and the subsequent change to a biofilm mode of growth makes eradication difficult. The most effective prevention strategy for reducing device-associated infections is a reduction of the use of indwelling biomaterials. However, people with spinal cord injury, multiple sclerosis, an enlarged prostate, chronic retention, or severe incontinence may need an indwelling urinary catheter to manage their urine output for the foreseeable future. As a result, they may suffer from repeated infections. Repeated courses of antibiotics for treatment are disadvantageous due to antibiotic-associated side effects and the increased risk of the development of resistance by bacteria that are not killed. For those without alternative bladder management options available to them such as clean intermittent catheterisation, they would benefit from preventative technology.

There are several commercial and experimental urinary catheters designed to reduce colonisation. However, clinical and experimental evaluation of these catheters has shown the protective duration of these catheters is short and unlikely to make a clinically important difference. For long-term catheter users there is no protective technology available. Therefore, there is a need for a biomaterial that offers protection for the lifetime of a catheter that may be in place for up to three months. Silicone urinary catheters impregnated with rifampicin, sparfloxacin, and triclosan were previously developed[77, 131]. This thesis aimed to further characterise the antimicrobial properties, surface characteristics, anti-mineral encrustation properties, safety, and patient acceptability of this antimicrobial impregnated catheter.

5.1 Statement of principal findings

Before investigation of the catheter properties, this thesis set out to firstly increase understanding of the aetiology of bacteria attached to the lumens and balloons of urinary catheters. Using a novel method of

investigating catheter colonisation, initial characterisation studies of 61 collected urinary catheters demonstrated almost universal catheter colonisation by 10 days with *E. coli* and *E. faecalis* being the two most commonly isolated organisms. The microbiological contents of the lumen and the balloon were in agreement for 65.6% of catheters highlighting that for 34.4% of urinary catheters the luminal and bladder contents differ which has implications for CSU results if it's sampling luminal bacteria that are not affecting the bladder. Correlation of available CSU results to the catheter contents did not show agreement between the two and highlighted how the CSU results did not influence antibiotic treatment. A significantly higher proportion of MDR organisms were found in catheters from patients known to be receiving antibiotics than those not receiving antibiotics. A longitudinal catheter collection study from one patient over one year determined that there is a stable catheter microflora in which the indistinguishable isolates, as determined by PFGE, continually re-colonised urinary catheters.

These findings influenced the following studies on the effect of the AUC on a broader spectrum of uropathogens, in which the AUC was tested against a greater spectrum of MDR organisms and enterococci to further assess its activity. In a clinically predictive in vitro flow model, the AUC prevented colonisation by MSSA, MRSE, *S. saprophyticus*, ESBL *E. coli*, and NDM-1 *E. coli* for 12-13 weeks and by MRSA for 10-12 weeks. This is the first urinary catheter, to the author's knowledge, that can prevent in vitro colonisation for 12 weeks by carbapenemase producing *E. coli*. The AUC was unable to prevent colonisation in the in vitro flow model by enterococci. HPLC demonstrated that sparfloxacin and triclosan remained in quantifiable concentrations in the AUCs at the end of 12-13 weeks of constant perfusion, whereas rifampicin was present in most AUCs, but was below the limit that could be quantified by HPLC.

This thesis presented a novel method of measuring mineral deposition in urinary catheters, by using a spectrophotocolourimetric method to detect phosphate. The catheter collection study showed that *P. mirabilis* was not present in all blocked catheters. Therefore, the ability of the AUC to resist mineral encrustation in the presence of bacteria such as *P. mirabilis* and *S. saprophyticus*, also with urease activity, and *E. coli* without urease activity,

and in the absence of bacteria was determined. In a static model phosphate deposition on the AUC when incubated with *P. mirabilis* was significantly reduced at 24, 48, and 72 hours compared to the control. There was no difference detected between phosphate deposition on the AUC when inoculated with other bacteria or in AU of varying pHs, most likely due to reduced mineral deposition under the conditions of the controls. In an in vitro flow challenge model all control catheters blocked before the end of 28 days when inoculated with *P. mirabilis*, whereas none of the AUCs blocked and had significantly reduced phosphate deposition according to the spectrophotocolourimetric assay and as visualised by SEM. AFM studies showed that soaking and antimicrobial impregnation did not affect the AUC surface characteristics which could predispose to increased mineral encrustation and bacterial attachment.

AUCs for patient use were produced and validated for sterility, drug content, and the removal of chloroform. Thirty long-term catheter users were recruited to a safety study of the AUC and received the AUC. Throughout 1681 days of catheterisation with the AUC only one AE was reported which was ‘probably’ related to the antimicrobial impregnation of the trial catheters. The description of this AE was increased stinging after catheter insertion that was not present with previous catheters. The AE resolved within 48 hours and there were no sequelae. The safety profile of the AUC is encouraging. Generally, the AUCs were acceptable to patients with the majority of participants stating the catheter was no different or better compared to their previous urinary catheters with several patients reporting being infection free and experiencing less pain with the AUC. As a secondary outcome measure, there were significantly fewer bacterial species attached to AUC balloons compared to the matched original catheters. There was a trend towards reduced bacterial burden in the AUC lumens but this was not statistically significant in this small sample.

5.2 Study caveats

The studies within this thesis have some limitations, although efforts were made to minimise these. In Chapter 2, CSU results as reported by the

Clinical Microbiology laboratory were compared to the findings from the lumens and balloons of the matching catheters. This was a retrospective review of the patient notes to review the CSU results. For many patients, there were no CSU reports available, which is a limitation of many retrospective studies. Additionally, the date of catheterisation for the catheters collected was unavailable for some patients if the patient was unable to self-report the date and it was recorded in a community records system. NOTIS does not speak with primary care and community systems so the information on catheterisation dates could not be retrieved retrospectively. However, these were accepted limitations of this intentionally pragmatic study. Additionally, the results from the 28 CSU results compared to the matched catheters were consistently inconsistent with the catheter findings, and it could be expected the unavailable data would be similar. One aim of this study was to validate a method of retrieving bacteria from catheter lumens and balloons and inform further studies presented in this thesis, which were successfully achieved.

In Chapter 3, the protective activity of the AUC was further characterised. This involved determining the susceptibility of many bacteria to the antimicrobials in the AUC. Interpreting the results was limited by the lack of agreed clinical breakpoints and therefore, alternative interpretive criteria such as ECOFF values, were used. However, the main limitation of ECOFF values is that they describe whether the MIC for a particular organism falls within the wild-type or acquired resistance phenotype for that particular antimicrobial. This does not necessarily correlate with clinical outcomes, unlike clinical breakpoints. In the absence of breakpoints, ECOFFS are recommended by EUCAST and do provide important information about if resistance mechanisms have been acquired.

In Chapters 2 and 3 antimicrobial susceptibility testing was described. Antimicrobial susceptibility testing is governed by guidelines so that the results are transferrable and interpretable. When the work described in this thesis began, antimicrobial susceptibility testing in the United Kingdom was governed by the British Society for Antimicrobial Chemotherapy. In January 2016, it was announced that they would support in preference the EUCAST guidelines on antimicrobial susceptibility testing[357]. To reflect current

practice, susceptibility testing in this thesis changed to support the most recent EUCAST guidelines which meant a change of media from Iso-Sensitest agar to Mueller-Hinton agar amongst other small changes. Both protocols were used in this thesis reflecting current practice at the time.

In Chapter 3, AU was used in the mineral encrustation studies to mimic the mineral content of urine. The recipe has been used previously in other mineral encrustation studies[77, 78] and was employed as a standardised and readily available media compared to urine donations. However, urine is complex and varies by person, time of day, diet and many other factors. The AU lacks the variability and some of the constituents that are present in human urine in small quantities such as hormones. However, its formulation is such that it provides adequate substrates for bacterial growth that mimic the contents of human urine and for crystallisation as seen in these studies and elsewhere[211].

In Chapter 4, the safety clinical trial was described. Several attempts to minimise bias in the trial design and recruitment were employed and were previously discussed. The trial was able to avoid recruitment bias of participants who had pre-existing infection problems as seen by the results of the feasibility questionnaire in which 41.7% of participants had problems with CAUTI. However, recruitment was biased towards patients that were able to travel. Restrictions on arrangements with REC did not allow catheterisation with the AUC in the community setting. Future trials will need to take this into account. While this was a single-centre study, there was an appreciable variety in the underlying causes for catheter use and how the patients were managed in the community. Although multi-centre trials provide a better generalisability of the results and therefore increased external validity, the participants represented many health conditions and were managed throughout Nottinghamshire and Derbyshire once catheterised with the trial catheter. Therefore, they were managed as standard according to their local policies and guidelines, which were not influenced by the clinical trial.

The overall recruitment target had to be revised during the clinical trial when it was apparent 60 participants could not be recruited within the year. The sample size was determined according to the catheter collection study in which 61 catheters were collected from long-term indwelling catheter users

within a year and also knowing that Nottingham CityCare had 487 potential participants on their database. However, neither calculation took into account that many participants were unable to travel, and that many were awaiting TURP or had recently had a successful TWOC following TURP and no longer required another catheter. The information on recruitment will be important for powering the next trial of efficacy of the AUC. However, the 30 participants that were included generated 1681 days of catheterisation with the AUC which are 1681 opportunities for the presentation of an adverse event, and there was a good consensus about the safety of the AUC for these participants at catheterisation and throughout the catheterisation duration.

5.3 Study strengths

The studies within this thesis also have some strengths. Several novel methods were developed within this thesis to fulfil the aims. These new methods included an improved method of quantifying bacteria attached to the lumens of urinary catheters without sampling the exterior surface and also of sampling the balloon separately to achieve information about microorganisms in the bladder and those only in the catheter. To quantify phosphate in the lumens of urinary catheters, a spectrophotocolourimetric was employed for this purpose. This is the first study to use spectrophotocolourimetry to measure phosphate deposition as an indicator of mineral encrustation. It was a beneficial method in that many samples could be analysed quickly using readily available equipment and reagents. It was not as sensitive as atomic absorption spectroscopy, which is often used in mineral encrustation quantification studies, but was able to detect 2.2 ppm differences and had the added benefit of the calcium and phosphate not interfering the readings. This sensitivity was sufficient for detecting significant and clinically relevant differences between AUCs and control catheters colonised with *P. mirabilis.* Significant method development went into optimising an HPLC method that was able to detect and quantify all three drugs in the AUC using the same extracted drug content. Finally, a method of quantifying chloroform content in the impregnated catheters was

developed by extracting chloroform into acetone and quantifying the chloroform by GC-MS.

Many findings from this thesis are unique. This was the first study to examine the catheter contents from consecutive catheters from a patient over one year and carry out intra-species genetic comparisons. The results from the in vitro flow model, indicate that the AUC is the first reported urinary catheter with protective activity for 12 weeks against a carbapenemase-producing *E. coli*. This is important clinically as CAUTI or subsequent pyelonephritis or urospepsis caused by carbapenemase-producing *E. coli* is costly in terms of economics and patient morbidity and mortality. It is also challenging to treat when required. This is the only urinary catheter which may offer some protection against mineral encrustation due to its ability to prevent colonisation by *P. mirabilis*. The clinical trial of safety presented in this thesis is the first use of the AUC in patients and it was met with positive outcomes.

5.4 Future work

The work of the longitudinal catheter collection study highlighted some interesting results in that indistinguishable isolates colonised subsequent catheters throughout the year. There was also a genetic event that caused a 2-3 band difference as detected by PFGE of the *Pseudomonas aeruginosa* isolates that bears further investigation. The genetic event is currently being investigated by several methods. A genetic event that altered a change in the virulence factor genes is being investigated, firstly, by pyocyanin production and quorum sensing molecule production which will be quantitatively determined by liquid chromatography-mass spectrometry. Single nucleotide polymorphisms in the genomes are being analysed by whole genome sequencing.

AUCs were sterilised by autoclaving and ethylene oxide for use in the laboratory studies and clinical studies, respectively. It may be prudent for future commercialisation requirements to further investigate whether sterilisation by either method impacts the drug content of the catheters. Initial studies demonstrated the method of sterilisation does not affect the ability of

the AUC to create zones of inhibition when serially exposed to a bacterial lawn, however, quantitative drug release studies using HPLC would be more robust.

The results of this thesis are currently underpinning the design and application of a larger, multi-centre randomised clinical trial of efficacy of the AUC in long-term catheter users. Due to the advantageous safety profile of the AUC, this planned future trial will be more inclusive to include those without sensation below the waist and adults who are unable to provide consent. The Nottingham Clinical Trials Unit is supporting the application for funding and the trial. The primary outcome measure will be the rate of CAUTI with secondary outcome measures including antimicrobial resistance monitoring, quality of life analysis, and a cost effectiveness analysis. There will be a nested study within which will analyse blood samples from a subsection of volunteers one hour and 24 hours after catheterisation to monitor if antimicrobials are detectable in the serum, and if so to quantify the amount.

The work herein has also generated the interest of a commercial company based in the UK. Through a collaboration agreement, they will source all-silicone urinary catheters, and arrange the manufacture of the AUCs for the clinical trial at a larger scale. The manufacturing process and results from this thesis will underpin the process of commercialisation, the first step of which is CE marking. An application for CE mark of a Class III medical device will be submitted moving forward.

Any future use of the AUC is intended as an adjunct to appropriate clinical management of the patient including appropriate infection prevention practices and timely removal of the catheter if warranted. The AUC is intended to enhance infection prevention practices and does not replace them. If the AUC proves in future trial trials to be clinically effective in reducing infection, reducing mineral encrustation, and possibly extending the life of the catheter, a significant impact could be made on urinary catheter care.

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356. Holmberg A, Mörgelin M, Rasmussen M. Effectiveness of ciprofloxacin or linezolid in combination with rifampicin against *Enterococcus faecalis* in biofilms. *J Antimicrob Chemother* 2012; **67**: 433-9.

357. Brown DFJ, Wootton M, Howe RA. Antimicrobial susceptibility testing breakpoints and methods from BSAC to EUCAST. *J Antimicrob Chemother* 2016; **71**: 3-5.

Appendices

Appendix 1: People in Research advertisement

People in Research

NHS National Institute for Health Research

Opportunity details

< Back to results

Add to favourites

Lay Member on Committee to Inform Antimicrobial Urinary Catheter Research

Longterm urinary catheter users and carers wanted to steer research on antimicrobial catheters

Link to organisation:

http://nottingham.ac.uk/research/groups/biomaterialsrelatedinfection/index.aspx

Full description:

We would like long-term urinary catheter users (those who have a catheter in place for >30 days) and carers to take part steering the NIHR funded research project 'Characterisation, commercialisation, and clinical studies of a longterm antimicrobial urinary catheter.' This will involve attending meetings in Nottingham 3-4 times a year over 3 years to steer the design and application for Ethics approval of a safety clinical trial. More information can be found in the Role Description attached.

Date from

27th May 2015

Date to

27th June 2015

Organisation

Biomaterials-Related Infection Group, University of Nottingham

The Biomaterials-Related Infection Group is embedded in the Queen's Medical Centre, Nottingham University Hospitals Trust, but is affiliated with the University of Nottingham. This allows for a close link between academia and the NHS, which is reflected by our multi-discipline group made up of scientists and clinicians. The focus of the research carried out in this group is to understand the causes of healthcare related infections and their prevention.

Attachment

Role description of lay panel member RPW (2).docx

Details

Topic:

Dementias
Infectious diseases
Neurological disorders
Stroke or cardiovascular disease

Payment:

Payment will be given for attending meetings at a rate of £45 for each 2-3 hour meeting. If you chose to review documents (optional) the payment will be £50 for documents less than 50 pages, £125 for documents

Location:

East Midlands

Involvement type:

Designing and managing
Disseminating
Implementing
Reviewing

What support is offered?

We can offer Ethics training (3 hours) as optional training. There will also be a dedicated Contact Person to get in touch with if you need any support or have any problems

50-200 pages, and £200 for documents over 200 pages.

Expenses:

All expenses (this includes travel, accompanying carer/personal assistant costs, and alternative child-care or carer costs) will be covered by reimbursement

Can the work be done from home?

No

Suitable for a beginner?

Yes

Name: Katie Belfield

Email: katherine.belfield@nottingham.ac.uk

Telephone: 01158231113

Web: http://nottingham.ac.uk/research/groups/biomaterialsrelatedinfection/index.aspx

Appendix 2: Role description for lay members

Role description of Lay panel member
Research Management Committee
'Characterisation, commercialisation, and clinical studies of a long-term antimicrobial urinary catheter'

Role Title

Lay panel member on the Research Management Committee (RMC) for the 'Characterisation, commercialisation, and clinical studies of a long-term antimicrobial urinary catheter' research project.

Background

Project Summary: Urinary tract infection is a major problem in long-term urinary catheter users and can result in repeated catheter removal, courses of antibiotics, and often obstruction (mineral **encrustation**) of the catheter. No **antimicrobial** catheter is currently available with the required long duration of protective activity for long-term catheter users, as existing ones lose activity after a few days. Therefore the aims of this project are to:

- Confirm results showing protective activity for 80-100 days against common infecting bacteria
- Assess whether the catheter minimises **antimicrobial resistance**
- Investigate the ability of the catheter to prevent mineral encrustation
- Conduct a safety trial in patients with a long-term urinary catheters
- Conduct a feasibility study to determine the number of sites needed to run an efficacy **clinical trial** in the future

Start Date: May, 2015
End Date: April, 2018
Please note, you are welcome to leave the RMC at any time if you feel you are no longer able to participate.

Funding: Three years of funding from the National Institute of Health Research Invention for Innovation (NIHR i4i) programme. The NIHR i4i funds late stage

project development whether further testing and clinical studies will prepare the technology for commercialisation and adoption by the NHS.

Academic Staff: This research project is sponsored by the University of Nottingham, but has involvement from both the University and Nottingham University Hospitals NHS Trust. These include:

- Professor Roger Bayston- Professor of Surgical Infection
  - University of Nottingham
- Mr. Richard Parkinson – Consultant Urologist
  - Nottingham University Hospitals NHS Trust
- Dr. Gillian Shuttleworth-Licensing Executive
  - Business Engagement and Innovation, University of Nottingham
- Miss Katherine Belfield-Research Associate
  - University of Nottingham

Aims and Objectives

Aims and objectives of Research Management Committee:

- Keep research focused on delivering a long-term antimicrobial urinary catheter designed with the users in mind
- Advise on preparation of the application for Ethics for safety and feasibility clinical study
- Review progress to date and plan for future work
- Advise on **dissemination** of results

Specific roles of the lay panel members:

- Provide expertise of real-life experience
- Highlight issues important to long-term catheter users
- Assist in preparation of patient information leaflets and consent forms for Ethics
- Advise on recruitment to the clinical study
- Provide feedback on progress of the research project
- Help in **dissemination** of results to relevant user groups at the end of the study

Roles and responsibilities the lay panel members

Duties:

- Have the time and ability to attend meetings in person

- Contribute to discussion within the Research Management Committee
- To be available to respond to emails in regards to meeting attendance and potentially in regards to preparing materials for **Ethics**.
- Optional: Outside of the meetings to help with reviewing patient information leaflets and consent forms. If you do choose to assist with this, you will be reimbursed for your time.

Qualities:

- Understanding the issues related to long-term urinary catheterization
- Ability to provide a broad view on behalf of patient and carer groups rather than a personal one
- Be able to maintain confidentiality

Matters for consideration by the lay panel members

Considerations	What this means for you
Confidentiality	As a representative of the 'Characterisation, commercialisation, and clinical studies of a long-term antimicrobial urinary catheter' at the University of Nottingham you are asked not to share confidential information you may have received as a result of your position. This should be discussed with the project group and / or contact person and you will be asked to sign a **confidentiality agreement**.
Internet Access	Meetings are likely to be arranged by email, and in preparation for **Ethics** approval, further emails may be circulated so internet access is preferable.
Frequency of meetings	The Research Management Committee meetings will be held 4 times a year over the research project period (May 2015-April 2018). This will be a total of 12 meetings. Each meeting will last approximately 2-3 hours and refreshments will be provided.
Location of meetings	Meetings will be held at the University Park campus of the University of Nottingham. Access requirements will be discussed privately beforehand.

Reimbursement and Payments

All travel expenses and carer expenses will be reimbursed and you will be paid a fee for your time in attending the meetings. If you would like we can arrange the travel (such as a taxi) for you.

Reviewing patient information leaflets and consent forms outside of the meetings will also be reimbursed according to the Reimbursement Policy, which will be provided at the first meeting.

It is important to note that, with regard to payments, recipients are responsible for notifying agencies such as Jobcentre Plus, the Department of Work and Pensions or Her Majesty's Revenue and Customs about additional income. For this reason, Patients and carers may also like to know about INVOLVE's benefits advice service: http://www.invo.org.uk/resource-centre/benefits-advice-service/

Training and Support

Lay panel members are able to access support from Katie Belfield (named contact person for this work) and other members of academic staff:

Katie Belfield
Email: Katherine.belfield@nottingham.ac.uk
Telephone: 01158231113

Professor Roger Bayston
Email: roger.bayston@nottingham.ac.uk
Telephone: 01158231115

Please let us know if you would like any access to literature, such as scientific journals, or NHS or University policies.

Training will be offered for those interested about why we have **Ethics** in research. This is optional and will last approximately 3 hours.

Glossary

Term	Definition
Antimicrobial	Agent that kills or inhibits the growth of bacteria, viruses, parasites, and/or fungi
Antimicrobial Resistance	**'**Antibiotics and similar drugs, together called antimicrobial agents…have greatly reduced illness and death from infectious diseases. However, these drugs have been used so widely and for so long that the infectious organisms the antibiotics are designed to kill have adapted to them,

	making the drugs less effective' (Centers for Disease Control and Prevention)
Clinical Trial	A study in humans intended to discover or verify the effects of a medical product, to identify adverse reactions and to examine safety and efficacy
Confidentiality Agreement	A legal agreement to protect confidential information revealed during discussions or negotiations with another party. It applies to both organisations and individuals and is likely to contain clauses covering protection of people against the copying or retention of confidential information, disclosing information that is not already in the public domain to a third party and remedy for a breach of the agreement
Dissemination	Communication of research findings to a wider audience through, for example, publication in medical journals, the media, and voluntary organisations' newsletters
Encrustation	Complication of indwelling catheters caused by deposition of minerals from the urine onto the surfaces of the urinary catheter that can cause blockage of the catheter, urinary retention, and trauma upon catheter removal
Ethics	The name given to the code of practice based on a set of decent, fair and moral principles and guidelines that researchers should abide by. Research that will seek to gain personal confidential information or to test a new intervention on people must get ethical approval from a **Research Ethics Committee (REC)**
Research Ethics Committee	**(REC)** groups of professionals and service users that review the ethical considerations of research studies

Appendix 3: Confidentiality agreement for lay members

Confidentiality Agreement
Research Management Committee
'Characterisation, commercialisation, and clinical studies of a longterm antimicrobial urinary catheter'

Introduction: Why do we have a confidentiality agreement?

It is a fundamental principle in health and social care research that ideas, research data and personal details are not disclosed without permission from the researchers and research participants involved. This agreement is to remind people that confidentiality is extremely important and will be respected by associates of the Research Management Committee of the project 'Characterisation, commercialisation, and clinical studies of a longterm antimicrobial urinary catheter'. The wording of this agreement may seem official and unnecessarily formal – this is because we need to include certain phrases for legal reasons. However, it is important that you understand the reason you are being asked to sign this agreement. The final paragraph in bold summarises the two passages below.

Confidentiality agreement statement

You may not, either during, or at any time after the termination of your partnership with this service disclose to anyone, other than in the proper course of your work, any information of a confidential nature relating to a project and shall further not use any such information in a manner which may either directly or indirectly cause loss to the project. Confidential information includes, but is not limited to, information about researchers/participants/subjects of the project, financial information, commercial information, technical information or intellectual property (i.e. ideas and plans developed by the researchers). This clause shall cease to apply to information once it comes into the public domain, for example, is published (except as a result of your unauthorised act or default).

Disposing of confidential information

All documentation provided to you throughout the course of your involvement with the Research Management Committee for the 'Characterisation,

commercialisation, and clinical studies of a longterm antimicrobial urinary catheter' project, be it in electronic format such as e-mail or in hard paper copy, is provided in the strictest confidence. It is important that this documentation is disposed of in accordance with the Data Protection Act http://www.ico.gov.uk/for_organisations/data_protection.aspx and with confidentiality in mind. Documents should not be stored on personal computers unless you are currently working on them and should always be password protected. In the event that you have destroyed or deleted documents that are still needed, your contact person of the Research Management Committee can resend documents to you. Hard copies of documents should be disposed of using agreed shredding methods, and if more convenient, you can send material back to your contact person of the Research Management Committee for disposal.

Summary

All documents associated with projects which you come into contact with during your work as a member of the Research Management Committee for the project 'Characterisation, commercialisation, and clinical studies of a longterm antimicrobial urinary catheter' sponsored by the University of Nottingham and funded by the National Institute of Health Research Invention for Innovation award, should be treated as confidential. Do not leave documents where they can be read or obtained by third parties. Do not duplicate the documents and, if stored on your computer, ensure they are password protected. Do inform us if you feel security may have been compromised.

Please destroy any files when you no longer need them, ideally by shredding or permanently deleting. Hard copies can be passed to the contact person for the Research Management Committee for safe disposal if necessary.

……

I agree to adhere to this agreement

Signed …….Date: ……./……../……

Name (print) ……..

If you have any questions regarding any aspects of this agreement, please contact Katie Belfield at Katherine.belfield@nottingham.ac.uk or you may contact the Research Design Service East Midlands PPI Lead, Raksha Pandya-Wood at rpandya@dmu.ac.uk .

Appendix 4: Conflict of interest statement for lay members

Conflict of interest statement

Research Management Committee

'Characterisation, commercialisation, and clinical studies of a longterm antimicrobial urinary catheter'

Please state whether you have any interests (financial, academic, clinical or personal) that might conflict with the project, its objectives or its execution, or with your role on the management committee.

I do / do not have any conflicts of interest to declare (please circle as appropriate).

If you do have a conflict of interest, please give details:

Signature:_____

Name (Please print):_____

Date:_____

Appendix 5: Statement of sponsorship

Research, Enterprise and Graduate Services
University of Nottingham
King's Meadow Campus
Lenton Lane Nottingham
NG7 2NR

Our reference: RGS 16060
IRAS Project ID: 206184

0115 8467906
sponsor@nottingham.ac.uk

Research Ethics Committee
Health Research Authority

Professor Roger Bayston
Professor of Surgical Infection
Department of Academic Orthopaedics, Trauma, an
Sports Medicine
School of Medicine
Queens Medical Centre
Derby Road Nottingham
NG7 2UH

13 July 2016

Dear Chair of the Ethics Committee,

Sponsorship Statement

Re: A novel antimicrobial urinary catheter for long-term catheter users: a study of its safety.

I can confirm that this research proposal has been discussed with the Chief Investigator and agreement to sponsor the research is in place.

An appropriate process of scientific critique has demonstrated that this research proposal is worthwhile and of high scientific quality.*

Any necessary indemnity or insurance arrangements will be in place before this research starts. Arrangements will be in place before the study starts for the research team to access resources and support to deliver the research as proposed.

Arrangements to allocate responsibilities for the management, monitoring and reporting of the research will be in place before the research starts.

The duties of sponsors set out in the NHS Research Governance Framework for Health and Social Care will be undertaken in relation to this research.**

* Not applicable to student research (except doctoral research).
** Not applicable to research outside the scope of the Research Governance Framework.

Yours faithfully

Angela Shone
Head of Research Governance
University of Nottingham

Appendix 6: Favourable opinion from Edgbaston Research Ethics Committee

NHS
Health Research Authority
West Midlands - Edgbaston Research Ethics Committee
The Old Chapel
Royal Standard Place
Nottingham
NG1 6FS

Please note: This is the favourable opinion of the REC only and does not allow you to start your study at NHS sites in England until you receive HRA Approval

12 September 2016

Professor Roger Bayston
Professor of Surgical Infection
University of Nottingham
Department of Academic Orthopaedics, Trauma, and Sports Medicine
Queen's Medical Centre
Nottingham
NG7 2UH

Dear Professor Bayston

Study title:	**A novel antimicrobial urinary catheter for long-term catheter users: a study of its safety**
REC reference:	**16/WM/0353**
Protocol number:	**16060**
IRAS project ID:	**206184**

Thank you for your letter responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to make a request to postpone publication, please contact the REC Manager at NRESCommittee.WestMidlands-Edgbaston@nhs.net.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

The REC favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission must be obtained from each host organisation prior to the start of the study at the site concerned.

NHS
Health Research Authority

Management permission should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).

Guidance on applying for NHS permission for research is available in the Integrated Research Application System, www.hra.nhs.uk or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of management permissions from host organisations

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database within 6 weeks of recruitment of the first participant (for medical device studies, within the timeline determined by the current registration and publication trees).

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to contest the need for registration they should contact Catherine Blewett (catherineblewett@nhs.net), the HRA does not, however, expect exceptions to be made. Guidance on where to register is provided within IRAS.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Non-NHS sites

NHS
Health Research Authority

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Copies of advertisement materials for research participants [Recruitment poster in A4 size]	1.0	13 July 2016
Copies of advertisement materials for research participants [Recruitment poster in A5 size]	1.0	13 July 2016
Covering letter on headed paper [Covering letter to NHS Research Ethics Committee]		13 July 2016
Covering letter on headed paper [Covering Letter to West Midlands Edgbaston REC]	2.0	26 August 2016
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only) [University of Nottingham Clinical Trials Insurance]		21 July 2015
GP/consultant information sheets or letters [Letter to GPs of participants in the study]	1.0	13 July 2016
Instructions for use of medical device [Instructions for Use of Unibal Urinary Catheter provided by the manufacturer]	1.0	13 July 2016
IRAS Application Form [IRAS_Form_27072016]		27 July 2016
Laboratory Manual [Standard Operating Procedure of the collection, transportation and laboratory analysis of trial devices removed from participants at the end of the study period]	1.0	13 July 2016
Laboratory Manual [Standard Operating Procedure of impregnating CE-marked silicone urinary catheters with three antimicrobials. This also includes a processing checklist and recording form to document the modification process]	2.0	26 August 2016
Letter from sponsor [Letter from Sponsor]		13 July 2016
Letters of invitation to participant [Recruitment letter to long-term catheter users from Nottingham CityCare (PIC)]	1.0	13 July 2016
Non-validated questionnaire [Questionnaire to assess feasibility and patient recruitment for a future RCT of the antimicrobial urinary catheter]	1.0	13 July 2016
Other [Letter of Support from Clinimed Ltd who will facilitate the supply of catheters for the study and the packaging and sterilisation of the trial devices]		14 July 2016
Other [Expert Opinion from Professor Guruprasad P Aithal on the use of the antimicrobial catheter (containing rifampicin) in patients with liver disease]		29 January 2016
Other [Telephone Interview Script and Record Form]	1.0	26 August 2016
Participant consent form [Participant consent form v2.0]	2.0	26 August 2016
Participant information sheet (PIS) [Participant information sheet v2.0]	2.0	26 August 2016
Referee's report or other scientific critique report [Outcome letter from the application to the NIHR Invention for Innovation grant call]	1.0	13 July 2016
Research protocol or project proposal [Protocol: Antimicrobial urinary catheter safety study]	2.0	26 August 2016
Summary CV for Chief Investigator (CI) [CV of Professor Roger Bayston]		13 July 2016
Summary CV for student [CV of Miss Katherine Belfield (PhD candidate and research assistant)]	1.0	13 July 2016

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

NHS
Health Research Authority

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website:
http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

HRA Training

We are pleased to welcome researchers and R&D staff at our training days – see details at
http://www.hra.nhs.uk/hra-training/

16/WM/0353	Please quote this number on all correspondence

With the Committee's best wishes for the success of this project.

Yours sincerely

pp Adam Cornett

Mr Paul Hamilton
Chair

Email:NRESCommittee.WestMidlands-Edgbaston@nhs.net

Enclosures: "After ethical review – guidance for researchers" *[SL-AR2]*

Copy to: *Miss Angela Shone*
Miss Joanne Thornhill, Nottingham Health Science Partners

Appendix 7: Health Research Authority Approval

NHS
Health Research Authority

Professor Roger Bayston
Professor of Surgical Infection
University of Nottingham
Department of Academic Orthopaedics, Trauma, and Sports Medicine
Queen's Medical Centre
Nottingham
NG7 2UH

Email: hra.approval@nhs.net

11 October 2016

Dear Professor Bayston,

Letter of HRA Approval

Study title:	**A novel antimicrobial urinary catheter for long-term catheter users: a study of its safety**
IRAS project ID:	**206184**
Protocol number:	**16060**
REC reference:	**16/WM/0353**
Sponsor	**University of Nottingham**

I am pleased to confirm that **HRA Approval** has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications noted in this letter.

Participation of NHS Organisations in England

The sponsor should now provide a copy of this letter to all participating NHS organisations in England.

Appendix B provides important information for sponsors and participating NHS organisations in England for arranging and confirming capacity and capability. **Please read *Appendix B* carefully**, in particular the following sections:

- *Participating NHS organisations in England* – this clarifies the types of participating organisations in the study and whether or not all organisations will be undertaking the same activities
- *Confirmation of capacity and capability* - this confirms whether or not each type of participating NHS organisation in England is expected to give formal confirmation of capacity and capability. Where formal confirmation is not expected, the section also provides details on the time limit given to participating organisations to opt out of the study, or request additional time, before their participation is assumed.
- *Allocation of responsibilities and rights are agreed and documented (4.1 of HRA assessment criteria)* - this provides detail on the form of agreement to be used in the study to confirm capacity and capability, where applicable.

IRAS project ID	206184

Further information on funding, HR processes, and compliance with HRA criteria and standards is also provided.

It is critical that you involve both the research management function (e.g. R&D office) supporting each organisation and the local research team (where there is one) in setting up your study. Contact details and further information about working with the research management function for each organisation can be accessed from www.hra.nhs.uk/hra-approval.

Appendices

The HRA Approval letter contains the following appendices:

- A – List of documents reviewed during HRA assessment
- B – Summary of HRA assessment

After HRA Approval

The document *"After Ethical Review – guidance for sponsors and investigators"*, issued with your REC favourable opinion, gives detailed guidance on reporting expectations for studies, including:

- Registration of research
- Notifying amendments
- Notifying the end of the study

The HRA website also provides guidance on these topics, and is updated in the light of changes in reporting expectations or procedures.

In addition to the guidance in the above, please note the following:

- HRA Approval applies for the duration of your REC favourable opinion, unless otherwise notified in writing by the HRA.
- Substantial amendments should be submitted directly to the Research Ethics Committee, as detailed in the *After Ethical Review* document. Non-substantial amendments should be submitted for review by the HRA using the form provided on the HRA website, and emailed to hra.amendments@nhs.net.
- The HRA will categorise amendments (substantial and non-substantial) and issue confirmation of continued HRA Approval. Further details can be found on the HRA website.

Scope

HRA Approval provides an approval for research involving patients or staff in NHS organisations in England.

If your study involves NHS organisations in other countries in the UK, please contact the relevant national coordinating functions for support and advice. Further information can be found at http://www.hra.nhs.uk/resources/applying-for-reviews/nhs-hsc-rd-review/.

If there are participating non-NHS organisations, local agreement should be obtained in accordance with the procedures of the local participating non-NHS organisation.

IRAS project ID	206184

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please email the HRA at hra.approval@nhs.net. Additionally, one of our staff would be happy to call and discuss your experience of HRA Approval.

HRA Training

We are pleased to welcome researchers and research management staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

Your IRAS project ID is **206184**. Please quote this on all correspondence.

Yours sincerely

Richard Hutson
Assessor

Email: hra.approval@nhs.net

Copy to: *Miss Angela Shone, University of Nottingham (Sponsor contact)*
Miss Joanne Thornhill, Nottingham Health Science Partners (Lead NHS R&D contact)

NIHR CRN Portfolio Applications Team

IRAS project ID	206184

Appendix A - List of Documents

The final document set assessed and approved by HRA Approval is listed below.

Document	*Version*	*Date*
Contract/Study Agreement [Research Agreement between the University of Nottingham and Nottingham University Hospitals NHS Trust]	n/a	20 July 2015
Copies of advertisement materials for research participants [Recruitment poster in A4 size]	1.0	13 July 2016
Copies of advertisement materials for research participants [Recruitment poster in A5 size]	1.0	13 July 2016
Covering letter on headed paper [Covering letter to NHS Research Ethics Committee]		13 July 2016
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only) [UoN Clinical Trials Insurance]		20 July 2016
GP/consultant information sheets or letters [Letter to GPs of participants in the study]	1.0	13 July 2016
Instructions for use of medical device [Instructions for Use of Unibal Urinary Catheter provided by the manufacturer]	1.0	13 July 2016
IRAS Application Form [IRAS_Form_27072016]		27 July 2016
Laboratory Manual [Standard Operating Procedure of the collection, transportation and laboratory analysis of trial devices removed from participants at the end of the study period]	1.0	13 July 2016
Laboratory Manual [Standard Operating Procedure of impregnating CE-marked silicone urinary catheters with three antimicrobials. This also includes a processing checklist and recording form to document the modification process]	2.0	26 August 2016
Letter from sponsor [Letter from Sponsor]		13 July 2016
Letters of invitation to participant [Recruitment letter to long-term catheter users from Nottingham CityCare (PIC)]	1.0	13 July 2016
Non-validated questionnaire [Questionnaire to assess feasibility and patient recruitment for a future RCT of the antimicrobial urinary catheter]	1.0	13 July 2016
Other [Schedule of Events for HRA approvals]	1.0	13 July 2016
Other [Statement of Activities for HRA approvals]	1.0	13 July 2016
Other [Letter of Support from Clinimed Ltd who will facilitate the supply of catheters for the study and the packaging and sterilisation of the trial devices]		14 July 2016
Other [Expert Opinion from Professor Guruprasad P Aithal on the use of the antimicrobial catheter (containing rifampicin) in patients with liver disease]		29 January 2016
Other [Telephone Interview Script and Record Form]	1.0	26 August 2016
Participant consent form [Participant consent form v2.0]	2.0	26 August 2016
Participant information sheet (PIS) [Participant information sheet v2.0]	2.0	26 August 2016
Referee's report or other scientific critique report [Outcome letter from the application to the NIHR Invention for Innovation grant call]	1.0	13 July 2016
Research protocol or project proposal [Protocol: Antimicrobial urinary catheter safety study]	2.0	26 August 2016
Summary CV for Chief Investigator (CI) [CV of Professor Roger Bayston]		13 July 2016
Summary CV for student [CV of Miss Katherine Belfield (PhD candidate and research assistant)]	1.0	13 July 2016

IRAS project ID	206184

Appendix B - Summary of HRA Assessment

This appendix provides assurance to you, the sponsor and the NHS in England that the study, as reviewed for HRA Approval, is compliant with relevant standards. It also provides information and clarification, where appropriate, to participating NHS organisations in England to assist in assessing and arranging capacity and capability.

For information on how the sponsor should be working with participating NHS organisations in England, please refer to the, *participating NHS organisations, capacity and capability* and *Allocation of responsibilities and rights are agreed and documented (4.1 of HRA assessment criteria)* sections in this appendix.

The following person is the sponsor contact for the purpose of addressing participating organisation questions relating to the study:

Angela Shone

angela.shone@nottingham.ac.uk

01158 467906

HRA assessment criteria

Section	HRA Assessment Criteria	Compliant with Standards	Comments
1.1	IRAS application completed correctly	Yes	No comments
2.1	Participant information/consent documents and consent process	Yes	A minor amendment has been made to add the IRAS ID to the Participant Information Sheet and Consent Form.
3.1	Protocol assessment	Yes	No comments
4.1	Allocation of responsibilities and rights are agreed and documented	Yes	A modified model non-commercial agreement will be used between the sponsor and research site.
4.2	Insurance/indemnity arrangements assessed	Yes	Where applicable, independent contractors (e.g. General Practitioners) should ensure that the professional indemnity provided by their medical defence organisation covers the activities expected of them for this

IRAS project ID	206184

Section	HRA Assessment Criteria	Compliant with Standards	Comments
			research study
4.3	Financial arrangements assessed	Yes	Study is funded by NIHR Research Invention for Innovation (i4i) award. Funding will be provided to Nottingham University Hospitals NHS FT as detailed in the agreement.
5.1	Compliance with the Data Protection Act and data security issues assessed	Yes	No comments
5.2	CTIMPS – Arrangements for compliance with the Clinical Trials Regulations assessed	Not Applicable	No comments
5.3	Compliance with any applicable laws or regulations	Yes	No comments
6.1	NHS Research Ethics Committee favourable opinion received for applicable studies	Yes	No comments
6.2	CTIMPS – Clinical Trials Authorisation (CTA) letter received	Not Applicable	No comments
6.3	Devices – MHRA notice of no objection received	Not Applicable	No comments
6.4	Other regulatory approvals and authorisations received	Not Applicable	No comments

IRAS project ID	206184

Participating NHS Organisations in England

This provides detail on the types of participating NHS organisations in the study and a statement as to whether the activities at all organisations are the same or different.
There will be one site-type for this study. Nottingham University Hospitals NHS Trust will identify and recruit participants and complete research procedures as described in the protocol. The Chief Investigator or sponsor should share relevant study documents with participating NHS organisations in England in order to put arrangements in place to deliver the study. The documents should be sent to both the local study team, where applicable, and the office providing the research management function at the participating organisation. For NIHR CRN Portfolio studies, the Local LCRN contact should also be copied into this correspondence. For further guidance on working with participating NHS organisations please see the HRA website. If chief investigators, sponsors or principal investigators are asked to complete site level forms for participating NHS organisations in England which are not provided in IRAS or on the HRA website, the chief investigator, sponsor or principal investigator should notify the HRA immediately at hra.approval@nhs.net. The HRA will work with these organisations to achieve a consistent approach to information provision.

Confirmation of Capacity and Capability

This describes whether formal confirmation of capacity and capability is expected from participating NHS organisations in England.
Participating NHS organisations in England **will be expected to formally confirm their capacity and capability to host this research**. • Following issue of this letter, participating NHS organisations in England may now confirm to the sponsor their capacity and capability to host this research, when ready to do so. How capacity and capacity will be confirmed is detailed in the *Allocation of responsibilities and rights are agreed and documented (4.1 of HRA assessment criteria)* section of this appendix. • The Assessing, Arranging, and Confirming document on the HRA website provides further information for the sponsor and NHS organisations on assessing, arranging and confirming capacity and capability.

Principal Investigator Suitability

This confirms whether the sponsor position on whether a PI, LC or neither should be in place is correct for each type of participating NHS organisation in England and the minimum expectations for education, training and experience that PIs should meet (where applicable).
A Principal Investigator will be required at the research site. The Research Nurse will be trained in the protocol and study-related procedures. GCP training is not a generic training expectation, in line with the HRA statement on training expectations.

IRAS project ID	206184

HR Good Practice Resource Pack Expectations

This confirms the HR Good Practice Resource Pack expectations for the study and the pre-engagement checks that should and should not be undertaken
Some, but not all, members of the research team have appropriate NHS contractual arrangements in place for the study. Non-NHS staff will require a Letter of Access to conduct research procedures for this study within NHS facilities.

Other Information to Aid Study Set-up

This details any other information that may be helpful to sponsors and participating NHS organisations in England to aid study set-up.
The applicant has indicated that they intend to apply for inclusion on the NIHR CRN Portfolio.

Appendix 8: Approval for Nottingham CityCare to act as a participant identification centre

positive

Nottinghamshire Healthcare NHS
NHS Foundation Trust

Research and Development
Nottinghamshire Healthcare NHS Foundation Trust
Duncan Macmillan House
Porchester Road
Mapperley
Nottingham
NG3 6AA

E-mail: Shirley.mitchell@nottshc.nhs.uk

Date of NHS permission for research: 14 October 2016

Professor Roger Bayston
University of Nottingham
Dept. of Academic Orthopaedics, Trauma and Sports Medicine
Queen's Medical Centre
Nottingham
NG7 2UH

Dear Prof. Bayston

Study title: A novel antimicrobial urinary catheter for long-term catheter users: a study of it's safety
IRAS/REC ID: 206184/16/WM/0353
Sponsor: University of Nottingham

Thank you for submitting your project to the Nottinghamshire Healthcare NHS Foundation Trust's Research Support Services. The project has now been given NHS permission for PIC activity by:

Tracy Tyrrell, R&D Lead, Nottingham CityCare Partnership

NHS permission for the above research has been granted on the basis described in the application form, protocol and supporting documentation. The documents reviewed were:

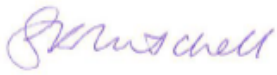
Document	Version	Date
Letters of invitation to participant (Recruitment letter to long-term catheter uses from Nottingham CityCare (PIC)	1.0	13/07/16
Participant Information Sheet	2.0	26/08/16
Participant Consent Form	2.0	26/08/16
Research Protocol	2.0	26/08/16

Permission is granted on the understanding that the study is conducted in accordance with the Research Governance Framework, ICH GCP [ONLY if applicable], and NHS Trust policies and procedures available http://www.nottinghamshirehealthcare.nhs.uk/contact-us/freedom-of-information/policies-and-procedures/

The research sponsor or the Chief Investigator, or the local Principal Investigator at a research site, may take appropriate urgent safety measures in order to protect research participants against any immediate hazard to their health or safety. The R&D office should be notified that such measures have been taken. The notification should also include the reasons why the measures were taken and the plan for further action. The R&D Office should be notified within the same time frame of notifying the REC and any other regulatory bodies. All amendments (including changes to the local research team) need to be submitted in accordance with guidance in IRAS.

Please note that the NHS organisation is required to monitor research to ensure compliance with the Research Governance Framework and other legal and regulatory requirements. This is achieved by random audit of research.

Yours Sincerely



Shirley Mitchell
Head of Research and Innovation

CC:

Sponsor

NCCP R&D Lead

Appendix 9: Confirmation of capacity and capability from NUHT

From: "Michalak Stefan (Research & Innovation)" <Stefan.Michalak@nuh.nhs.uk>
Date: Friday, 2 December 2016 14:06
To: Belfield Katherine <mszklb@exmail.nottingham.ac.uk>, "Parkinson Richard (Urology)" <Richard.Parkinson@nuh.nhs.uk>, "Betts Helen (Urology)" <Helen.Betts@nuh.nhs.uk>, Roger Bayston <mszrb2@exmail.nottingham.ac.uk>
Cc: 'Bb-Sponsor' <sponsor@nottingham.ac.uk>
Subject: RE: IRAS 206184 Confirmation of Capacity and Capability at Nottingham University Hospitals NHS Trust

Dear study team,

RE: IRAS 206184 Confirmation of Capacity and Capability at Nottingham University Hospitals NHS Trust

R&I Ref: 16UR005 (Grant Ref 14UR001)

Full Study Title: A novel antimicrobial urinary catheter for long-term catheter users: a study of its safety

Sponsoring Organisation: University of Nottingham

This email confirms that **Nottingham University Hospitals NHS Trust** has the capacity and capability to deliver the above referenced study. Please find attached our signed agreement as confirmation.

We agree to start this study on a date to be agreed when you as sponsor give the green light to begin.

Please be aware this confirmation of capacity is provided on the understanding and provision that you will follow the conditions set out in the attached document (NUH R&I of Confirmation of Capacity and Capability Conditions, v1).

The following documents were reviewed:

Document	Dated	Version
Protocol	20/10/16	3.0
Consent Form	26/08/16	2.0
CityCare Recruitment Letter.	13/07/16	1.0
GP Letter	13/07/16	1.0
PIS	28/08/16	2.0
QUESTIONNAIRE to assess feasibility of a future RCT	13/07/16	1.0

Recruitment Poster A4	13/07/16	1.0
Recruitment Poster A5	13/07/16	1.0
SOP of antimicrobial impregnation of silicone urinary catheters	26/08/16	2.0
SOP of collection, transport, and analysis of trial devices when removed from participants enrolled in the Antimicrobial Urinary Catheter Safety Study	13/07/16	1.0
Telephone Interview Script and Record Form	26/08/16	1.0
Instructions for Use of Unibal Catheter from Yushin Medical Co, Ltd.	13/07/16	1.0

If you wish to discuss further, please do not hesitate to contact me.

Kind regards
Nottingham University Hospitals NHS Trust

Dr Maria Koufali
Deputy Director Research and Innovation

Please note that the R&I department maintains a database containing study related information, and personal information about individual investigators e.g. name, address, contact details etc. This information will be managed according to the principles established in the Data Protection Act.

Kind regards,

Stefan

Stefan Michalak
Research Project Manager
Research & Innovation | Nottingham Health Science Partners | Nottingham University Hospitals NHS Trust | C Floor, South Block | Queens Medical Centre Campus | Derby Road | Nottingham | NG7 2UH

Phone: 0115 924 9924 x70259
R&D Admin line: 0115 970 9049
Stefan.Michalak@nuh.nhs.uk
www.nuhrise.org

research & innovation

Appendix 10: Authorisation of use of trial catheter

Nottingham University Hospitals NHS
NHS Trust

DEVICE R&D FILE NOTE

Ref No: 16UR005 (Grant reference 14UR001)

Date	Comments
	R&D ref: 16UR005 (Grant reference 14UR001) **Study Title:** A novel antimicrobial urinary catheter for long-term catheter users: a study of its safety **Principal Investigator:** Mr Richard Parkinson **Sponsor:** University of Nottingham As NUH R&D Director/ Deputy Director I am satisfied that the following research governance checks have been completed for the above study: -Participant Information and Consent documents explaining the use of a medical device and any risks associated with it. -Emergency arrangements are in place -PI/research team are suitable and fully trained and supported for the use of the medical device -appropriate ad adequate insurance and indemnity are in place -risks to the PI and research team are acceptable -the device is CE marked for it use According to article 4.3 of the Managing Loan Medical Devices Procedure, v.1.0, I therefore authorise the use of the medical device for the purposes of research study listed above only. Name: MAZIA KOUFALI - DEPT. DIRECTOR OF R+I Signature: Date: 06 DEC 2016

MESU R&D Director sign off v1 14/04/11

Appendix 11: Trial consent form

The University of Nottingham
UNITED KINGDOM · CHINA · MALAYSIA

Nottingham University Hospitals NHS
NHS Trust

CONSENT FORM
Final version 3.5: 11/09/17

Title of Study: A novel antimicrobial urinary catheter for long-term catheter users: a safety study

REC ref: 206184

Names of Researchers:
Professor Roger Bayston, Mr. Richard Parkinson, Miss Katherine Belfield, and Research Nurse

Name of Participant:

Please initial box

1. I confirm that I have read and understand the information sheet version number 3.5 dated 11 September, 2017 for the above study and have had the opportunity to ask questions. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, and without my medical care or legal rights being affected. I understand that should I withdraw then the information collected so far cannot be erased and that this information may still be used in the project analysis. ☐

3. I understand that relevant sections of my medical notes and data collected in the study may be looked at by authorised individuals from the University of Nottingham, the research group and regulatory authorities where it is relevant to my taking part in this study. I give permission for these individuals to have access to these records and to collect, store, analyse and publish information obtained from my participation in this study. I understand that my personal details will be kept confidential. ☐

4. I understand and agree that I will be contacted on the telephone number I provide for telephone interviews throughout the length of my participation in the study. The telephone interview schedule can be found in the information sheet. ☐

5. I agree to complete the questionnaire entitled ‘A new antimicrobial urinary catheter for long-term use: a feasibility study for planning a future randomised controlled trial.’ I understand that this questionnaire asks about my interest in a randomised controlled trial, but by completing the questionnaire I will not be enrolled into such a study. ☐

6. I understand and agree that at the end of the study period, the antimicrobial urinary catheter will be collected, rather than disposed of, for laboratory analysis. ☐

7. I agree to my GP being informed of my taking part in this study. ☐

8. I agree to take part in the above study. ☐

9. **(Optional)** Consent for storage of data and use in possible future research

Please initial

I agree that the information gathered about me can be stored by the University of Nottingham at the Biomaterials-Related Infection Group, Department of Academic Orthopaedics, Trauma, and Sports Medicine for possible use in future studies. I understand that some of these studies may be carried out by researchers other than the current team who ran the first study, including researchers working for commercial companies. Any data used will be anonymised, and I will not be identified in anyway.

☐ Yes ☐ No

Please initial

10. **(Optional)** Consent for storage of personal details for possible future contact

I agree that the personal details I have given about me can be stored securely by the University of Nottingham in a locked cupboard in the Department of Academic, Orthopaedics, Trauma, and Sports Medicine for possible future contact about future clinical trials. Your personal details will be stored according to the principles of confidentiality explained in the information sheet.

☐ Yes ☐ No

_____	_____	_____
Name of Participant	Date	Signature
_____	_____	_____
Name of Person taking consent	Date	Signature

3 copies: 1 for participant, 1 for the project notes and 1 for the medical notes

Appendix 12: Trial telephone interview script

The University of Nottingham
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Nottingham University Hospitals NHS
NHS Trust

Telephone Interview Script and Record Form
To be kept in case report form
Version 1.0 26/08/2016
Antimicrobial urinary catheter safety study

Telephone Interview ____: _____ days after antimicrobial catheter insertion

Date of Assessment: __ __ / __ __ / __ __ __ __
(dd/mm/yyyy)

1. Time of call: Did participant answer?	Yes ☐ No ☐ If no, call a second time
2. Time of call: Did participant answer?	Yes ☐ No ☐ If no, call a third time
3. Time of call: Did participant answer?	Yes ☐ No ☐

Skip the following section if contact was made within 24 hours and three phone calls or less. If unable to reach the participant after three calls, get in contact with emergency contact in case of medical problem

1. Able to reach emergency contact?		Yes ☐ No ☐
	1a. If **yes**, was there a medical problem that prevented the participant from answering?	Yes ☐ No ☐
	1b. If **no**, was contact made to the emergency contact or participant in 24 hours since first call?	Yes ☐ No ☐
2. If 'yes' in response to question 1a please details the reason here and consider filling out the adverse events form if necessary		

Question Script	Participant's answers (please tick the appropriate box or add free text)
1. Have you had any pain from the catheter?	☐ More than I would normally expect ☐ About the same as I would normally expect ☐ Less than I would normally expect
2. Where is the pain?	☐ No pain ☐ Water pipe/urethra ☐ Penis ☐ Bladder/abdominal ☐ Other: _____
3. Any rashes?	Yes ☐ No ☐
4. Has the catheter bypassed (leaked around the outside)	☐ Yes, a lot ☐ Yes, but not much ☐ No
5. Has the catheter blocked?	Yes ☐ No ☐
6. Have you had a urinary infection?	☐ Yes, needed admission to hospital ☐ Yes, got antibiotics ☐ Yes, but it has settled on its own ☐ No
7. Does the catheter need to be changed?	Yes ☐ No ☐
Continued on next page	

8. Any other problems with the catheter? (comment)	
9. How would you rate this catheter compared to your usual catheter?	☐ Much better ☐ A bit better ☐ No different ☐ A bit worse ☐ Much worse
10. Reason (if any) for answer to question 9:	

Please tick here if an appointment was made during this interview for catheter removal.

Yes ☐ No ☐

If **yes**, when is the appointment? __ __ / __ __ / __ __ __ __
(dd/mm/yyyy)

If **yes**, was the reason for removal related to an adverse event? Please consider filling in the adverse events form, if **yes**.

Yes ☐ No ☐

Is this the last telephone interview?

Yes ☐ No ☐

Signature of person completing page data: _____
Printed Name: _____

CI or Clinician Signature: _____

Appendix 13: Participant information sheet

The University of Nottingham
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Nottingham University Hospitals NHS
NHS Trust

Participant Information Sheet
Final version 3.5: 11/09/2017

IRAS Project ID: 206184

Title of Study: A novel antimicrobial urinary catheter for long-term catheter users: a study of its safety

Names of Researchers: Professor Roger Bayston, Mr. Richard Parkinson, Miss Katherine Belfield, Research Nurse

We would like to invite you to take part in our research study. Before you decide we would like you to understand why the research is being done and what it would involve for you. One of our team will go through the information sheet with you and answer any questions you have. Please talk to others about the study if you wish. Ask us if there is anything that is not clear.

Background

Long-term urinary catheter users are at risk of catheter-associated urinary tract infections (CAUTI). This can mean courses of antibiotics, early removal of the catheter, blockage of the catheter, and other serious complications for patients. We have added to the normal urinary catheters three antimicrobial drugs (rifampicin, sparfloxacin, and triclosan) which prevent microorganisms, such as bacteria, from attaching to the catheter to prevent infection. Laboratory studies show that this antimicrobial catheter can prevent infection by the main bacteria that cause CAUTI for 7-12 weeks. In order to bring this catheter to patients, we need to understand if there are any side effects associated with the antimicrobial urinary catheter. The drugs in the catheter have been in use for many years and we do not expect any side effects, but we need your opinion.

What is the purpose of the study?

The purpose of the study is to determine if the addition of three antimicrobials to a silicone urinary catheter produces any side effects when used in patients. This study will also form part of a PhD academic qualification.

Why have I been invited?

You are being invited to take part because you have had a urinary catheter in place for over 28 days and will require another urinary catheter for 28 days or longer. We are inviting 60 participants like you to take part.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. This would not affect your legal rights or your treatment.

What will happen to me if I take part?

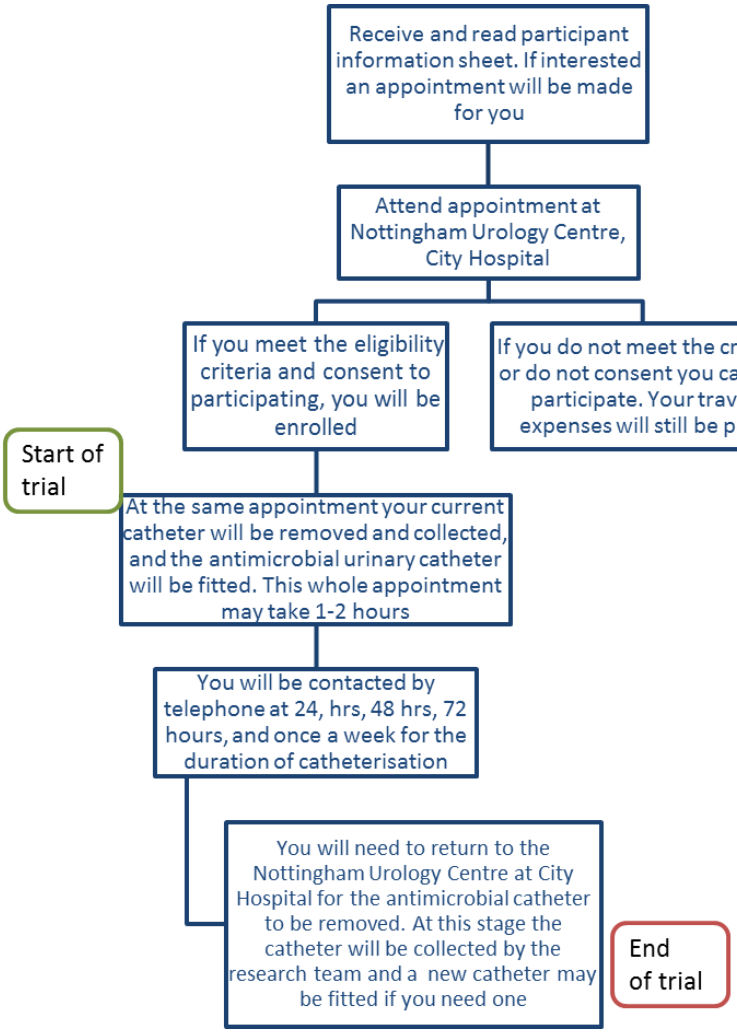
This study does not involve taking any new medicines or drugs. The authorised members of the research team will have to look at your medical notes to ensure you are eligible to be part of the study. If so, at your next catheter insertion date, the antimicrobial urinary catheter will be fitted by the clinical research team at the Urology Centre at City Hospital, Nottingham University Hospitals Trust. If you currently are fitted with a urinary catheter, it will be removed and collected for laboratory analysis, and the antimicrobial urinary catheter will be fitted in its place. The new catheter will remain in place for your normal catheterisation schedule length (ie if you normally have your catheter changed every 6 weeks, the antimicrobial catheter will remain in place for 6 weeks).

You will be asked to be aware of any sensations or side effects of the antimicrobial urinary catheter unusual to urinary catheterisation. The research nurse will speak to you by telephone at 24 hours, 48 hours, 72 hours, and then once a week for the time the antimicrobial catheter remains in place. Each telephone call may take up to 15 minutes. We will call up to three times on the date to get in touch with you. If the antimicrobial urinary catheter needs to be removed earlier than the expected trial end date, the research nurse will arrange an appointment with you for its removal.

At the first appointment you will also be given a short questionnaire that may take up to 20 minutes to complete. You may choose to complete this at your appointment or complete it at home and return to the research team via a pre-addressed and stamped envelope. The questionnaire will ask questions regarding your current catheter care and also interest in a future randomised controlled trial of the antimicrobial urinary catheter. You will NOT be enrolled into such a trial by answering this questionnaire. The purpose of the questionnaire is to help us determine how many participants in the Nottingham area are eligible and interested in taking part in such a study to plan the future study.

You will need to return to the Urology Centre at City Hospital to have the antimicrobial catheter removed. At this point the antimicrobial catheter will be collected for laboratory analysis, and another normal urinary catheter will, if needed, be fitted.

Please see the following page for a flow diagram of the study:



Expenses and payments

Travel expenses and replacement carer/child-care costs will be offered for any visits incurred as a result of taking part. A taxi can be arranged and paid for in advance if that is convenient. Receipts/invoice/tickets will be required for care arrangements and other travels costs such as car parking fees. If travelling in your own vehicles, please take note of the mileage and travel will be reimbursed at 45p per mile.

What are the possible disadvantages and risks of taking part?

Risks

Several adverse events of urinary catheterisation are well documented, such as risk of CAUTI, leakage of urine around the catheter, blockage of the catheter, or injury to the urethra (if urethral catheterisation), bladder or rectum. These are risks of all types of urinary catheterisation. In addition, as the antimicrobial urinary catheter will contain three antimicrobials, which are rifampicin, triclosan, and sparfloxacin, there is a small risk of a local reaction (hypersensitivity) to this antimicrobial catheter. You will be assessed for allergy to the antimicrobials, before you are enrolled in the trial. As with all drugs, it is possible that allergy may result from the antimicrobials in the catheter, and you should inform us of any previous allergic reactions to any of these three drugs. There may also be side-effects associated with

their interaction with other medications you are taking. This same technology has previously been shown to be effective in catheters used to treat hydrocephalus (water on the brain) and catheters used for dialysis. The antimicrobials are not new and have been used in clinical practice and in many clinical applications.

Disadvantages
Taking part will also mean taking time out of your normal activities to attend two appointments at the Nottingham Urology Centre, City Hospital, and also speaking on the phone for approximately 15 minutes, once a week. Travel expenses will be paid and we will telephone you so as to not incur extra costs to your phone bill.

What are the possible benefits of taking part?

We cannot promise that the study will help you but the information we get from this study may help the process of regulatory approval for this antimicrobial urinary catheter for use by patients in the community and hospitals, so it will hopefully help patients in the near future. It will also help us calculate how many participants we will need for a future study of the effectiveness (how well it works) of the antimicrobial urinary catheter to prevent infection. The catheter is designed to prevent attachment of bacteria that cause infection, so you may see no signs or symptoms of an infection (CAUTI) during your catheterisation. The catheter also prevents infection by some bacteria that may cause mineral encrustation, which can cause catheter blockage, so you may see reduced catheter blockage. However, this study is not designed to test the effectiveness of the antimicrobial catheter, only to assess the likelihood of side-effects or discomfort.

What happens when the research study stops?

Your involvement in the study will end once you have the antimicrobial urinary catheter removed. The use of another antimicrobial urinary catheter will not be available after the study finishes if you require additional catheterisation. The antimicrobial urinary catheter is currently only available for use as part of this study and cannot be used outside of the trial. If you do require re-catheterisation, at the appointment at which the catheter is removed, a new standard catheter will be fitted for you.

What if there is a problem?

If you have a concern about any aspect of this study at any time, you should ask to speak to the researchers who will do their best to answer your questions. The researchers contact details are given at the end of this information sheet. If you remain unhappy and wish to complain formally, you can do this by contacting the Patient Advice and Liaison Service (PALS):

Visit: Queen's Medical Centre, Derby Road, Nottingham NG7 2UH
B Floor, Main Corridor behind the Main Reception desk at the Derby Road Entrance on B Floor
Phone: 0800 183 0204 or 0115 924 9924 for mobile phone users (Please note that 0800 numbers are now free to call from mobile telephones)
Email: pals@nuh.nhs.uk

Please also be aware that trial participants are covered by appropriate insurance and indemnity provisions. However, there are no special compensation arrangements in place. You may have recourse through NHS complaints procedures.

Will my taking part in the study be kept confidential?

We will follow ethical and legal practice and all information about you will be handled in confidence.

If you join the study, some parts of your medical records and the data collected for the study will be looked at by authorised persons from the University of Nottingham who are organising the research. They may also be looked at by authorised people to check that the study is being carried out correctly. All will have a duty of confidentiality to you as a research participant and we will do our best to meet this duty.

All information which is collected about you during the course of the research will be kept **strictly confidential**, stored in a secure and locked office, and on a password protected database. Any information about you which leaves the hospital will have your name and address removed (anonymised) and a unique code will be used so that you cannot be recognised from it.

Your personal data (address, telephone number) will be kept for 4 years after the end of the study so that we are able to contact you about the findings of this study and possible follow-up studies (unless you advise us that you do not wish to be contacted). We would like to keep your personal details for this length of time as securing funding and planning the follow-up studies may be a lengthy process. All research data will be kept securely for 7 years. After this time your data will be disposed of securely. During this time all precautions will be taken by all those involved to maintain your confidentiality, only members of the research team will have access to your personal data.

Although what you say in the interview is confidential, should you disclose anything to us which we feel puts you or anyone else at serious risk of harm, we may feel it necessary to report this to the appropriate persons.

What will happen if I don't want to carry on with the study?

Your participation is voluntary and you are free to withdraw at any time, without giving any reason, and without your legal rights or treatment being affected. If you withdraw then the information collected so far cannot be erased and this information may still be used in the project analysis.

Involvement of the General Practitioner/Family doctor (GP)

We will need to notify your GP of your participation in this research study. This ensures that if you visit your GP during the trial they are aware of any side-effects that may be attributable to the antimicrobial urinary catheter.

What will happen to the results of the research study?

The results will be disseminated, that is communicating the findings, to interested patient groups, published in relevant journals, and will be submitted as part of a PhD thesis. You will not be identified in any report or publication. If you provide us your contact details, we can send you a summary of the trial results and any publications when they are publicly available. The full results will likely be available in May 2018.

Who is organising and funding the research?

This research is being organised by the University of Nottingham in collaboration with Nottingham University Hospitals Trust and is being funded by the National Institute of Health Research Invention for Innovation award programme.

Who has reviewed the study?

All research in the NHS is looked at by independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by West Midlands-Edgbaston Research Ethics Committee.

Further information and contact details

Miss Katie Belfield
Research Assistant
Biomaterials-Related Infection Group, University of Nottingham
Tel: 0115 8231113
Email: Katherine.belfield@nottingham.ac.uk

Mr. Richard Parkinson
Consultant Urologist
Nottingham University Hospitals Trust
Email: Richard.parkinson@nuh.nhs.uk

Or the Chief Investigator:

Professor Roger Bayston
Biomaterials-Related Infection Group
Department of Academic Orthopaedics, Trauma, and Sports Medicine
QMC Campus, Derby Road, Nottingham NG7 2UH
Phone: 0115 8231115
Email: roger.bayston@nottingham.ac.uk

Appendix 14: Nottingham CityCare letter of interest

Dear Service User,

We are contacting you because we are currently supplying you with bladder catheters. We are working closely with Nottingham University Hospitals NHS Trust and the University of Nottingham, who doing research into how to reduce the risk of urine infections for people with catheters. For example, there is a study being planned to try a new catheter which could reduce the risk of infections.

We would like to be able to pass on your contact details to one of the doctors at Nottingham University Hospitals NHS Trust working with the University of Nottingham on this project. This would allow them to send you some more details about the project and what it involves. There is absolutely no obligation to take part in any study and we will continue to supply your normal catheters as usual. Your medical records will not be shared with anyone.

Please could you reply using the slip below and the pre-paid envelope to let us know if you are happy for us to share you details with the trial research team. If we do not hear from you we will not be able to pass on your information to the research team.

Best wishes,
Nottingham CityCare Team

- -

Name:
_____

Address:
_____

[ ] I am happy to have my contact details shared with the University of Nottingham research team. These details will not be shared with anyone else.

[ ] I am not happy to have my contact details shared.

Appendix 15: Feasibility questionnaire

The University of Nottingham
UNITED KINGDOM · CHINA · MALAYSIA

Nottingham University Hospitals NHS
NHS Trust

A new antimicrobial urinary catheter for long-term use: a feasibility study for planning a future randomised controlled trial

Questionnaire to understand recruitment rates at Nottingham University Hospitals Trust

Chief Investigator: Professor Roger Bayston
Sponsor Reference: 16060
REC Reference: 16/WM/0353
Questionnaire version number: 1.0, 13/7/2016

For assistance or any queries, please contact the research team:

Phone: 0115 8231113

Email: Katherine.belfield@nottingham.ac.uk

For office use only:

Trial Identity Code Number:

Date returned:

Instructions
Please complete this questionnaire enquiring about your interest in and understanding of a clinical trial of antimicrobial urinary catheter. Please note that you will not be enrolled into such a trial by filling out this questionnaire. The purpose of the questionnaire is to understand how many long-term urinary catheter users could be recruited from Nottingham University Hospitals Trust for a large clinical trial.

Background Information Section		
How old are you today? _____ years		
Are you:	☐ Male	☐ Female

In the past year have you had a urinary catheter in place for 28 days or longer?

☐ Yes ☐ No

Are you fitted with a urinary catheter today?

☐ Yes ☐ No

Which type of catheter best describes the urinary catheter you are most often fitted with?

☐ Urethral ☐ Suprapubic

Where do you usually have your catheter changed?

☐ Hospital

☐ GP's/Family Practitioner's surgery

☐ Your own home

☐ Other

Please specify if 'Other': _____

Issues with Your Urinary Catheter

Please answer every question by ticking the appropriate box, and tick only one box per question. The following questions relate to your urinary catheter use in the past year.

1. In the past year, have you experienced a urinary tract infection when a catheter has been in place?

☐ Yes ☐ No

If NO, please go to question number **2**.

1a. If YES, have you had more than one urinary tract infection when the catheter has been in place?

☐ Yes ☐ No

1b. If YES, have you received antibiotics for a urinary tract infection in the past year?

☐ Yes ☐ No

1c. If YES, were you hospitalised as a result of a urinary tract infection when the catheter was in place?

☐ Yes ☐ No

2. In the past year, have you sought medical attention for your catheter being blocked?

☐ Yes ☐ No

If NO, please go to question number **3**.

2a. If YES, has blockage of your catheter resulted in it being removed earlier than expected?

☐ Yes ☐ No

3. In the past year has urine leaked around the outside of your urinary catheter?

☐ Yes, often ☐ Yes, but not often ☐ No

4. In the past year have you been told you have bladder stones?

☐ Yes ☐ No

5. Do you have pain when catheterised?

☐ Yes, often ☐ Yes, but not often ☐ No

Situation Questions

In this section, the questions will ask you about your responses to the following situation:

Situation: A member of your usual care team tells you about a research study that you could agree to take part in. The research study wants to find out if a new catheter is better than your usual one. It is a new antimicrobial urinary catheter which prevents many bacteria that cause bacteria from attaching to the catheter. It is being used in a randomised controlled trial, which means you may receive the new antimicrobial urinary catheter or you may receive a catheter without antimicrobials. The purpose of the trial is to determine if the urinary catheter with antimicrobials can reduce the rate of catheter-associated urinary tract infections compared to standard urinary catheters.

6. After hearing this information from your usual care team, would you like to receive the participant information sheet to learn more about the trial?

☐ Yes ☐ No

If YES, please go to question **7** If NO, please go to question **8**

Please leave these questions blank if you responded NO to the question **6**

7a. If YES, which reason(s) best describes why you might be interested in learning more about the trial? Please tick all that apply

☐ I believe this trial is relevant to me

☐ I have been looking for a way to get involved with research

☐ I think this research could benefit future patients

☐ Catheter-associated urinary tract infections are a problem for me

☐ I am unhappy with my current catheter care

☐ Other

Please specify if 'Other': _____

_____

7b. If YES, do you understand that the nature of randomisation means that you may not be allocated to the group that receives the antimicrobial urinary catheter?

☐ Yes ☐ No

Please leave this question blank if you responded YES to question **6**

8. If NO, which reason(s) best describes why you are not interested in learning more about the trial? Please tick all that apply.

☐ I do not think this trial is relevant to me

☐ I do not have time for commitment to a trial

☐ Catheter-associated urinary tract infections are not a problem for me

☐ I am happy with my current catheter care and do not want to change it

☐ I would not be happy being randomised to the 'control' catheter group (ie the one not containing antimicrobials, and only receiving my normal catheter)

☐ Other

Please specify if 'Other':

_____

Optional:

Please provide your name and address (or email) **only** if you wish to be contacted about future studies:

Name: _____

Address: _____

_____

Email: _____

Thank you very much for completing this questionnaire.

If you have any queries, please contact:

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Phone: 0115 8231113

Appendix 16: SOP: Manufacture of trial catheters

SOP of antimicrobial impregnation of silicone urinary catheters
Version 2.0 Date: 26/08/2016

Biomaterials-Related Infection Group
Division of Academic Orthopaedics, Trauma, and Sports Medicine
University of Nottingham
Laboratory Protocol

Title
Impregnation of silicone urinary catheters with antimicrobial agents

Summary
The procedure described below is to be used when impregnating silicone urinary catheters with antimicrobial agents. A record of the impregnation process will be created for traceability purposes.

Safety
Lab coat, gloves, and safety glasses must be worn throughout the procedure. The procedure must be carried out in the extractor/fume hood.

Equipment
- Fume hood
- Forceps
- Laboratory glassware (beakers, conical flasks, measuring cylinders)
- Bench scale
- Timer
- Impregnation container
- Conical flask (volume 2.5L)

Materials and Reagents
- Chloroform
- Ethanol
- Rifampicin (R3501) CAS: 13292-46-1
- Sparfloxacin (56968) CAS: 110871-86-8
- Triclosan (Irgacare MP) CAS: 3380-34-5
- 205 all-silicone long-term urinary catheters in five lumen sizes and two lengths:
    - 12 CH (5.0 ml balloon)
        - Female length – 30 catheters
        - Standard length – 74 catheters
    - 14 CH –
        - Female length – 24 catheters
        - Standard length – 34 catheters
    - 16 CH
        - Female length – 10 catheters
        - Standard length – 14 catheters
    - 18 CH – standard length -10 catheters
    - 20 CH – standard length - 9 catheters

Procedure (refer to antimicrobial processing record check form 1.0)

1. Measure out 2.5 litres of chloroform into a conical flask
2. Weigh out 1 x 25 g of sparfloxacin (1.0% w/v)
3. Weigh out 1 x 25 g of triclosan (1.0% w/v)
4. Weigh out 1 x 5 g of rifampicin (0.2% w/v).
5. Add sparfloxacin all in one go to the chloroform and swirl flask (dissolves rapidly).
6. Next add triclosan all in one go and swirl (dissolves rapidly).
7. Finally add rifampicin all in one go and swirl (dissolves rapidly).
8. Now pour 2.5 litres of the antimicrobial solution into the impregnation container.
9. Now place the catheters into the solution tank making sure the catheters are fully in contact with the antimicrobial solution.
10. Allow the catheters to soak in the solution for 60 ± 5 minutes agitating the solution for 30 seconds each 15 ± 5 minute interval.
11. After soaking, remove catheters, drain, segregate, and allow drying for a minimum of 12 hours under constant air flow (fume hood).
12. After drying, briefly rinse the catheter samples with ethanol on the exterior and interior of the lumens (5 seconds) by submerging the catheters vertically in a cylinder of ethanol for 5 seconds, to remove the dried antimicrobial chemical spots, and then shake the excess ethanol off and leave to dry in the fume hood.
13. One out of 20 catheters will be removed for batch analysis. Drug content will be analysed by high – performance liquid chromatography and verification of removal of chloroform will be verified by gas chromatography.
14. Label catheters with solution number and send to Flexible Medical Packaging, Ltd for sterilisation and packaging.

Antimicrobial processing record check list (form 1.0)

Date Time (Hr/min)	To impregnate antimicrobials into 100 urinary catheters under documented conditions for further testing Solution lot number ☐	Performed √
	Measure out 2.5 litres of chloroform into a conical flask	☐
	Weigh out 1 x 25 g of sparfloxacin (1.0% w/v) ☐ ☐	☐
	Weigh out 1 x 25 g of triclosan (1.0% w/v) ☐ ☐	☐
	Weigh out 1 x 5 g of rifampicin (0.2% w/v). ☐ ☐	☐
	Add sparfloxacin all in one go to the chloroform and swirl flask (dissolves rapidly)	☐
	Next add triclosan all in one go and stir (dissolves rapidly).	☐
	Finally add rifampicin all in one go and stir (dissolves rapidly).	☐
	Now pour 2.5 litres of antimicrobial solutions into the impregnation container.	☐
	Now place the catheters into the solution tank making sure the catheters are fully in contact with the antimicrobial.	☐
	Allow the catheters to soak in the solution for 60 ± 5 minutes agitating the solution for 30 seconds each 15 ± 5 minute interval.	☐
	After soaking, remove catheters, drain, segregate, and allow drying for a minimum of 12 hours under constant air flow (fume hood).	☐
	After drying, briefly rinse the catheter samples with ethanol on the exterior and interior of the lumens (5 seconds) by submerging the catheters vertically in a cylinder of ethanol for 5 seconds, to remove the dried antimicrobial chemical spots, and then shake the excess ethanol off and leave to dry in the fume hood.	☐
	Package and label with the assigned Solution Lot number.	☐
	Fill out and complete processing section of the antimicrobial record form 2.0.	☐

Antimicrobial processing record form 2.0

Solution mixing section				
Solution Lot number:			**Room Temp**	**ºC**
Date	**Start time**		**Stop time**	
Material	**Manufacturer**	**Part number**	**Lot Number**	**Weight/ Volume**
Chloroform				
Rifampicin				
Sparfloxacin				
Triclosan (Irgacare MP)				

Mixing performed by: _____ Date:_____
Verified by: _____ Date:_____

Processing section				
Material	**Manufacturer**	**Part number**	**Lot Number**	**Volume/quantity**
Antimicrobial solution	**University of Nottingham**	**N/A**		
Urinary catheters				
Ethanol				
Start soak time	**End soak time:**	**End post soak drying time:**	**Ethanol rinse elapsed time:**	**End post rinse drying time:**

Processing performed by: _____ Date: _____
Verified by: _____ Date: _____

Appendix 17: Trial catheter sterilisation documentation

Bioburden test report

Company: Yushin Medical Co., Ltd.
Document No.: HDD-161020-01
Department: Quality Assurance
Inspector: Gi Ppeum, Kim
Date: 20TH. Oct, 2016

	Test Staff	Approver
Approval	10/20	10/20

1. Test Objective

This test is performed to check bioburden of Foley Balloon Catheters impregnated with antimicrobials from the post-washing to prior process of sterilization in accordance with ISO 11737-1. The products used for test were chosen by random sampling

2. Test Instruments

Autoclave Sterilizer, Incubator, Clean Bench, Electronic Balance, pH meter, Membrane Filter, Forceps, Scissors, Alcohol Ramp, Media Bottle

3. Test Product

Foley Balloon Catheters, 2-Way, Silicone, Standard Type
(Model No. 1002-12 / Lot No. A16777)

4. Test Method

4.1 Preparation of test solution Treatment – Vortex mixing (by ISO 11737-1 Annex B.2.2.3)
1) After cutting the samples, insert them into the sterilized test tube, and then fill the buffer into the sterilized test tube till the samples to be soaked.
2) Elute the germs from the products by vortex mixing method from the test tube.
(Buffer: phosphate - buffered saline)

4.2 Membrane filtration
1) Germ is transferred from products to solvent.
2) Pass the solvent through the sterilized membrane filter.
(Membrane filter size: 47 mm, 0.45 ㎛)

4.3 Culture
1) Put the filter membrane passed through solvent on the solid medium coated Petri-dish and then to culture Aerobic bacteria for 2~5day at 30~35℃.
2) Culture condition : TSA medium (Tryptic Soy Agar) 30 ℃ ~ 35 ℃

5. Result

5.1 Test result

Tester	Gi Ppeum, Kim		Test date	2016.10.17 ~ 2016.10.19	
Test product	Lot no.	Exposure place	Exposure time		Germ (CFU/Unit)
Foley Balloon Catheter 12FR	A16777	Cleanroom	16.10.17. AM 10:00	16.10.17. PM 17:00	0
Foley Balloon Catheter 12FR	A16777	Cleanroom	16.10.17. AM 10:00	16.10.17. PM 17:00	0
Foley Balloon Catheter 12FR	A16777	Cleanroom	16.10.17. AM 10:00	16.10.17. PM 17:00	0
Foley Balloon Catheter 12FR	A16777	Cleanroom	16.10.17. AM 10:00	16.10.17. PM 17:00	0
Foley Balloon Catheter 12FR	A16777	Cleanroom	16.10.17. AM 10:00	16.10.17. PM 17:00	0
Remark			* Germ (CFU/Unit) 0~70 : Pass 71~100 : Conditional acceptance 100 ≥ : Fail		

* Incubation Condition : 35℃, 48hrs.

6. Conclusion

Bioburden of Foley Balloon Catheter impregnated with antimicrobials from the post-washing to prior process of sterilization was checked in accordance with ISO 11737-1 and we confirm that the CFU(Colony-forming unit) was within criteria.

7. Reference

* ISO 11737-1:2006, Sterilization of health care products – Microbiological methods – Part1 : Estimation of the population of microorganisms on product.
* Sterility Test of the Korean Pharmacopoeia 10TH

(주)유 신 메 디 칼
Yushin Medical Co., Ltd.

36, Jomaru-ro 427 beon-gil, Wonmi-gu, Bucheon-si, Gyeonggi-do, 420-387, Korea
TEL:(032)668-0323 FAX: (032)668-0329 E-mail:ysmed01@yushinmed.com http:// www.yushinmed.com

Date : 23-Nov-16

VitalCare UK

Essex Technology & Innonvation Centre,

The Gables, Fyfiled Road,

Ongar, Essex, CM5 OGA, UK

STERILITY CERTIFICATE

Yushin Medical co., Ltd. certifies that the below mentioned products have been sterilized by Ethylene Oxide gas in accordance with "Sterilization Work Standard(YMS-F701)". Biological and chemical indication test was shown negative results.

*** Order No.: 24134** *** Invoice No.: YM-161107-02**

Prod. Name	Prod. No	Lot No.	Quantity(PCS)		Production date.
All Silicone, 2-way Foley Normal Balloon Catheter.	1002S-12	A16777	30	PCS	10-2016
	1002S-14		23	PCS	
	1002-12		22	PCS	
	1002-14		31	PCS	
All Silicone, 2-way Foley UniBal® Balloon Catheter	1002UBS-16	A36777	8	PCS	
	1002UB-16		14	PCS	
	1002UB-18		9	PCS	
	1002UB-20		8	PCS	

Yushin Medical Co., Ltd

K.H Shin / Quality Management Representative

2016-11-23

Amount of Remaining EO Gas Test Report

Inspector	Manager
10/27	10/27

File No. ; HDD-161027-01

Product (component)	Impregnated Catheter 12Fr, 400mm	Product. No.	1002-12
LOT NO.	A16777	Test date	2016. 10. 26.
Test Room	Lab (Yushin medical Co.,Ltd)	Inspector	K. B. Kim
Sterilization Lot no.	C-16-10-22	Sampling date	2016. 10. 24.
Extraction Condition			
Weight of Product or Component(SPw)	10553.2 ㎎	Sample weight(SEw)	1016.7 ㎎
Solvent volume (EWw)	4187.6 ㎎	Extraction Temp./ Time	37 ℃ / 24 hr
GC injection			1 ㎕
Amount of remaining (EO)			
GC response(R_{EO})			2.0805 ppm
EO_{RS}(R_{EO}×EWw)			0.0087 ㎎
Criterion		Standard	Result
ISO10993-7 Criterion(㎎)	EO_{RSP}(EO_{PPM}×SPw)	< 0.1 ㎎	0.0904 ㎎
Amount of remaining (ECH)			
GC response(R_{ECH})			- ppm
ECH_{RS}(R_{ECH}×EWw)			- ㎎
Criterion		Standard	Result
ISO10993-7 Criterion(㎎)	EO_{RSP}(EO_{PPM}×SPw)	< 0.4 ㎎	- ㎎

● Remark :

1. Test sample which is passed 72hrs after Sterilization
2. Attachment : EO chromatogram Report (2 Sheet)