Mitigating the harm caused by *Pseudomonas aeruginosa* in Cystic Fibrosis

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Candidate statement

I confirm that the work presented in this thesis is all my own with the following exceptions:

Oli Rayner devised and conducted the patient survey in Chapter 3 and supported the analysis. Paul Leighton devised the analysis plan and acted a second reviewer when analysing the data. Prof. Alan Smyth designed the figures used in the final paper, one of which I have reproduced here.

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Abstract

Cystic fibrosis (CF) affects up to 100,000 people worldwide. Defects in the Cystic Fibrosis Transmembrane Regulator (CFTR) gene cause abnormally viscid secretions at epithelial surfaces. In the lungs the abnormal secretions reduce clearance of both pathogens and sputum, predisposing to recurrent lung infections. Ultimately recurrent infections can cause respiratory failure and death.

Pseudomonas aeruginosa is a major cause of pulmonary infections and morbidity in CF. Preventing infection with *P. aeruginosa* is, therefore, an important goal in maintaining good health in people with CF. The source of most infection is unclear, though in some cases is through person-to-person spread.

Once infection has occurred eradication therapy can be used to delay the onset of chronic infection. People with CF chronically infected with *P. aeruginosa* are exposed to additional treatment to suppress the organism and treat additional pulmonary exacerbations. They are therefore at risk of additional side effects. Aminoglycoside antibiotics, commonly used in the treatment of pseudomonal infection, cause ototoxicity as one of their major side effects.

This thesis will aim to understand the additional harms caused by *P. aeruginosa* in CF, over and above direct pulmonary damage. In addition it will examine strategies which may help mitigate these harms.

The first study qualitatively examined how the CF community feels about *P. aeruginosa*. This identified significant, negative emotional impact on both patients and families. One identified theme was the fear engendered by the lack of knowledge about the source if infection and how it can be prevented.

The second study therefore, systematically reviewed the evidence for strategies to prevent initial infection. The only strategies which were found to be potentially successful were CFTR modulator therapy, vaccination against *P. aeruginosa* and cohort segregation.

Since prevention is challenging the third study systematically reviewed strategies to delay recurrence of infection after eradication. Only a single study was included but gave moderately good evidence that cycled antibiotic therapy delays recurrence of *P. aeruginosa* infection.

Finally, the utility of a novel hearing test, the High Frequency Digit Triplet test, was assessed in adults and adolescents with CF, when well and unwell, and in children. Testing was feasible but the test was insufficiently sensitive and specific to be used as a screening tool.

In this thesis I have shown additional ways in which *P. aeruginosa* can cause harm in CF and found some strategies which may help ameliorate this harm. The need for internationally recognised definitions surrounding *P. aeruginosa* infection and hearing loss were highlighted.

Contents

Abbreviations

AAA	American Academy of Audiology
ABC	ATP-binding cassette transporter
ABPA	Allergic bronchopulmonary aspergillosis
AS	Professor Alan Smyth, School of Medicine, University of Nottingham
ASHA	American Speech-Language-Hearing Association
ASL	Airway surface liquid
ATP	Adenosine triphosphate
BAL	Bronchoalveolar lavage
Bcc	*Burkholderia cepacia* complex
BCH	Birmingham Children's Hospital
BSA	British Society of Audiology
cAMP	Cyclic AMP
CBAVD	congenital bilateral absence of the vas deferens
CF	Cystic fibrosis
CFFPR	CF Foundation Patient Registry
CF-D	CF-diabetes
CFTR	Cystic fibrosis transmembrane conductance regulator
CHL	Conductive hearing loss
CI	Confidence interval
CRISS	Chronic Respiratory Infection Symptom Score
CSM	Cell surface membrane
dB	Decibels
dB HL	Decibels Hearing Level

dB SPL	Decibels – Sound pressure level
DEXA	Dual energy X-ray absorptiometry
DIOS	Distal intestinal obstruction syndrome
DNA	Deoxyribonucleic acid
DP	Distortion product
DPOAE	Distortion-product otoacoustic emissions
DTT	Digit triplet test
ECM	Extracellular matrix
eDNA	Extracellular deoxyribonucleic acid
EHF	Extended high frequency
EHF-PTA	Extended high frequency pure tone audiogram
ENaC	Epithelial sodium channel
ENT	Ear, nose and throat
ETI	Elexacaftor-tezacaftor-ivacaftor
FEV_1	Forced expiratory volume in one second
GFR	Glomerular filtration rate
GRADE	Grading of Recommendations, Assessment, Development and Evaluation
HFDT	High Frequency Digit Triplet
HFDTT	High Frequency Digit Triplet Test
HL	Hearing loss
HR	Hazard ratio
Hz	Hertz
IHC	Inner hair cell
IL	Interleukin

IP&C	Infection prevention and control
IPD	Individual patient data
IV	Intravenous
JLA	James Lind Alliance
kHz	Kilohertz
LES	Liverpool Epidemic Strain
M. abscessus	*Mycobacterium abscessus*
MA	Manchester Epidemic Strain
MI	Meconium ileus
MLST	Multilocus sequence typing
MLVA	Multiple-locus variable-number tandem repeat analysis
MRSA	Methicillin resistant *Staphylococcus aureus*
MSSA	Methicillin sensitive *Staphylococcus aureus*
NAC	N-acetylcysteine
NAG	N-acetyl-β, D-glucosaminidase
NBD1	Nucleotide binding domain 1
NBS	Newborn screening
NCH	Nottingham Children's Hospital
NETs	Neutrophil extracellular traps
NF-κB	Nuclear factor-κB
NICU	Neonatal intensive care unit
NIHR	National Institute of Health Research
NPV	Negative predictive value
OAE	Otoacoustic emissions
OCR	Oli Rayner, CF Trust Patient Representative

OHC	Outer hair cell
OPC	Oropharyngeal culture
PCD	Primary Ciliary Dyskinesia
PA	*Pseudomonas aeruginosa*
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
PERT	Pancreatic enzyme replacement therapy
PES	Prairie Epidemic Strain
PFGE	Pulsed field gel electrophoresis
PL	Dr Paul Leighton, School of Medicine, University of Nottingham
$ppFEV_1$	Percent predicted forced expiratory volume in one second
PPI	Patient and Public Involvement
PPV	Positive predictive value
PSP	Priority Setting Partnership
PTA	Pure tone audiogram
pwCF	People with CF
QS	Quorum sensing
RCT	Randomised controlled trial
RfPB	Research for patient benefit
RoB2	Cochrane Risk of Bias Tool, version 2 (for RCTS)
ROBINS-I	Risk of Bias in Non-randomised Studies of Interventions
ROC	Receiver-operating characteristic
ROS	Reactive oxygen species
RR	Risk ratio

rRNA	Ribosomal ribonucleic acid
RSV	Respiratory syncytial virus
S. aureus	*Staphylococcus aureus*
SNHL	Sensorineural hearing loss
SNP	Single nucleotide polymorphism
SNR	Signal (speech)-to-noise ratio
SP	Dr Sally Palser, School of Medicine, University of Nottingham
SPL	Sound pressure level
SPLUNC1	Short palate, lung and nasal epithelial clone 1
spp.	Species
SRT	Speech reception threshold
ST	Sequence type
TB	Tuberculosis
TLR	Toll-like receptor
TM	Tympanic membrane
UK	United Kingdom
US	United States
UV	Ultraviolet
VNTR	Variable-number tandem repeat
WCFC	Wolfson Cystic Fibrosis Centre, Nottingham
WGS	Whole genome sequencing
WMACFC	West Midlands Adult Cystic Fibrosis Centre, Birmingham

List of Figures

List of Tables

Chapter 1: Introduction

1.1 Cystic fibrosis

Cystic fibrosis (CF) is a multi-system disorder characterised by pulmonary infection, pancreatic disease and high sweat chloride concentrations. Much of the morbidity and mortality in CF is caused by pulmonary disease (1).

1.1.1 A history of CF

The first description of CF was made in 1938 by Andersen (2) in her seminal paper, “Cystic fibrosis of the pancreas and it’s relation to celiac disease”. Discovery of abnormally high sweat sodium and chloride concentrations in people with CF compared to control subjects (3) formed the basis of testing for the condition (4). It was established that CF was an autosomal recessive condition, with the gene, the cystic fibrosis transmembrane conductance regulator (CFTR), finally identified in 1989 (5-7).

1.1.2 Epidemiology

CF is the commonest life-limiting autosomal recessive disorder in Caucasian populations, affecting between 70,000 (8) and 100,000 (9) people worldwide, with this number growing as life expectancy increases. In the United Kingdon (UK) there are more than 10,000 patients (10), with a birth incidence of approximately 1:2,400 live births (11).

Median age of death in CF has increased from under ten years in the early 1970s to approximately 36 in 2020 (10, 12). In the United States (US), children with CF born in 2000 have a predicted survival of over 50 years (13).

1.1.3 Genetics

The CFTR gene is located on the long arm of chromosome 7, comprises approximately 190kb of genomic DNA, and codes for a 1480-amino acid protein, CFTR (14). CFTR is an ATP-binding cassette transporter (ABC); a class in which CFTR is unique in also being an ion channel (15). It comprises two transmembrane domains, two cytosolic nucleotide binding domains which bind and hydrolyse ATP, and a regulatory domain which requires phosphorylation in order that the channel can open (15).

Identification of the CF-gene was a major milestone in the history of CF, facilitating improved diagnostic techniques and providing a basis for treatment of the condition at a fundamental level. There are over 2000 known variants of the CFTR gene; as of April 2023, 719 CF-causing variants have been identified, with a further 49 variants of varying clinical consequence and 11 of unknown significance (16). By far the commonest variant worldwide is p.phe508del. In 2020 in the UK 89.0% of patients carried at least one copy of this variant (10); in the US in

2020 44.5% of CF patients were homozygous and 41.2% were heterozygous for p.phe508del (12).

The p.phe508del variant causes loss of a phenylalanine residue at amino acid position 508 in the CFTR protein. Loss of this amino acid causes instability in nucleotide binding domain 1 (NBD1) and abnormal folding of the protein (17). The misfolded protein is retained in the endoplasmic reticulum then transferred to the cytosol for degradation via the ubiquitin/proteasome pathway (18).

p.phe508del is postulated to confer some heterozygote advantage, with suggestions including resistance to tuberculosis (12, 19), cholera (20) and protection against asthma (21), though these have never been conclusively proven. The high prevalence of this variant in people of Northern European descent accounts for the preponderance of the disease in Caucasian populations (22). CF is however seen in all races, with other variants predominant in other racial groups, for example p.Gly551Asp in French Canadians and p.Trp1282X in Ashkenazi Jews (22).

Different variants have variable effects on protein production; these effects have been classified into six classes, though some variants, including p.phe508del, have features of more than one class. The classes of variants are summarised in Figure 1-1.

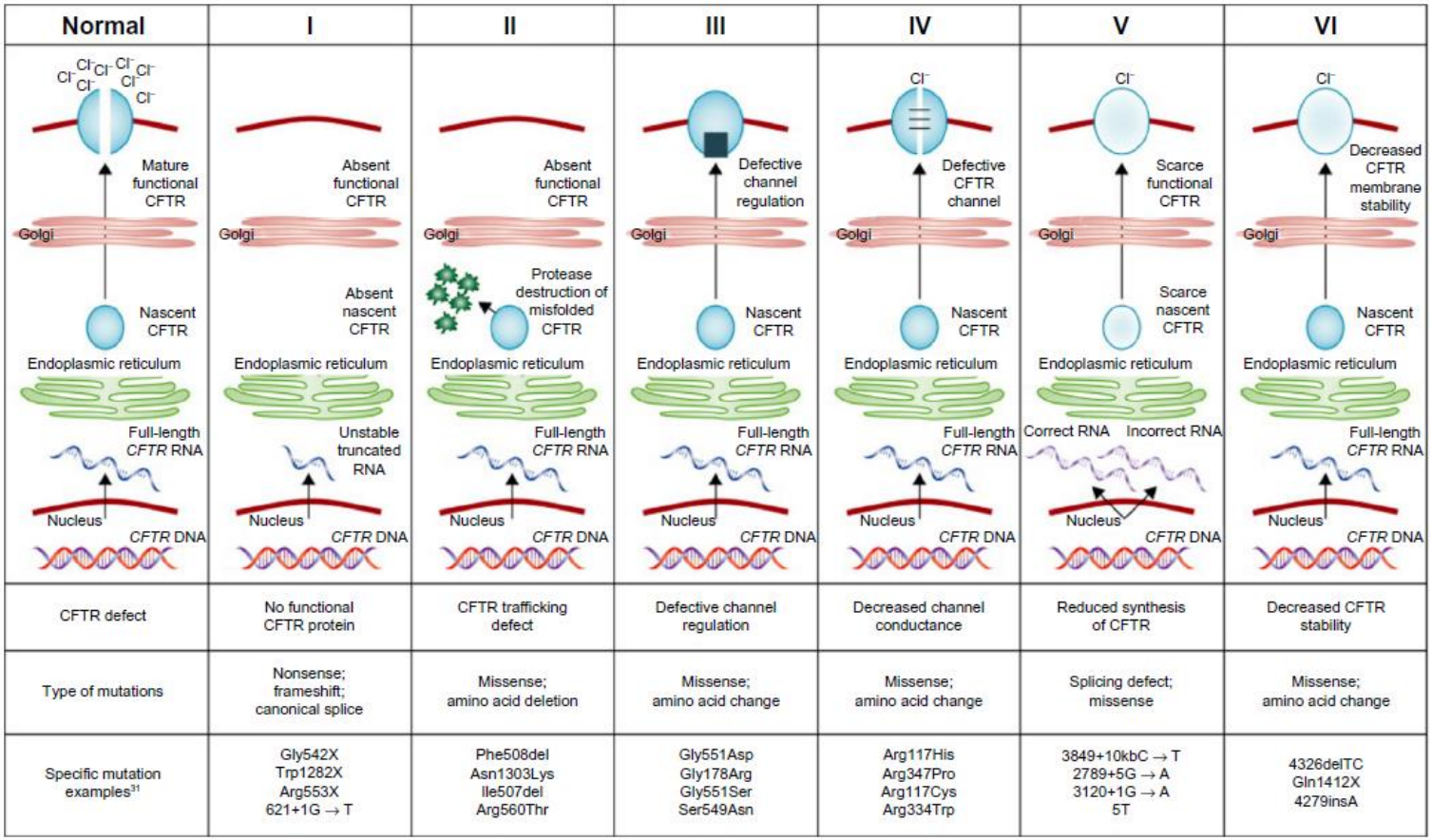


Figure 1-1. CFTR variant classes. Summarising normal CFTR cellular production and processing and the defects caused by different classes of CFTR gene variants. Reproduced with permission from Boyle and De Boeck 2013. Elsevier/Copyright Clearance Centre license number 5224400739167 (23).

Variants in classes IV-VI tend to provide some residual CFTR function. Patients with at least one variant from these classes may have a milder CF phenotype (24). Some specific genotypes are associated with pancreatic sufficiency (25) however aside from this there is a lack of genotype-phenotype correlation (26); patients with the same CF genotype may demonstrate a very different clinical course. It is therefore likely that other genetic modifiers have a bearing on disease severity and clinical manifestations (27, 28).

1.1.4 Pathophysiology

CFTR is a cAMP-regulated anion channel, present on the apical surface of epithelial cells of the respiratory tract, pancreas, biliary tract, gastro-intestinal tract, sweat ducts and vas deferens (29, 30). CFTR has also been identified in immune cells including lymphocytes (31), monocytes (31), alveolar macrophages (32) and neutrophils (33).

CFTR facilitates chloride and bicarbonate transport at epithelial membranes. It has a number of other functions, including inhibition of the epithelial sodium channel (ENaC) (34). Combined, loss of these functions affects the quality of secretions at epithelial surfaces, which become dehydrated and viscid.

Increased viscidity of epithelial secretions underlies much of the pathophysiology of CF. Ductal structures, including bronchi in the lung,

pancreatic and hepatic ducts, become blocked, eventually causing end-organ damage.

In her original description of the pathology of CF, Andersen described concretions of various sizes within the pancreatic acini. The acini were surrounded by, "…moderate to large amounts of fibrous tissue, the quantity varying roughly with the age of the child." In the lungs she found purulent exudate filling the bronchial lumens (2). Bjarnsholt *et al.* propose that while, in the presence of intensive antibiotic treatment, much of the lung disease is focussed on the conducting zones, small foci of damage in the respiratory zones build up over time, eventually causing end-stage lung damage (35).

1.1.4.1 Airway surface liquid

In the lungs the ciliated epithelial surface is lined with airway surface liquid (ASL). ASL occurs in two distinct layers. An outer mucus layer traps bacteria and particles, whilst an inner periciliary layer surrounds the cilia, enabling them to beat (see Figure 1-2). In health airway mucus traps inhaled debris and pathogens, while beating cilia remove this from the lungs via the mucociliary escalator.

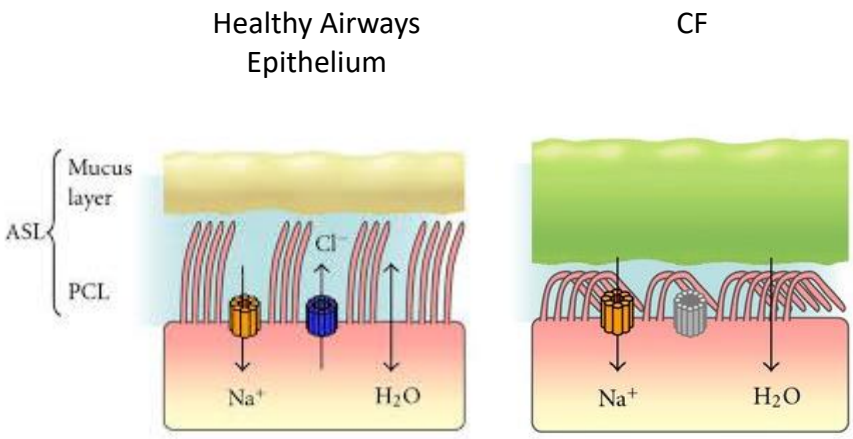


Figure 1-2. Airway surface liquid in health and CF. In health CFTR allows chloride transport to the airway lumen. ENaC is inhibited, preventing hyperabsorption of sodium (Na+). Water moves freely into the periciliary fluid down the osmotic gradient. In CF abnormal CFTR prevents movement of Cl- into the periciliary fluid. ENaC is uninhibited leading to hyperabsorption of Na+. Water moves into the cells down the osmotic gradient. Dehydrated mucus becomes thick and tenacious while dehydration of the periciliary layer leads to collapse of cilia and consequential reduction in mucociliary clearance. Reproduced from Reeves et al. 2012 (36) under a Creative Commons Attribution 3.0 International license (37) https://creativecommons.org/licenses/by/3.0/.

Loss of functional CFTR prevents secretion of chloride and bicarbonate anions into the ASL (38). In addition, lack of inhibition of ENaC causes hyperabsorption of sodium into the epithelial cells (39). Water is drawn down the osmotic gradient, out of the ASL. The gel-on-brush theory proposes that appropriate hydration of the tethered mucins within the periciliary layer supports the mucus layer and prevents crushing of the cilia. Excessive dehydration of the ASL initially affects the mucus layer rendering it viscid. Eventually the periciliary layer thins, collapsing the cilia which can no longer beat (40). Damage to the mucociliary escalator prevents clearance of pathogens from within the lung.

Bicarbonate secretion is also necessary for normal mucus formation. Mucins are secreted from airway epithelial cells in aggregations;

bicarbonate is necessary for them to disaggregate and detach from the epithelial surface. In the absence of bicarbonate mucus is left viscid and strongly adherent to the epithelial membrane (38). Further, loss of bicarbonate secretion affects the pH of the ASL, rendering it acidic (41). pH-dependent airway anti-microbial peptides are inactivated, impairing the innate host response to bacterial infection (42).

1.1.4.2 Inflammation and immunity

In addition to abnormalities at epithelial surfaces, CF is associated with abnormalities in inflammatory pathways and the immune response to lung pathogens.

1.1.4.2.1 The normal inflammatory response

Microbial invasion of tissue causes an acute inflammatory response. Blood flow to damaged tissue increases, bringing an influx of cells and proteins, the acute inflammatory exudate. The vascular endothelium becomes leaky, allowing these cells and proteins to access the damaged tissue. Opsonic proteins in the exudate bind to bacterial surface receptors, facilitating neutrophil phagocytosis and killing of infecting organisms. Once infecting microbes are eliminated, damaged tissue is broken down by neutrophil proteases and the debris removed. If tissue damage is minimal resolution occurs; where structural damage is present healing occurs with the generation of a collagen scar (43).

If the infecting microbes cannot be cleared the response matures to a process of chronic inflammation. Neutrophilic infiltration gives way to macrophages and lymphocytes. Continuing immune reaction, tissue damage and attempts to repair this damage occur concurrently. This process will continue unless and until the infection is cleared (43).

1.1.4.2.2 Aberrant response to pathogens in CF

CF lung disease is characterised by an aberrant response to pathogens with failure of the process to evolve into “normal” chronic inflammation.

Debate remains as to whether the primary pathology in CF is inflammation or infection (44). Studies in a porcine model of CF found defects in innate immunity within hours of birth and suggest early failure to clear pathogens sets up a cascade of infection and inflammation (45). In humans Rosenfeld *et al.* found increased neutrophil influx and higher concentrations of pro-inflammatory cytokines, particularly the neutrophil chemokine interleukin (IL)-8, in bronchoalveolar lavage (BAL) samples taken from children aged under three years (46). Yonker *et al.* propose that early pathogens, such as *Staphylococcus aureus* (*S. aureus*), cause inflammation and structural lung damage which then paves the way for colonisation with *Pseudomonas aeruginosa* (*P. aeruginosa*) (47).

Defects in innate immunity are important in the pathogenesis of CF lung disease, with abnormalities in neutrophil function central to the process. Inflammatory dysregulation appears to be driven, in part, by neutrophilic

inflammation in response to IL-8. IL-8 expression by epithelial cells is promoted by activation of the nuclear factor (NF)-κB pathway, itself triggered by binding of ligands, including *P. aeruginosa* pili, to epithelial surface receptors. However, it is suggested that, for those genotypes in which the mutant CFTR is diverted to the endoplasmic reticulum for degradation, accumulation of mutant protein causes a cellular stress response with additional, endogenous, activation of the NF-κB pathway (48, 49). Intrinsic activation of pro-inflammatory pathways, in the absence of bacterial pathogens, may contribute to the abnormal inflammatory response seen in CF lungs.

Neutrophils isolated from people with CF have reduced ability to kill infecting organisms (33). Despite ongoing neutrophilic recruitment, the immune response is insufficient (47), failing to clear the infection. CFTR-deficient neutrophils are unable to transport chloride ions into the phagolysosome, preventing formation of hypochlorite, an important reactive oxygen species (ROS) for the killing of phagocytosed bacteria (50). The persistent inflammatory response does not mature and the continuing attempts of neutrophils to clear pathogens are deleterious. In addition, neutrophils carrying CF-causing CFTR variants, are more prone to degranulation, with release of ROS, proteases (including neutrophil elastase), and peroxidases. These enzymes degrade collagen and elastin in the lung parenchyma, leading to structural damage. Formation of Neutrophil Extracellular Traps (NETs) also

promotes release of proteases and leads to the release of large quantities of DNA, which further increases sputum viscosity. They may also further deplete innate antimicrobial peptides (38) and contribute to the cleavage of other molecules such as SPLUNC1 (short palate, lung and nasal epithelial clone 1), loss of which further potentiates ENaC activation and dehydration of the ASL.

Adaptive immunity is also impaired in CF. T-helper lymphocytes are more likely to differentiate into Th-17 lymphocytes which promote a neutrophilic response to infection. Whilst this is beneficial in acute infection, longer-term it promotes ongoing neutrophilic inflammation and subsequent tissue damage (51, 52).

CFTR may itself be important in the immune response to *P. aeruginosa* since wild-type protein has been shown *in vitro* and in mouse models, to bind *P. aeruginosa*-specific lipopolysaccharide (LPS) leading to internalisation of the organism, a mechanism that may be important in clearance of the pathogen in non-CF populations (53). This mechanism however, has never been proven in humans (54).

1.1.5 Clinical manifestations of cystic fibrosis

1.1.5.1 Lung disease

1.1.5.1.1 Natural history of CF lung disease

Respiratory disease accounts for much of the morbidity and mortality in people with CF. Pulmonary infections begin in childhood. Most children are asymptomatic (55) unless they have a superadded respiratory infection, but the prevalence of daily symptoms, including cough, sputum production and shortness of breath, increases with increasing age.

Disease progression is frequently described in terms of lung function. The most commonly used measure of lung function in CF is the percentage of the predicted Forced Expiratory Volume in one second (FEV_1). Predicted FEV_1 is calculated from a healthy population, adjusted for sex, race, height and age (56). Decline in percent-predicted (pp)- FEV_1 is a marker of disease progression (57) and mortality increases as FEV_1 declines (58). A number of factors, including genotype (with type IV-VI variants relatively protective), sex, pancreatic status, chronic *P. aeruginosa* infection status, presence of symptoms and socio-economic status have all been found to affect this rate of progression (59-62).

Recurrent infection, and the abnormal response to chronic infection, cause progressive damage to lung parenchymal tissue. Bronchiectasis

is evident in some children in their first year of life, with the prevalence increasing with age (63). Accumulated parenchymal damage ultimately leads to a loss of alveoli and reduced surface area for gas exchange. Eventually patients develop respiratory failure, which is the commonest cause of death in people with CF (10, 12).

1.1.5.1.2 Common respiratory pathogens in CF

The lung microbiome in CF evolves with increasing age. In infants and young children the most prevalent respiratory pathogens are *S. aureus* and *Haemophilus influenzae*. Intermittent *P. aeruginosa* infection occurs in throughout life, while the prevalence of chronic *P. aeruginosa* infection increases in adolescence and early adulthood (see Figure 1-3) (10, 12).

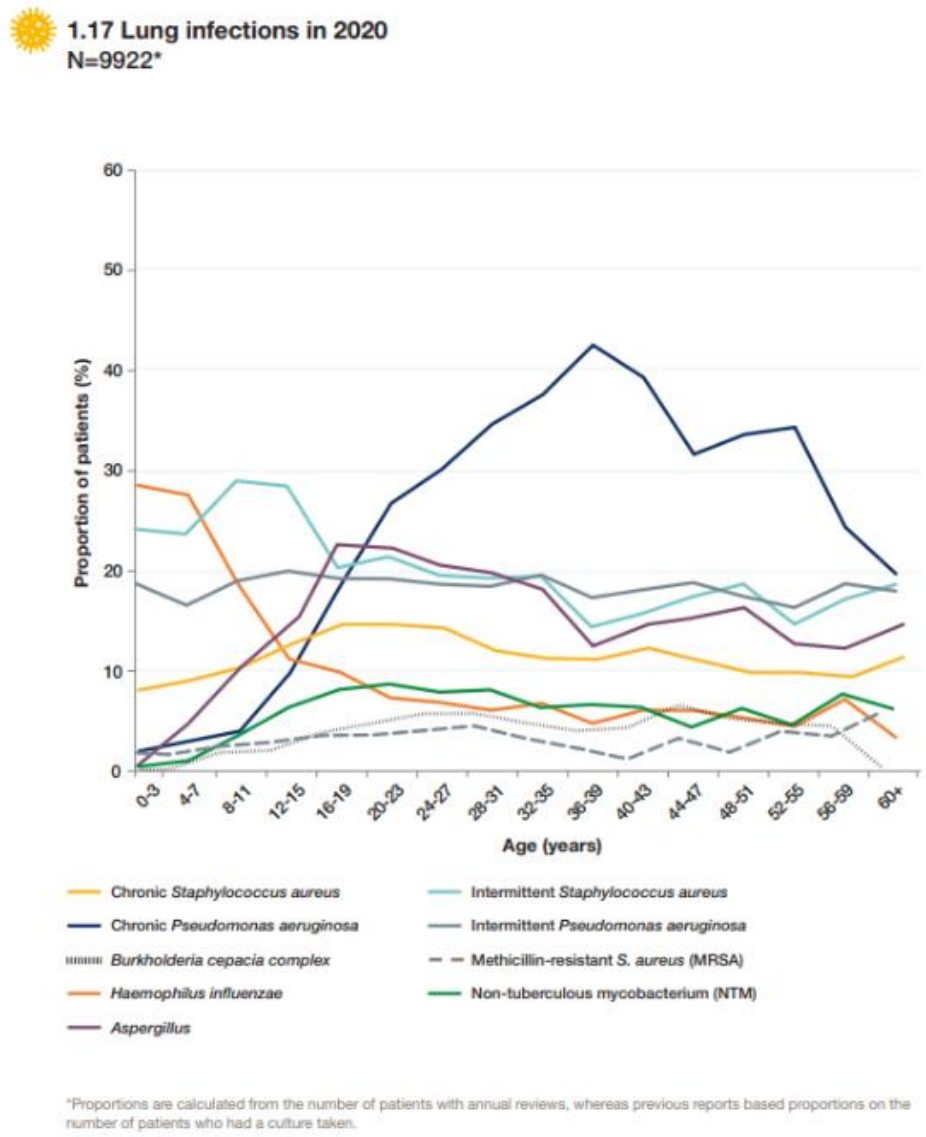


Figure 1-3. Proportion of patients in different age brackets infected with common CF pathogens in 2020. Reproduced with permission from the UK CF Registry 2020 National Report (10).

With increasing age, the prevalence of more unusual organisms, including *Stenotrophomonas maltophilia, Achromobacter species* and non-tuberculous mycobacteria increases. For some of these organisms, for example *Mycobacterium abscessus* (*M. abscessus)* and *Burkholderia cenocepacia*, there is clear evidence that the organism is

pathogenic (64), whilst the impact of other organisms, including *Achromobacter* spp. remains less clear. These organisms are commonly resistant to multiple antibiotics and treating them requires complex antibiotic regimens with an increased risk of side effects (65).

Some organisms, notably *P. aeruginosa, M. abscessus* and the *Burkholderia cepacia* complex (Bcc) have a particular tendency to cause chronic infection. The development of chronic pulmonary infection is a hallmark of cystic fibrosis lung disease and has an additional, detrimental effect on lung function (64) as described in section 1.2.4. Factors influencing the ability of *P. aeruginosa* to persist within the CF lung will be further discussed in section 1.2.5.

1.1.5.1.3 Acute infection and pulmonary exacerbations

Acute pulmonary exacerbations are episodes of increased respiratory symptoms, which may be associated with constitutional symptoms, deterioration in pulmonary function and radiographic changes. An increased frequency of pulmonary exacerbations is associated with an increased rate of decline in lung function (66).

A number of diagnostic criteria for defining a pulmonary exacerbation have been described (67, 68), though clinically the opinion of the treating physician is usually the guide for treatment. Pulmonary exacerbations are treated aggressively with therapy aimed at controlling infection, clearing the airways and maintaining nutrition.

Control of infection is achieved predominantly through antibiotic therapy, the aim of which depends on the patient's infection status. Novel isolations of pathogens known to cause chronic infection, such as *P. aeruginosa*, are treated aggressively to reduce the chance of chronic infection developing. Eradication of novel *P. aeruginosa* infection is discussed in section 1.2.3.

For patients with known chronic infection and no evidence of a new pathogen, treatment aims to suppress a presumed overgrowth of bacteria. In both circumstances the objectives are a reduction in respiratory symptoms and maintenance of pulmonary function. Oral antibiotics are commonly used first line with timely review to ensure improvement. If the patient is more unwell, oral treatment has failed, or the organism is unlikely to be sensitive to an oral option, intravenous antibiotics are required.

Antibiotic choice depends on the organism to be treated. A single agent may be sufficient prior to *P. aeruginosa* acquisition but afterwards a dual regimen is preferred to reduce the risk of resistance developing, though the evidence for this practice is inconclusive (69). Results of sensitivity testing are of limited use (70, 71). Within the CF lung multiple strains of individual bacterial species may be present and may not all be reflected in the cultured sample (72).

1.1.5.1.4 Management of CF lung disease

Since respiratory failure remains the commonest cause of death in people with CF (10, 12) aggressive maintenance treatment to prevent lung function decline is essential (73). Treatment is based on three key pillars: control of infection, airway clearance and maintenance of nutrition (1).

CF airway mucus provides a breeding ground for bacteria and contributes to airway obstruction. Strategies to reduce sputum viscosity include inhaled mucolytics and airway hydrators. Nebulised recombinant human DNase, which breaks down some of the excess DNA extruded from dead neutrophils and infecting bacteria (67), has been shown to improve lung function and decrease the rate of pulmonary exacerbations (74). Nebulised hypertonic saline and inhaled dry powder mannitol reverse the abnormal osmotic gradient, drawing water into the airways and loosening secretions (75, 76). Both may slightly improve lung function (76, 77) and hypertonic saline may reduce pulmonary exacerbations (77).

A number of physiotherapy techniques are available for chest clearance. In the UK the most commonly used strategies are those which use positive expiratory pressure (10), whilst in the US, “the vest,” which uses high-frequency chest wall oscillation, is the most frequently used modality (12). Both strategies use vibration to loosen secretions;

positive airway pressure further splints open the airways allowing sputum to be cleared by coughing.

In the UK exercise is also a commonly used method of chest clearance, either as the primary physiotherapy technique or as an adjunct (10). Exercise programs can improve lung function and quality of life (78) and should be incorporated in physiotherapy regimens regardless of the other techniques used (73).

Where patients have developed chronic airways infection (further discussed in sections 1.2.4 and 1.2.5.2), long-term suppressive antibiotic therapy has been shown to have a beneficial effect on lung function, pulmonary exacerbations and sputum *P. aeruginosa* density (79). Inhaled antibiotics are delivered directly into the lungs, allowing increased antibiotic concentrations at the site of need, whilst minimising systemic side effects. Commonly used agents include colistin, tobramycin and aztreonam. These agents may be nebulised or given as dry powder inhalers; the latter are quicker to use and may improve treatment concordance (80).

Oral azithromycin, a macrolide antibiotic, has been shown to have a beneficial effect in patients with chronic *P. aeruginosa* infection with improvements in lung function and reduction in pulmonary exacerbations (81). This effect may be due to interference with biofilm formation; azithromycin has been shown to reduce biofilm formation *in*

vitro and improve clearance of *P. aeruginosa* biofilms in a rat model of infection (82). In patients without chronic *P. aeruginosa* infection azithromycin has been shown to reduce exacerbation frequency, though it did not have an impact on lung function (83). It is unclear whether this benefit is due to its anti-microbial or anti-inflammatory properties, or both.

1.1.5.2 CFTR modulators

The advent of CFTR modulators has had a significant impact on CF care. Modulation of CFTR aims to restore, at least partially, the function of the CFTR channel at epithelial surfaces.

Modulation strategies vary according to the class of variant present. Modulation of class I variants requires read-through of the premature translation codons to allow translation of full length CFTR mRNA. A number of molecules, including ataluren and aminoglycosides, have been trialled for this purpose but so far have not shown clinical benefit (84, 85).

Class II variants cause a defect in protein trafficking due to misfolding of the CFTR protein product, which is then degraded prior to reaching the cell surface membrane (CSM). Corrector molecules facilitate correct folding of the protein, increasing the quantity transported to the cell surface (86). Corrector molecules include Lumacaftor, Tezacaftor and Elexacaftor.

Variants in classes III and IV, including p.Gly551Asp, require potentiation of the function of CFTR at the CSM (87) to increase the probability of an open channel (86). Ivacaftor, the first modulator licensed for treatment of CF, is a CFTR potentiator.

Modulators for class V and VI variants are still in development. These variants will require amplification of the CFTR protein product and stabilisation at the CSM (86).

Modulation of p.508del, which has features of both class II and III variants, requires correction in addition to potentiation. A number of compounds have been trialled with increasing efficacy. Currently the most commonly used modulator is Elexacaftor/Tezacaftor/Ivacaftor (ETI), a combination of two correctors and a potentiator (88).

Modulation improves CFTR function at epithelial surfaces. Rehydration of the ASL and reduced density in the structure of ASL mucins (89, 90), and improved ciliary beat frequency (91) improve mucociliary clearance (92). The effect on ASL pH is less clear; increased pH as a consequence of improved bicarbonate transport is predicted but not consistently seen (89, 90). In studies of patients on ivacaftor some have found a reduction in markers of airway inflammation (93), while others have found no effect (92, 94).

CFTR modulation also appears to have an immunomodulatory effect. Treatment with ivacaftor improved neutrophil degranulation and

bacterial killing (95), though the impact on macrophages is less clear (96).

There is some effect of CFTR modulation on microbiology, at least initially, with a reduction in the sputum density of *P. aeruginosa* in those chronically infected (97). However, after a year on therapy this may rebound (93). The proportion of chronically infected patients with positive sputum cultures for *P. aeruginosa* has been shown to fall with modulation (92, 98, 99) but, even where patients became culture negative, *P. aeruginosa* could be isolated from samples by PCR (97). Nor does modulation completely prevent novel *P. aeruginosa* infection (94, 97, 99), though the rate of new infections may be improved (100, 101). This lack of prevention may be related to underlying lung disease; patients with non-CF bronchiectasis and COPD are also prone to *P. aeruginosa* infection, which may associate with more severe lung disease (102).

Despite incomplete microbiological reversal, clinically modulators have had a profound effect on patients, with significant improvements in the $ppFEV_1$ in patients with amenable CFTR-variants. Ivacaftor resulted in a mean increase of 10.4% from baseline $ppFEV_1$ in patients with p.Gly551Asp (103). The efficacy of compounds to modulate the p.phe508del variant have increased with each iteration (104-106); ETI produced an increased $ppFEV_1$ of 10-11% from baseline in p.phe508del homozygotes (107, 108) and approximately 14% in

p.phe508del heterozygotes (108, 109). The long-term impact of these treatments is still unclear but it likely that they will have a beneficial effect on CF prognosis.

1.1.6 Extra-pulmonary manifestations of cystic fibrosis

1.1.6.1 Pancreatic insufficiency

Pancreatic exocrine insufficiency occurs in 85-90% of CF patients (110). Damage to the pancreatic acini prevents secretion of the pancreatic enzymes necessary for the digestion of fat. Without replacement of pancreatic enzymes CF patients are unable to properly digest food. This leads to steatorrhoea (fatty, offensive-smelling stools) and failure-to-thrive. Prior to the advent of newborn screening for CF, failure of weight gain and linear growth were common presenting features.

Pancreatic enzyme replacement therapy (PERT) was introduced in the 1940s (111). PERT revolutionised CF care and its introduction coincided with an increase in life expectancy (112). Initially PERT consisted of enzymes sprinkled on to food, which was essentially digested prior to consumption. It now comprises microgranules (for babies and young children) and capsules (for older patients) which are taken with any fat-containing food. Use of enteric coated microspheres has improved the delivery of enzymes to the duodenum, overcoming neutralisation by stomach acid, however the optimum timing for taking

PERT with respect to meals is yet to be determined (113). PERT dosing is based on the fat content of meals and is assessed clinically, looking for signs and symptoms of malabsorption. Care is needed as excessive PERT can lead to constipation and abdominal pain (73).

Inability to digest fat also leads to deficiency of the fat-soluble vitamins, A, D, E and K. Monitoring of levels of these vitamins should be undertaken at least yearly and used to guide replacement therapy. Vitamin deficiencies can be clinically significant. Vitamin A is needed for night vision and is important in immune function. Vitamin D is also important for the immune system and for bone health (see section 1.1.6.3). Vitamin E is necessary for normal neurological development and is an antioxidant. Vitamin K is required for blood clotting. It is monitored using prothrombin time, rather than as a direct measurement of the blood level (114).

Poor nutritional status is associated with lower lung function and a worse clinical outcome (59, 115) and has additional causes beyond maldigestion. Chronic infection and increased work of breathing, which occurs as lung function declines, increase the basal metabolic rate leading to increased caloric requirements (116). Thus, as lung disease progresses, nutritional requirements increase while the ability to meet these needs reduces. Pro-inflammatory cytokine levels, increased in CF and particularly during pulmonary exacerbations (116), cause anorexia (117, 118). Swallowing of infected sputum, production of

which increases with progression of lung disease, may further contribute to anorexia.

Dietetic input with close monitoring of macro- and micro-nutrient intake and absorption is key (119). Where diet is insufficient to maintain a healthy BMI, food supplementation is required. This may be in the form of high calorie oral supplement drinks or additional enteral feed regimens.

1.1.6.2 CF-related diabetes

Pancreatic endocrine function is initially preserved but with increasing age the presence of CF-diabetes (CF-D) increases; in the UK in 2020 10.4% of 10-15 year olds with CF were treated for CF-D, increasing to 35.3% of those aged 16 and over (10). The condition is characterised by a reduction in insulin production due to destruction of pancreatic acini. Insulin production is rarely totally eliminated and the small amounts of circulating native insulin prevent CF patients developing ketoacidosis in the manner of type I diabetes mellitus. However, lack of insulin prevents cellular uptake of glucose, further contributing to weight loss. CF-D is associated with a faster rate of decline in FEV_1 (59).

Annual screening for diabetes by HbA1C should be performed after the age of 10 years, or in those with symptoms, for example in patients losing, or failing to gain, weight. Treatment should be in conjunction with an endocrinologist with a special interest in CF-D (120).

1.1.6.3 CF bone disease

People with CF are at greater risk of osteopaenia than the general population. This is in part related to vitamin D malabsorption but is likely to be multifactorial. Malnutrition, delayed puberty, pro-inflammatory cytokines, low body weight, steroid use and decreased physical activity may all be important (121). Guidelines vary with respect to the monitoring required but most combine monitoring of blood parameters, including calcium, phosphate and vitamins D and K, with bone mineral density (dual energy X-ray absorptiometry, DEXA) scanning at intervals determined by the degree of bone loss (122). Replacement of deficient vitamins and minerals is sufficient in many cases though treatment with bisphosphonates may sometimes be required (73).

1.1.6.4 Gastrointestinal symptoms

Motility in the CF gut is abnormal with prolonged oro-caecal transit times compared to non-CF subjects (123). This predisposes patients to a number of GI complications and may cause bloating, nausea and abdominal pain. Constipation may be the most common manifestation of dysmotility in CF (124). Management is based on ensuring adequate hydration and use of osmotic laxatives where indicated.

Gastro-oesophageal reflux may occur in 30% of people with CF (10), with silent reflux in up to 90% of patients (124). Reflux is associated with worse lung function though causal evidence is lacking (125).

Symptomatic reflux should be treated with a proton-pump inhibitor; in severe, refractory, cases fundoplication may be indicated (124).

Meconium ileus is a neonatal complication of CF. Abnormally thick, sticky meconium causes bowel obstruction in the neonatal period and may require surgical treatment and ileostomy (which is subsequently reversed in most cases). It occurs in 15-25% of patients at initial presentation (10, 12) .

In older children and adults, distal intestinal obstruction syndrome (DIOS) is probably caused by a similar mechanism to meconium ileus (126). Layers of dehydrated intestinal secretions and viscid faecal matter adhere to the bowel wall, most commonly in the terminal ileum. Patients present with an acute or subacute history of pain in the right lower quadrant, abdominal distension and, depending on the degree of obstruction, vomiting. DIOS can usually be managed conservatively with a combination of oral rehydration and osmotic laxatives containing polyethylene glycol (PEG). A nasogastric tube may be indicated where the patient is vomiting and, where oral treatment fails, a gastrografin enema, performed by a specialist radiologist may be considered (73, 126). Risk factors for DIOS include pancreatic insufficiency, inadequate PERT dosing, CF-related diabetes, and a history of meconium ileus or a previous episode of DIOS (126). Organ transplantation may also be a risk factor, particularly in the post-operative period (126). Prevention of

future episodes of DIOS, using PEG-containing laxatives, is important after an initial presentation.

1.1.6.5 CF-related liver disease

Abnormalities of liver function tests and sub-clinical liver disease are common in CF, with reported prevalence of non-cirrhotic liver disease ranging from 3.6% in the US (12) to 15.7% in the UK (10). Clinically significant liver disease with cirrhosis is less common, occurring in 3.1% (12) and 2.1% (10) of patients in the US and UK respectively.

CF-related liver disease (CFLD) is caused by biliary obstruction, which variably progresses to focal and multi-lobular biliary cirrhosis as a result of unknown factors (127). Incidence of CFLD is highest in the first and second decades and is virtually unknown after the age of 20 (127). In those affected, CFLD can progress to portal hypertension and hepatic failure necessitating liver transplant. Liver disease accounts for approximately 3% of deaths in people with CF (10, 12). Monitoring for liver disease through clinical examination, liver function tests and ultrasound scanning allows early identification and commencement of ursodeoxycholic acid which may delay disease progression (127).

1.1.6.6 Infertility, subfertility and pregnancy

Male infertility occurs in over 98% of men with CF due to congenital bilateral absence of the vas deferens (CBAVD) causing obstructive

azoospermia (128). The condition may also be seen in CFTR heterozygotes (129) and is considered a CFTR-related condition.

Infertility and subfertility are more common in women with CF, with rates of approximately 35%, compared to 5-15% in the non-CF population (130) and pregnancy rates approximately one-third that of the non-CF population (131). The mechanisms for this include anovulation, related to physiological stress and poor nutrition, abnormalities of the cervical mucus and endometrium, as a consequence of defective CFTR, and lower ovarian reserve in women with CF (132).

With increasing life expectancy family planning has become a vital part of good CF care. For males with CF, sperm production is usually normal so biological fatherhood may be achieved using assisted reproduction techniques (128). In women with CF, it is important to consider the effect of pregnancy on maternal health. Pregnancy may be associated with an increase in the rate of pulmonary exacerbations (133) and decline in lung function (134). Women with CF who became pregnant were found to have better 10 year survival rates than those who did not become pregnant, though this may be due to better baseline health (135).

Ideally pregnancies would be planned. This allows pre-conceptual genetic counselling to assess the risk of the baby developing CF,

important for both male and female potential parents. The baseline health status of the mother can be maximised and potential risks to her health discussed. Regardless of this a pregnant woman with CF requires close monitoring by both the CF and obstetric teams (136).

The advent of CFTR modulators has been associated with improved female fertility in CF (131, 137, 138). The effects of these drugs on the foetus and neonate are as yet unknown, though Nash *et al*. report no adverse reactions in an international survey of pregnant women with CF taking ivacaftor, ivacaftor-lumacaftor and tezacaftor-ivacaftor (139).

1.1.6.7 Sinus disease

Chronic rhinosinusitis is common in people with CF, with a prevalence approaching 100% (140). The paranasal sinuses may be colonised with pathogens prior to their identification in respiratory samples. These are poorly penetrated by systemic or inhaled antibiotics and may provide a reservoir for recurrent lower respiratory tract infection (141, 142).

1.1.7 Prognosis

Life expectancy in CF is increasing, with the rate of increase far outpacing the general population (143). The median predicted survival for babies born today is 50.6 years in the UK (10) and 50.0 years in the US (12). CFTR modulator use is likely to increase this further.

This success has brought new challenges to the management of CF (144). New pathologies are emerging which require even more treatment. For example it is becoming evident that people with CF are at increased risk of gastrointestinal malignancy (124). Diabetic complications may become more frequent as patients survive longer.

Increased longevity also increases patients' exposure to medications. Higher cumulative dose of medications, such as antibiotics, increases the chance of side effects (145, 146).

1.2 *Pseudomonas aeruginosa*

P. aeruginosa is a gram–negative bacillus found ubiquitously in the environment. It has a preference for moist conditions and is commonly found in soil and water (147). It is also present as a coloniser of plants, animals and humans. Typically, *P. aeruginosa* is an aerobic organism but it can act as a facultative anaerobe in microaerophilic and fully anaerobic conditions. The organism is readily able to adapt to environmental stressors (142) and has been found growing in, "…environments as diverse as jet fuel and distilled water." (148). In dry conditions, such as on the surface of medical equipment, it can produce extracellular polysaccharides which protect it from desiccation and has been found to survive in a desiccated state for 125 days (149).

Environmental *P. aeruginosa* are commonly free-living and motile due to the presence of a unipolar flagellum. The organism is also capable

of forming adherent biofilms (150) which, once established, become virtually impossible to eradicate (151).

P. aeruginosa is an opportunistic human pathogen, rarely causing disease in healthy people (152). Susceptible populations include neonates, burns patients and those who have undergone chemotherapy (153, 154). *P. aeruginosa* colonisation of chronic wounds, such as leg ulcers, impairs healing (155) and colonisation of medical devices, such as catheters and joint replacements, is a source of significant morbidity (156).

1.2.1 Sources of *P. aeruginosa* infection in CF

The source of pulmonary infection in CF is usually unknown (157). Pathogenic bacteria have been identified in the environment (158) and transmission in nosocomial settings (159) or between patients has been described (160, 161).

For most patients the source of *P. aeruginosa* infection is probably environmental (162). In the home it has been found in drains, toilets, sinks, taps and nebulisers (163-167) but temporal evidence that these represent the source of infection, rather than contamination from an infected patient, is lacking (168).

P. aeruginosa has been found in soil (169) and both fresh and salt water (170, 171). Living closer to an open body of water has been

associated with an increased risk of developing *P. aeruginosa* infection in people with CF (172). Few recommendations for patients and families about strategies to avoid infection at home exist but case reports have linked incident *P. aeruginosa* to use of hot tubs (173) therefore patients are advised to avoid these and hydrotherapy pools (174, 175).

Definitive, molecular, evidence for the role of cross-infection in the acquisition of *P. aeruginosa* was provided from Liverpool in 1996. Genomic fingerprinting studies confirmed that of 92 patients infected with *P. aeruginosa* 55 had identical strains on DNA testing (176). Cross-infection may be through direct person-to-person contact or through contact with contaminated surfaces. Siblings have been found to share *P. aeruginosa* strains since the 1980s (177-179) but the level of contact needed to facilitate transmission is unknown and probably multifactorial.

Despite thorough investigations of hospital environments (159), including at the time of an outbreak (176, 180, 181), no definitive source has been identified, though the finding of viable *P. aeruginosa* in air samples (157) suggested the possibility of airborne transmission in the manner of other respiratory pathogens (182). Subsequently it has been shown that *P. aeruginosa* can survive not only in respiratory droplets, produced when coughing and sneezing, but also in droplet nuclei, the microscopic (≤5 μm diameter) remnants of evaporated

droplets (183, 184). Droplet nuclei can remain suspended for hours depending on the flow of air and are small enough to deposit in the lower airways (182). In these droplets viable *P. aeruginosa* can spread at least 4m from a coughing patient and survive at least 45 minutes in the air (185).

At present the mechanism(s) of *P. aeruginosa* acquisition in people with CF remain unclear. Lack of knowledge has led healthcare professionals to recommend a variety of strategies to avoid infection, most with no evidence basis (186). Patients and parents have also developed time-consuming practices in the hope of avoiding infection (187, 188). Potential sources of *P. aeruginosa* infection and strategies to reduce the risk will be discussed further in chapter 4.

1.2.2 Difficulties in assessing *P. aeruginosa* infection in CF

1.2.2.1 Sampling

Regular respiratory tract sampling is recommended to allow early detection of new organisms, including *P. aeruginosa*, and facilitate early treatment. US infection control guidelines recommend three-monthly respiratory sampling (174), in the UK the recommendation is to sample every eight weeks (146) whilst the Danish CF Centre samples every month (189). Sampling frequency will affect the chance of finding a pathogen (190, 191). Rogers *et al*. found that a single sputum sample

identified only 58% of the bacterial diversity identified through more intensive sampling (72); too few samples may be falsely reassuring.

Practically, where patients are productive of sputum and able to expectorate, this is the most efficient method of sampling the lower respiratory tract (146). Not all patients however are productive of sputum; those with limited lung disease may not be productive and young children may not be able to expectorate, even in the presence of a wet cough. A number of alternative methods are available including cough swabs (coughing while a swab is held in the oropharynx), cough plates (coughing directly onto an agar plate) or oropharyngeal swabs. Unfortunately, all these methods have lower sensitivity for lower respiratory tract infection than sputum culture (146, 192, 193). The gold standard investigation is bronchoalveolar lavage (BAL) (146), however this technique is invasive and may miss bacteria only present in non-sampled lobes of the lung (162). A reasonable substitute may be induced sputum following administration of nebulised hypertonic saline, which has been shown to be superior to cough swab in assessing children with CF (55). In some CF centres microbiological screening for *P. aeruginosa* is augmented by monitoring for serum antibodies to microbial proteins (190).

1.2.2.2 Strain resolution

In order to understand how *P. aeruginosa* is acquired, comparison of a patient isolate with a potential source is essential. Techniques for identifying related strains of *P. aeruginosa* have evolved since the possibility of patient-to-patient transmission was first suspected. Prior to the advent of genetic techniques phenotypic features were used to type *P. aeruginosa* strains.

In 1975, Zierdt and Williams used two series of antisera to assess 173 *P. aeruginosa* isolates obtained from 144 patients with CF. Of these strains 104 belonged to one serotype which they termed the CF serotype, and which they believed was transmitted between CF patients. They noted phenotypic variation between isolates demonstrating the same serotype, concluding that phenotypic features could not be used to assess strain-relatedness. However the study was limited by the limited number of antisera available (seven in the Fischer system and 18 in the Homma system) and the high percentage of non-typable isolates (194).

Phage-typing, whereby isolates are distinguished on the basis of the pattern of lysis they undergo on contact with bacteriophages, is similarly limited by the number of phages available. These methods are limited, not only by the number of reagents, but also because variable gene

expression leads to limited correlation of the phenotype with a given genotype (195-197).

Molecular techniques enabling identification of *P. aeruginosa* at the genetic level revolutionised the study of its acquisition. For many years the gold standard was pulsed field gel electrophoresis (PFGE). Samples are digested using a restriction enzyme, commonly SpeI, which cuts at a limited number of locations within the genome. The negatively charged DNA fragments are separated through an agarose gel subject to an electric current. Variations in the genome affect the size of the DNA fragments leading to unique band patterns. Strains identified by this method are referred to as pulsotypes.

Polymerase chain reaction (PCR)-based strategies amplify random, or targeted, segments of bacterial DNA which are then electrophoresed for comparison of the banding pattern. Reproducibility of these techniques between laboratories is limited which makes comparing samples in outbreak settings challenging (198). Multiple-locus variable-number tandem repeat (VNTR) analysis (MLVA) amplifies tandem-repeat sections of the *P. aeruginosa* chromosome; the allele identified at each site is coded and these codes can be compared between laboratories (199).

Sequencing of all, or part of the *P. aeruginosa* genome far increases the ability to assess strain relatedness. Multilocus sequence tying

(MLST) compares the sequences of seven highly conserved housekeeping genes. Polymorphisms in these genes are equivalent to separate alleles. The allele present at each locus is coded; isolates with seven matching alleles comprise an individual sequence type (ST). Where 6/7 alleles are matched the strains are presumed to be related. Caveats must be made where there are mutations in DNA repair genes which may lead to a higher frequency of mutation in the studied genes (the hypermutator phenotype) and can lead to strains of the same pulsotype demonstrating different STs (198).

Whole genome sequencing (WGS) is rapidly becoming less expensive. The ability to compare the entire genome of an isolate and identify changes down to the level of a single nucleotide polymorphism (SNP) has revolutionised the study of strain relatedness. Sequencing techniques allow comparisons of strains between laboratories. It affords much greater resolution in comparing isolates from different sources to determine strain relatedness (200).

1.2.2.3 Definitions

There is still no universally agreed definition of chronic *P. aeruginosa* infection. The first definition of chronic *P. aeruginosa* infection was proposed in Denmark in 1974, in which *P. aeruginosa* positive cultures for 6 consecutive months was defined as chronic, with less than this labelled intermittent (201). Definitions varied worldwide until in 2003

Lee *et al*. proposed categorising patients according to four *P. aeruginosa* categories:

- Chronic infection – more than 50% of months, when samples had been taken, are culture positive for *P. aeruginosa*.
- Intermittent infection – When 50% or less of months, where cultures had been taken, are culture positive for *P. aeruginosa*.
- Free of infection – no growth of *P. aeruginosa* for 12 months, having previously been *P. aeruginosa* culture positive.
- Never – *P. aeruginosa* never cultured from sputum or cough swab.

This definition did not specify how often samples should be taken though the authors recommended respiratory tract sampling at least every 3 months. Using months positive rather than samples positive this definition goes some way towards ensuring that patients who had more frequent sampling, for example during a pulmonary exacerbation, were not biased towards a diagnosis of chronic *P. aeruginosa* (191). The Leeds criteria were validated in 2006 by a Belgian group who found good agreement between this definition of *P. aeruginosa* status and clinical disease severity, particularly in children. They also noted agreement between the definitions of *P. aeruginosa* status and *P. aeruginosa* serology (201).

Rates of chronic *P. aeruginosa* infection compiled by patient registries are commonly cited and the definition used in these is variable. The UK CF Registry defines chronic *P. aeruginosa* infection as, “three or more isolates in the last 12 months.” (10). The US CF Foundation Registry Report reports only whether patients have grown *P. aeruginosa* or not and makes no distinction about the chronicity of infection (12). The lack of a universally agreed definition causes difficulties for patient care – cohort segregation of patients depends on an understanding of microbial status. It also leads to difficulties in research as the endpoint of chronic *P. aeruginosa* infection may vary between studies.

Microbiological definitions are further limited by treatment success. Chronic suppressive anti-microbial treatment may lower the burden of *P. aeruginosa* infection below the limit of detection so that patients are incorrectly categorised as intermittently infected or even free from infection, “It is a paradox in CF that as treatment of pulmonary infection improves, diagnosis of such infection becomes more difficult.” (146).

1.2.3 Acute *P. aeruginosa* infection in CF

New *P. aeruginosa* infection may be found incidentally on routine respiratory tract sampling or identified during a pulmonary exacerbation.

Once infection with *P. aeruginosa* is confirmed, early aggressive treatment has been shown to improve outcomes (202). A variety of regimens have been proposed but few trials have compared different

regimens (see Table 3-2). In a landmark study Valerius *et al*. showed that 3 weeks of oral ciprofloxacin and nebulised colistin was superior to placebo in delaying the onset of chronic *P. aeruginosa* infection (203). Further trials have supported the benefit of eradication (204-207) but the optimal regimen is as yet unknown (208). The TORPEDO study compared a two-week course of intravenous antibiotics with a three-month course of oral ciprofloxacin; both arms were also treated with three months of inhaled colistin. The study found that the primary end-point, eradication at three months, sustained to 15 months, was significantly better in the oral ciprofloxacin group but noted that the population was overwhelmingly paediatric which may affect the real-world applicability of the finding (209). More evidence is needed to understand the optimal regimen for eradication.

1.2.4 Chronic infection with *P. aeruginosa*

1.2.4.1 Clinical features and consequences of chronic *P. aeruginosa* infection

The natural history of *P. aeruginosa* infection in CF is of recurrent, intermittent infection which may occur over many years. Ultimately the organism cannot be cleared, and chronic infection is said to have developed (210). Recurrence may be with a new strain, or with the same strain of *P. aeruginosa* - indicating either ineffective eradication or re-infection from a common source (211).

The development of chronic *P. aeruginosa* infection is among the most significant events in the life of a person with CF (212). Both Lee *et al.* and Proesmans *et al*. found that patients categorised by the Leeds criteria as “chronic” were clinically worse than those in other groups (190, 191). Chronic *P. aeruginosa* infection has been associated with worse lung function (210, 213-215), increased rate of lung function decline (215, 216), increased frequency of hospitalisation or pulmonary exacerbation (214, 217, 218) and increased mortality (214, 217, 219).

Chronic *P. aeruginosa* increases treatment burden in CF (220); infection with an epidemic strain further increases this burden (221). As discussed in section 1.1.5.1 nebulised therapy has been shown to improve pulmonary function and reduce the chance of hospitalisation (79), however nebulised therapy is time-consuming (222). Worsened clinical status associated with chronic infection may necessitate other additional, non-specific treatments including invasive nutritional support and increased need for physiotherapy.

Pulmonary exacerbations are more common, necessitating more frequent courses of systemic, often intravenous, antibiotics. Some Centres advocate regular, three-monthly, courses of intravenous antibiotics (223), which, while having a positive effect on lung health, may be burdensome to both patient and family (220). Current guidelines recommend dual therapy with two agents of differing mechanisms of action (146, 224). Evidence for this is weak due to a

lack of robust studies (69), though both the UK CF Trust and the CF Foundation suggest a combination of an anti-pseudomonal β-lactam in combination with an aminoglycoside as an appropriate first line (146, 225). The corollary to this recommendation is the increased chance of side effects with more than one agent (224).

More recently the association of chronic *P. aeruginosa* and poorer outcome has been questioned. Burkett *et al.* found no clinical benefit in patients who cleared *P. aeruginosa* over a three-year follow-up compared to those who failed to clear the organism (226). Sampling in these patients was however only conducted annually, therefore patients falsely labelled as clearing *P. aeruginosa* may have influenced their conclusion. Mayer-Hamblett *et al*. followed up patients who received *P. aeruginosa* eradication as part of the EPIC trial (227). They found that clinical outcomes were similar in patients with sustained eradication compared to non-sustained eradication (a group similar to the Leeds Intermittent infection category) but did not further compare these to patients who developed chronic *P. aeruginosa* infection. They noted that increased anti-microbial usage in non-sustained eradicators may have a beneficial effect on the natural history of *P. aeruginosa* infection compared to older cohorts in whom treatment was less aggressive.

It was previously accepted that by adulthood up to 80% of patients would have chronic *P. aeruginosa* (226, 228) however the proportion of patients with chronic *P. aeruginosa* at any given age is falling. UK CF

Trust data show that in every age bracket the percentage of patients with chronic *P. aeruginosa* infection has fallen between 2010 and 2020 (10); for example in 2010 60.7% of 24-27 year olds with CF had chronic *P. aeruginosa*; in 2020 this had fallen to 30.7% (10). US data is presented in graphical form only but the trend is similar; in 2013 approximately 61% of 18-24 year olds had *P. aeruginosa* infection, dropping to approximately 32% in 2020 (12, 229). It should be noted that there is no breakdown between chronic and intermittent *P. aeruginosa* infection in the US registry data.

1.2.4.2 Psychological effects of *P. aeruginosa* infection

Given the potentially severe consequences of *P. aeruginosa* infection it is therefore unsurprising that *P. aeruginosa* engenders fear in the CF community. Work in Germany by Ullrich *et al.* (188) showed that *P. aeruginosa* is a source of anxiety for parents of children with CF. Some parents developed maladaptive practices in the hope of avoiding infection (187, 188). This was found to be further compounded by conflicting advice given to families by healthcare professionals (186). Some of the practices outlined may themselves be detrimental to the development and mental well-being of people with CF (230). This is discussed further in chapter 3.

1.2.5 *P. aeruginosa* adaptations in cystic fibrosis

Initial *P. aeruginosa* infection is usually with environmental organisms which are relatively antibiotic sensitive (162, 231). Up to 90% of individuals will clear their first infection (151) but studies have shown that eradication therapy with nebulised antibiotics, either with or without oral antibiotics, is better than no treatment at ensuring this occurs (208).

1.2.5.1 Establishment of initial *P. aeruginosa* infection

1.2.5.1.1 Adhesion

Environmental *P. aeruginosa* organisms are commonly motile, with a unipolar flagellum. The flagellum is important in facilitating bacterial penetration of airway mucus in the CF lung (232).

Initial *P. aeruginosa* infection requires adhesion of the organism to the airway epithelial cell wall. A number of components, including flagella, pili, LPS, adhesin and Psl polysaccharide are involved in adhesion of *P. aeruginosa* microorganisms to airway epithelial cells. Binding of these structures and molecules to airway surface receptors, including Toll-like Receptors (TLRs) and asialoGM1, stimulates the production of pro-inflammatory cytokines, including IL-8, and activation of the immune response as described in section 1.1.4.2.2 (54).

P. aeruginosa adherence may be increased in the presence of other microorganisms, including respiratory syncytial virus (RSV) (233).

1.2.5.1.2 Virulence factors

P. aeruginosa possesses a wide array of virulence factors, including outer membrane proteins, flagella, pili, enzymes (proteases, catalases and lipases), rhamnolipids, pyocyanin, exotoxins and extracellular polysaccharides. Virulence occurs both through direct damage to host tissues and interference with the host immune response (234).

P. aeruginosa can be cytotoxic, particularly in acute infection. Pilin-mediated adherence facilitates the injection of exotoxins directly into airway epithelial cells (54) leading to cellular death and interference with the epithelial barrier. Expression of these exotoxins is associated with increased mortality (235).

Exotoxin secretion can also cause lysis of neutrophils which both reduces phagocytosis of the invading organisms and provides extracellular DNA (eDNA), important in biofilm formation (see section 1.2.5.2.2). Proteases breakdown innate antimicrobial peptides and complement while elastases degrade matrix proteins within the lung architecture. Pyocyanin, in addition to being pro-inflammatory, causes neutrophil apoptosis, while rhamnolipids lead to neutrophil necrosis (35). Necrotic neutrophils release ROS and enzymes, further contributing to tissue damage (236).

1.2.5.2 Development of chronic infection

The unique environment of the CF lung facilitates *P. aeruginosa* evolution into a phenotype fitted for chronic infection. Stressors include competition from other organisms for space and nutrients, the anaerobic environment (secondary to thick airway mucus), high antibiotic concentrations and excess neutrophil recruitment. This provides both a selection pressure for mutation and a stimulus to adopt newly favourable phenotypic features.

1.2.5.2.1 Quorum sensing

Many microorganisms, including *P. aeruginosa,* employ a method of cell-to-cell signalling, termed quorum sensing (QS), to facilitate adaptation to their environment. This signalling is dependent on the population density. Secretion of small, diffusible molecules regulates transcription and the expression of traits to promote survival.

Activation of QS is promoted by nutrient scarcity, particularly iron and phosphate, low oxygen tensions and host factors (236). *P. aeruginosa* demonstrates four known QS systems which promote biofilm formation and regulate virulence factor production (237).

1.2.5.2.2 Biofilm development

Evolution from planktonic to sessile growth provides protection from the effect of antibiotics and neutrophil phagocytosis (47, 238). A biofilm consists of microorganisms growing within an extracellular matrix

(ECM). For *P. aeruginosa* this matrix comprises exopolysaccharides (Psl, Pel and alginate), eDNA and proteins (including flagella and pili).

P. aeruginosa within a biofilm is significantly more resistant to the effect of antimicrobial agents, due to reduced penetration of the antibiotics into the ECM and to specific effects of the ECM which can directly sequester cationic antimicrobials, including aminoglycosides (239, 240).

Biofilm components further stimulate neutrophil recruitment however the physical barrier means phagocytosis is further inhibited. Additionally, defence mechanisms are subverted; NETs are equally unable to penetrate the matrix to kill the bacteria, but their constituent DNA is used to support the biofilm ECM.

In vivo biofilms consist of multiple species of microorganism. Multi-species biofilms are only beginning to be understood but interactions with other microorganisms may increase *P. aeruginosa* virulence. *In vitro* experiments have shown that in a mixed biofilm with *Aspergillus fumigatus* production of elastase by *P. aeruginosa* is increased (241).

1.2.5.2.3 Mucoidy

Alginate is one of three exopolysaccharides which contribute to *P. aeruginosa* biofilm. Overproduction of alginate, beyond that required for biofilm formation, leads to the development of mucoid colonies (242). Mucoidy is a feature characteristically associated with *P. aeruginosa* isolated from CF lungs (243, 244).

Mucoidy occurs following mutations in the *mucA* gene. The *mucA* product is an inner membrane bound protein, MucA, that sequesters the transcription factor AlgT. Loss of MucA frees AlgT, activating genes required for alginate biosynthesis. It has been shown that mutations in *mucA* are a common response to situations of sublethal oxidative stress (245); again *P. aeruginosa* subverts the host immune response to facilitate favourable development within the CF lung.

Overproduction of alginate provides a physical barrier. In addition, alginate can scavenge ROS (246, 247), and mucoid variants are less susceptible to complement mediated killing and phagocytosis (44).

Alginate overproduction also affects the physical development of the biofilm, with increased microcolony formation, greater thickness and a more heterogenous structure (242). This provides increased protection from antimicrobial agents, with resistance up to 1000 times greater to tobramycin than non-mucoid biofilms (242). Conversion to mucoidy may further promote *P. aeruginosa* persistence in the CF lung.

In addition, AlgT has a promoter effect on genes beyond those in the alginate pathway. Transcription of genes which code for lipoproteins is enhanced in mucoid *P. aeruginosa* (248). Lipoproteins have a further immunostimulatory effect, leading to increased production of NF-κB and IL-8, with greater effects seen in cells carrying CF-causing CFTR variants (248). Regions of the lung in which *P. aeruginosa* colonies are mucoid or mixed are associated with increased levels of pro-

inflammatory cytokines than those without *P. aeruginosa* or with non-mucoid colonies. This may in part explain the detrimental effect of mucoid *P. aeruginosa* conversion on prognosis in CF (210, 219).

Despite the benefits of mucoidy, co-existence of mucoid and non-mucoid organisms is common in CF cultures. Production of alginate has a high metabolic cost (44). Reversion to a non-mucoid phenotype may occur via mutations in *algT*, among other mechanisms. The presence of mixed cultures may further improve antimicrobial resistance; in co-culture mucoid *P. aeruginosa* cells shield non-mucoid cells via alginate, while non-mucoid cells produce high levels of catalase which inactivate ROS (249).

1.2.5.2.4 Changes in virulence

Classically the evolution of *P. aeruginosa* in CF was thought to be associated with a loss of virulence factors. It is now apparent that the production of virulence factors adapts to those most fitted for survival in the CF lung.

Downregulation of the exotoxins associated with the type III secretory system is likely due to the requisite high energy costs and the type IV pili needed for injection of these substances into host cells (147). Indeed, isolates from CF sources are much less likely to produce any of the type III secretory proteins, with associated reduction in acute mortality (235).

Loss of flagella production is associated with reduced motility. Flagellum-associated motility is of less benefit to sessile organisms and inhibition of flagellum-production is mediated by AlgT (147). Loss of the flagellum may have a two-fold benefit; reduction in immunostimulation and reduced phagocytosis, which is mediated by binding of the flagellum to TLR5 on phagocytic cells. Other adherence factors are down-regulated; in *P. aeruginosa* retrieved from explanted lungs very few adherent cells were identified with little direct epithelial damage (54).

QS mutants are common in CF isolates. Mutations in the QS-regulator *lasR* may reduce the production of virulence factors such as the elastase, LasB (147) but these mutants have also been shown to increase the production of IL-8 and other pro-inflammatory cytokines *in vivo* (250).

P. aeruginosa LPS evolves in chronic CF infection, with loss of O-side chains and loss of serotypability (251). Modifications of the lipid A part of LPS are common in CF and may increase host inflammatory response and reduce bacterial susceptibility to antibiotics (252, 253).

Thus, evolution of *P. aeruginosa* in the CF lung does not simply reduce virulence but converts factors associated with virulence from those associated with acute mortality to those fitted for persistence within the CF lung.

1.2.5.2.5 Metabolic changes

It's large genome (5.5-7Mb) makes *P. aeruginosa* highly adaptable to diverse environmental habitats. Unlike many habitats, the CF lung provides a rich source of nutrients, including amino acids, fatty acids and lactic acid (254), though oxygen tension is low.

The abundance of amino acids within the CF lung drives development of auxotrophy, particularly for methionine, leucine and arginine (255). Furthermore, evolution towards increased efficiency of use is greater for higher energy cost amino acids (256).

Low oxygen levels initially promote the use of nitrate as an alternative electron acceptor but *P. aeruginosa* also adapts to ferment acetate and arginine, bypassing most of the TCA cycle through the glyoxylate shunt (254, 256). These processes yield less energy than oxidative phosphorylation and contribute to reduced growth rates (257).

While many nutrients are in good supply in the CF lung iron and zinc, important enzymatic co-factors, are sequestered by the host. Production of iron and zinc scavengers, including siderophores and haem are upregulated compared to environmental *P. aeruginosa* isolates (254).

1.2.5.2.6 Antibiotic resistance

As described above a number of *P. aeruginosa* adaptations in the CF lung assist with increasing bacterial tolerance to antimicrobial agents.

In addition to the physical barrier provided by the biofilm ECM and the specific effects of, amongst others, alginate and LPS, *P. aeruginosa* has high intrinsic resistance to antimicrobials which is augmented by adaptation, and acquisition of drug-specific mechanisms promote antimicrobial resistance.

The *P. aeruginosa* cell wall is significantly less permeable than other bacteria, for example that of *Escherichia coli* (258). Access of antibiotics is mediated either by membrane bound porins (β-lactams, quinolones) or by interaction of the antibiotic with surface structures, such as LPS (aminoglycosides, colistin). Efflux pumps extrude β-lactams, quinolones and aminoglycosides while constitutively expressed enzymes such as β-lactamases and aminoglycoside modifying enzymes reduce the antibiotic concentration within a cell (232). All these features may be induced through altered translation, or acquired, either through mutations or acquisition of externally derived DNA (258).

Mutations may reduce the expression or function of porins, thereby reducing access of antibiotics into the cell (259) or increase the expression of efflux pumps (260, 261), preventing sufficient antibiotic concentrations occurring within the organism. Modification of antibiotic targets prevents binding of the agent with subsequent functional reduction. Examples include alterations to the ribosome to prevent binding of aminoglycosides (262) and changes to the structures of DNA

gyrase to prevent binding of fluoroquinolones (261). Mutational increase in the production of enzymes, such as β-lactamases (259), further reduces intracellular antibiotic concentration.

Acquisition of resistance mechanisms can occur by horizontal transfer of DNA. Plasmids, transposons, integrons and prophages provide sources of one or multiple resistance genes through intra- or inter-species transfer (258).

Within a biofilm a small proportion of cells, termed persister cells, become dormant, with dramatically reduced metabolic activity. These cells are highly resistant to the effects of antibiotics and can repopulate a biofilm after cessation of antibiotic treatment (254).

1.2.5.2.7 Evolution of persistence

Mechanisms of persistence are likely multifactorial and dependent on both patient and microbial factors. In a study examining mechanisms of *P. aeruginosa* persistence in CF no bacterial phenotypic feature was found to correlate with development of chronic infection; indeed, the only significant factor found was patient age at acquisition, with an older age associated with a greater chance of persistence (263). Potential reasons for this include a higher burden of lung damage, unrecognised persistence of a previously acquired *P. aeruginosa* infection or improved ease of sampling in older patients (either through ability to expectorate or increased sputum production).

1.3 Aminoglycoside antibiotics

Aminoglycosides are bactericidal antibiotics with a broad-spectrum of action. They are highly efficacious against gram-negative aerobic organisms, including *P. aeruginosa*. Clinically they are most commonly used in the treatment of severe gram-negative infections, such as urosepsis, and sepsis of unknown origin. They have some gram-positive activity and can be used in the treatment of neonatal group B streptococcal infection. Because they are bactericidal, aminoglycosides are also used in the treatment of bacterial endocarditis.

Streptomycin, the first aminoglycoside antibiotic was isolated from a strain of *Streptomyces* in 1944 (264). It was one of the first antibiotics to be developed with activity against *P. aeruginosa* (264). Gentamicin was identified in 1963 and tobramycin in 1967 (265). Other agents in the class include netilmicin, kanamycin and amikacin.

Chemically aminoglycosides consist of several amino sugars (aminoglycosides) linked to a dibasic cyclitol (see Figure 1-4). They are water soluble but positively charged (266) and highly polar so must be administered parentally (267). The positive charge comes from the multiple amine groups which are protonated at physiological pH (268).

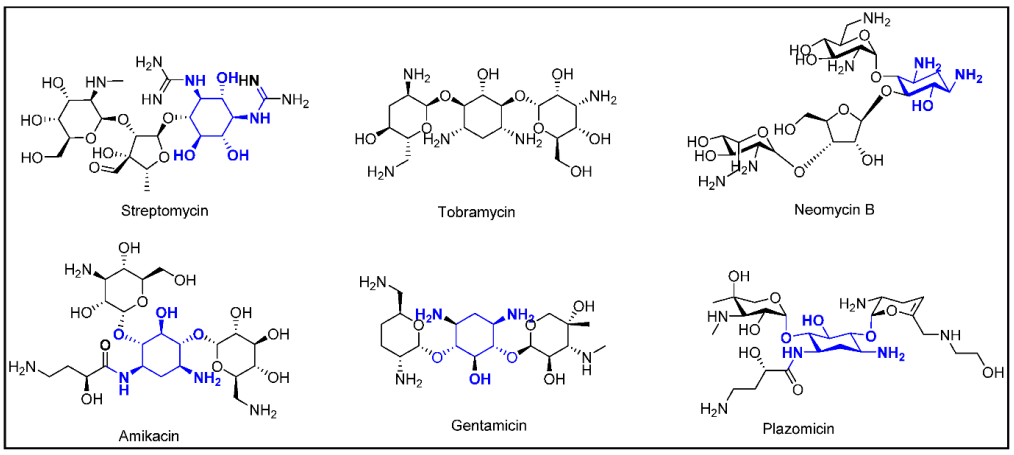


Figure 1-4: Chemical structure of aminoglycosides, with structural differences highlighted in blue. Reproduced from Childs-Kean et al (2019) (269) under a Creative Commons Attribution 4.0 International license (270) Creative Commons — Attribution 4.0 International — CC BY 4.0.

1.3.1 Mechanism of action

Aminoglycosides act in a concentration-dependent manner. The positive charge on aminoglycoside molecules allows them to interact with negatively charged lipopolysaccharide (LPS) in the bacterial cell membrane, displacing magnesium ions which act to stabilise the membrane (271). Transport across the cell membranes is an energy-dependent process which can be interrupted by hyperosmolarity, low pH and anaerobic conditions (265), important considerations in the CF lung. Blocking transport into the cell is an important mechanism of bacterial resistance (265).

Once inside the bacterial cell the positive charge gives the aminoglycoside an affinity to nucleic acids, in particular prokaryotic ribosomal RNA (rRNA) (268). They bind to the bacterial ribosome, in

both the 30S and 50S subunits (272), blocking protein synthesis. In combination damage to the cell wall and blockage of protein synthesis cause rapid bacterial death (265).

1.3.2 Pharmacokinetics

Aminoglycosides are administered parenterally or topically and are renally excreted with minimal prior metabolism. They are filtered at the glomerulus with approximately 15% reabsorbed into the proximal tubule via a saturable, calcium-dependent, active process (265, 273).

Historically aminoglycosides were administered in divided doses three times a day. A large multi-centre randomised controlled trial, in people with CF, showed no difference in efficacy in once vs. three-times daily tobramycin dosing, with once-daily dosing associated with a reduced risk of nephrotoxicity in children (274).

Once daily aminoglycoside-dosing is now preferred for a number of reasons. Firstly, aminoglycoside-induced bacterial killing is dose-dependent; giving a single, larger dose results in a higher peak concentration (275) and therefore improved bactericidal action. Secondly, aminoglycosides show a post antibiotic effect whereby inhibition of bacterial growth continues after the plasma concentration has fallen below the minimum inhibitory concentration (MIC). This effect is prolonged following a higher peak (276). Thirdly, gram-negative organisms, including *P. aeruginosa*, show adaptive resistance

to aminoglycosides. By this phenomenon prolonged exposure to the drug (> 2 hours) results in reduced efficacy of bacterial killing for several hours after exposure (277); without a drug-free break *P. aeruginosa* can survive in an aminoglycoside concentration many times the original MIC. Finally, a once-daily regimen may be more acceptable to patients and less intrusive in their lives (274).

1.3.2.1 Altered pharmacokinetics in CF

People with CF have altered pharmacokinetics for antibiotics compared to the general population. This is believed to relate to an increased volume of distribution and increased renal clearance of aminoglycosides (278). Airway aminoglycoside concentrations are dependent on serum concentrations; when this is reduced their concentration-dependent bactericidal activity will be impaired. Higher doses of aminoglycosides, compared to the general population, are therefore recommended in CF (146, 279). It is possible that the altered pharmacokinetic effects are related to an increase in fat-free mass in people with CF. Hennig *et al.*, using a model of pharmacokinetics, compared CF and non-CF populations and found no difference once subject age, fat-free mass, sex and renal function are accounted for (280).

With the advent of CFTR modulators the body composition of many people with CF is changing (281). Some of these effects may therefore

be mitigated and further research will be needed to assess whether the altered pharmacokinetics remain.

1.3.3 Aminoglycosides in cystic fibrosis

Aminoglycoside antibiotics have a number of uses in CF:

- Intravenous treatment of pulmonary exacerbations in patients chronically infected with *P. aeruginosa*.
- Long-term suppressive, inhaled, therapy for patients chronically infected with *P. aeruginosa*.
- Eradication regimens for new *P. aeruginosa* infection (may be intravenous or nebulised).
- Treatment of atypical organisms, such as non-tuberculous mycobacteria (may be intravenous, inhaled or a combination).

In contrast to the general population, in which gentamicin is the most commonly used aminoglycoside, in CF tobramycin is preferred. Several studies have shown that tobramycin is less nephrotoxic than gentamicin (282, 283) and 47% of *P. aeruginosa* samples tested were resistant to gentamicin compared to only 10% resistant to tobramycin in a UK survey (284).

Atypical CF pathogens may require aggressive treatment with combination antibiotic regimens which include prolonged courses of aminoglycosides. *M. abscessus* acquisition causes a steep decline in

lung function (64). Aggressive treatment of this organism usually comprises an induction phase of intravenous amikacin in combination with another IV agent and azithromycin for 3-12 weeks. This is then followed by combination therapy including nebulised amikacin (65, 146).

Aside from the altered pharmacokinetics, the tenacity of infection in CF, coupled with difficulties in antibiotic penetration of thickened respiratory secretions, necessitate longer courses, typically 14 days, compared to 5-7 days in a non-CF patient. Chronic infection with *P. aeruginosa* causes an increased frequency of pulmonary exacerbations (214). People with CF are therefore exposed to higher doses of aminoglycosides, for longer and more frequently than the general population. In the 1980s a Danish group showed that aggressive, regular antibiotic treatment was beneficial in improving survival (223). Many CF patients will undergo three to four courses of IV antibiotics per year, though this may change in light of CFTR modulator therapy (10).

1.3.4 Side effects of aminoglycosides

Aminoglycosides have two main classes of adverse effects, nephrotoxicity and ototoxicity; both were described during the first clinical trials of streptomycin (267). There is no significant relationship between nephro- and ototoxicity (285). Ototoxicity will be discussed in detail in chapter 2.

1.3.4.1 Nephrotoxicity in CF

Nephrotoxicity associated with aminoglycoside administration is usually acute and reversible (266, 267). A survey by Bertenshaw *et al.* in the UK found that the incidence of acute renal failure (defined as raised plasma creatinine for age with or without oliguria) in CF patients was 4.6-10.5 cases per 10,000 CF patients per year. Eighty-eight per cent of these cases had received an aminoglycoside in the preceding week (283). Of these patients 13 required dialysis and 2 had ongoing renal impairment.

Plasma markers of renal function may be insufficiently sensitive to identify early injury (286). N-acetyl-β, D-glucosaminidase (NAG) is found in lysosomes of the cells of the proximal renal tubule and may be a more sensitive marker for early renal damage (287). Treatment with a 14-day course of tobramycin caused an eight-fold increase from baseline in the levels of urinary NAG in patients with CF despite normal plasma markers; the increase was greatest in patients with CF-D (286). In children once daily tobramycin administration was associated with reduced increase in urinary NAG levels compared to three-times daily administration (274).

Chronic renal impairment as a consequence of aminoglycoside therapy is less well-established. In 1987 Pedersen *et al.* studied nephrotoxicity before and after a course of tobramycin in 46 people with CF. They

found no correlation between creatinine clearance and cumulative tobramycin dose. Although 18/46 patients had a creatinine clearance less than 1.6 ml/s per 1.73m^2 body surface area (their cut-off for chronic nephrotoxicity) they concluded that since all had a serum creatinine within the normal range any toxicity was slight (288). In contrast Al-Aloul *et al.* examined 80 CF outpatients and found a strong correlation between cumulative aminoglycoside exposure and diminished renal function (289).

1.3.4.2 A brief outline of renal physiology

The functional unit of the kidney is called the nephron. Each nephron comprises a renal corpuscle (made up of a tuft of capillaries, the glomerulus, surrounded by Bowman's capsule) and a renal tubule. Filtration of blood from the glomerulus to produce ultrafiltrate within Bowman's capsule is dependent on the pressure and concentration gradient between these spaces. Pressure in the glomerulus is controlled by the relative pressures in the afferent and efferent glomerular arterioles, which in turn affect renal blood flow. Increased renal blood flow and greater pressure gradient equate to a higher glomerular filtration rate (GFR). GFR is a measure of renal function, with falling GFR eventually leading to renal failure.

Ultrafiltrate generated in Bowman's capsule flows through the renal tubule. Active reabsorption of electrolytes and solutes with passive

reabsorption of water along the concentration gradient leads to the formation of concentrated urine. In addition, some molecules, including aminoglycosides, are actively secreted by the proximal tubule. These processes allow for water and electrolyte homeostasis, acid-base balance and excretion of toxins.

1.3.4.3 The mechanism of aminoglycoside nephrotoxicity

The mechanism of aminoglycoside nephrotoxicity is not fully established but probably represents a combination of damage to the glomeruli and proximal renal tubules, and vascular effects leading to reduced renal blood flow. Much of the work has been done in-vitro, in animal models and using gentamicin so care must be taken in extrapolating effects to humans and for other aminoglycosides (273).

Gentamicin is taken up into the epithelial cells of the proximal tubule by the cubulin-megalin complex (273). Accumulation within lysosomes and the endoplasmic reticulum triggers apoptosis of proximal tubular cells. In addition, within the cytosol, gentamicin complexes with iron to form ROS which can indirectly trigger apoptosis. At higher concentrations cell necrosis may be triggered. Death of proximal tubular cells causes obstruction of the affected tubule and reduced reabsorption of water and electrolytes distally. Reduced reabsorption of water and electrolytes would lead to rapid, potentially fatal fluid shifts thus the tubuloglomerular feedback mechanism is rapidly triggered.

This reflex causes rapid reduction in renal blood flow, which together with the increased backpressure caused by blocked renal tubules, reduces the ultrafiltration pressure and consequently the glomerular filtration rate (290).

Gentamicin has further direct effects on the glomerulus, primarily causing contraction of the supporting mesenchymal cells which reduces the surface area available for ultrafiltration, further reducing the GFR (291).

1.3.4.4 Monitoring for nephrotoxicity

Higher individual and cumulative aminoglycoside doses leave people with CF at greater risk of side effects. The therapeutic window for aminoglycosides is small (292). Renal function should be checked prior to starting a course of aminoglycosides, with the dose reduced if this is impaired (293). Monitoring to ensure plasma levels have dropped (for example to <1mg/l in the case of tobramycin) prior to administration of the next dose should be undertaken (146). Monitoring of serum levels does not however, prevent all aspects of toxicity as some patients may develop adverse effects despite levels within the therapeutic range (294, 295).

1.4 Harm

“First do no harm,” as paraphrased from the Hippocratic Oath (296), is widely taught to medical professionals early in their training. It informs the principle of non-maleficence, one of the Four Principles of medical ethics defined by Beauchamp and Childress (297) which underpin modern clinical medicine.

1.4.1 What is harm?

A simple definition of harm is, “a negative effect, whether or not it is known to the patient.” (298). A more revealing definition is, “Physical or psychological injury (including increased anxiety), inconvenience (such as prolonged treatment), monetary loss, and/or social impact etc. suffered by a person.” (299).

Since both action and inaction can cause harm, treatment decisions are commonly a balance between the probability of achieving the least harm for the greatest health benefit.

This is particularly true in the case of historically fatal diseases which, with advances of modern medicine, may instead be chronic and life-limiting. Altering the natural history of such diseases requires increasingly complex treatment regimens, to either better control symptoms or mitigate those caused by essential therapies.

Polypharmacy will often decrease the patient's quality of life and the chance of adherence to the regimen (300).

Patient harm may be predictable, and potentially preventable. Failing to monitor potentially toxic medications can lead to serious side effects. Failing to effectively separate patients infected with multi-resistant organisms may lead to transmission of these pathogens to other patients, with potentially life-threatening consequences. Equally the provision of important information about a health condition can generate anxiety. This could also be seen to cause harm but may not be preventable. Idiosyncratic reactions to medications and known side effects can also cause harm which cannot be completely mitigated.

1.4.2 Differences in perception of harm between healthcare professionals and patients/carers

Medical definitions of harm do not always correspond to the harm felt by patients; a patient may be far more affected by time taken up by treatment (for example nebulised antibiotics) than an arbitrary numerical endpoint (for example drop in lung function), yet clinical trials may not take this factor into account.

Cystic fibrosis (CF) is a life-long illness and many patients become experts in managing their own disease. Treatment, and all its attendant risk of harm, must therefore be a collaborative process between a multi-professional team, the patient and those close to them to provide an

individually tailored regimen which takes into account all aspects of the patient's life.

Harm cannot be completely prevented but by remaining vigilant, providing appropriate monitoring and information, making changes to care where necessary and by doing all this in concert with the patient, much can be done to mitigate it.

1.5 Preventative medicine

1.5.1 Primary prevention

Primary prevention is the attempt to prevent an illness or event occurring by putting in place mechanisms which apply to the whole population or a targeted, at-risk population. Examples include vaccination, to prevent potentially lethal or life-changing infections, fortification of basic foodstuffs to prevent diseases of nutritional deficiency and folic acid supplementation, recommended for women considering pregnancy to reduce the chance of neural tube defects.

In CF primary prevention for the acquisition of *P. aeruginosa* may reduce the morbidity and mortality associated with chronic infection. Heinzl *et al*. proposed the use of nebulised gentamicin in CF patients deemed at increased risk of *P. aeruginosa* acquisition. This retrospective study found that none of the 12 patients who remained on treatment for the duration of the study became colonised with *P.*

aeruginosa. Of the 16 patients who discontinued treatment, seven became chronically infected with *P. aeruginosa*. These patients had significantly worse lung function compared to non-infected patients (301). This strategy involves a high burden of treatment and the risk of side effects (indeed in the above study 3/16 patients stopped treatment due to side effects).

For many parents of people with CF the lack of knowledge regarding *P. aeruginosa* source is a major source of frustration [51]. Greater understanding of the sources of *P. aeruginosa* infection and the mechanism(s) of acquisition might bring reassurance and perhaps allow a less invasive method of primary prevention.

1.5.2 Secondary prevention

Secondary prevention is the early identification of a health problem prior to the occurrence of symptoms. Examples in the general population include identification and treatment of hypertension before the development of end-organ damage or the provision of aspirin to prevent a further myocardial infarction (302). Screening programs, including cervical smear testing and mammography are also examples of secondary prevention.

In CF secondary prevention can take many forms. In patients who have acquired *P. aeruginosa,* successful eradication may prevent or delay the onset of chronic infection and the attendant clinical

deterioration. Secondary prevention, achieved by eradication, may be augmented by further treatment to prevent recurrence of *P. aeruginosa*.

Screening for ototoxicity in people with CF undergoing treatment with aminoglycosides is currently sub-optimal. The implementation of a screening program which could pick up hearing loss at an early stage, allowing identification of early hearing damage and the modification of treatment to prevent further deterioration would have a positive effect on quality of life.

1.5.3 Tertiary Prevention

Tertiary prevention occurs in patients with established disease, with the intention to minimise the impact of illness and improve quality of life, for example rehabilitation programs in patients who have had a stroke (302).

Examples of tertiary prevention in CF include inhaled anti-pseudomonal antibiotics in people with chronic *P. aeruginosa* infection to reduce ongoing lung damage and provision of hearing amplification devices to those with established hearing loss.

In Chapter 2 I will now consider the problem of ototoxicity as a consequence of antibiotic therapy in CF.

Chapter 2: Ototoxicity in Cystic Fibrosis

2.1 What is ototoxicity?

Ototoxicity is defined as damage to the inner ear following exposure to drugs or chemicals. Damage may be to the cochlea (cochleotoxicity), the vestibular system (vestibulotoxicity) or both. A variety of therapeutic agents can cause ototoxicity; commonly implicated classes of drugs including antibiotics, particularly the aminoglycosides, anti-cancer drugs and diuretics (303).

In this chapter I will be examining the effect of medications on hearing in people with CF so will use ototoxicity to mean cochleotoxicity unless specifically stated.

2.2 What is hearing?

The human ear converts sound waves to electrochemical impulses; hearing however, is more complicated than simple sound transduction. It requires an ability to detect and recognise sounds, localise them to the source and interpret their meaning.

Hearing is affected as much by surroundings as by the hearing ability of the participants. The presence or absence, and character of background noise, as well as the acoustics of the space and any competing signals all have an effect. Other intrapersonal qualities, including the speech characteristics of the speakers and the

participants' cognitive abilities, have a bearing on successful hearing (304, 305).

The conjunction of these factors means that even where there is functional impairment, where other circumstances are favourable hearing may be good. Conversely even people with normal hearing may struggle in challenging conditions.

Human auditory pathways are designed to fulfil the functions of hearing; damage at any point in the pathway can lead to hearing impairment. Where this impairment is sufficient to compromise activities of daily living a person can be said to have a hearing handicap, while disability reflects an inability to complete job tasks as a consequence of hearing handicap (304).

2.3 Anatomy of the ear and physiology of hearing

2.3.1 The outer ear

The external ear, also called the auricle or pinna, is a cartilaginous structure comprising a helix and an anti-helix. It is shaped to catch sounds and the cartilaginous ridges can modify these to aid localisation (306).

Sound waves are funnelled into the external auditory canal at the external auditory meatus. The canal is a skin-lined, sigmoid shaped tube running posteromedially in the temporal bone. The outer one-third

is cartilaginous with some flexibility while the inner two-thirds are bony and fixed. Resonance properties within the canal improve sensitivity of the human ear to sound frequencies between 1000 and 6000 Hz, where most speech sounds are pitched (see Figure 2-6) (306). The external auditory canal ends at the tympanic membrane (TM).

2.3.2 The middle ear

The TM is a three-layered structure eight to ten mm in diameter which is stretched across the external auditory canal. Medial to the TM is the middle ear cavity which contains the ossicles and auditory muscles. The inner wall of the cavity has two openings in continuity with the inner ear, the oval window and the round window.

The ossicles are three small bones, the malleus, the incus and the stapes, which transmit sound waves to the inner ear. The handle of the malleus is in continuity with the TM while its head is attached to the body of the incus. The long process of the incus attaches to the head of the stapes, the footplate of which is in contact with the oval window. The short process of the incus is attached to the middle ear cavity via a ligament.

The ossicles function as a transducer to improve the efficiency of sound transduction between the air-filled middle ear cavity and the fluid filled cochlea. Differing movement of sound waves through air and water would cause significant loss of signal with simple air-cochlear fluid

transmission. Reduction in the surface area from the TM to the stapes footplate leads to an equivalent increase in the pressure transduced which offsets the different impedance properties of air and water (306).

Two auditory muscles attach to the bones. The tensor tympani attaches to the malleus; contraction of this causes increased tension of the TM to reduce vibration and therefore sound transmission. The stapedius muscle attaches to the stapes, reducing vibration of the ossicles and again reducing transmission of sound to the inner ear. These muscles have a protective effect from loud noise but act too slowly to protect against sudden noises.

The middle ear cavity also contains the superior opening of the Eustachian tube which connects the middle ear to the nasal cavity to allow equalisation of pressure, for example at altitude.

2.3.3 The cochlea

The inner ear is situated within the petrous part of the temporal bone. It has two parts, the cochlea and the vestibular apparatus (see Figure 2-1). The vestibular semi-circular canals detect angular motion and are important as the organs of balance.

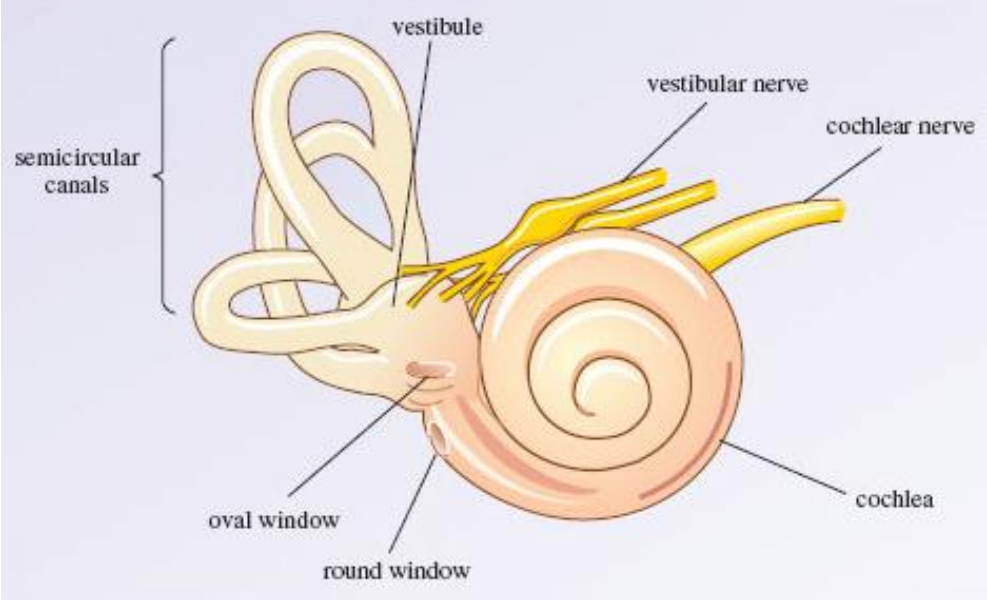


Figure 2-1. A diagrammatic representation of the human cochlea. Reproduced unchanged under the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 Licence from The Open University, available at https://www.open.edu/openlearn/science-maths-technology/biology/hearing/content-section-3.1 (307).

The cochlear is a 3.5cm long, seven mm diameter tube. It is membrane lined and sits within a bony canal. The tube forms a spiral helix with two-and-a-half turns. A central bony canal, the modiolus, contains blood vessels and the auditory nerve fibres.

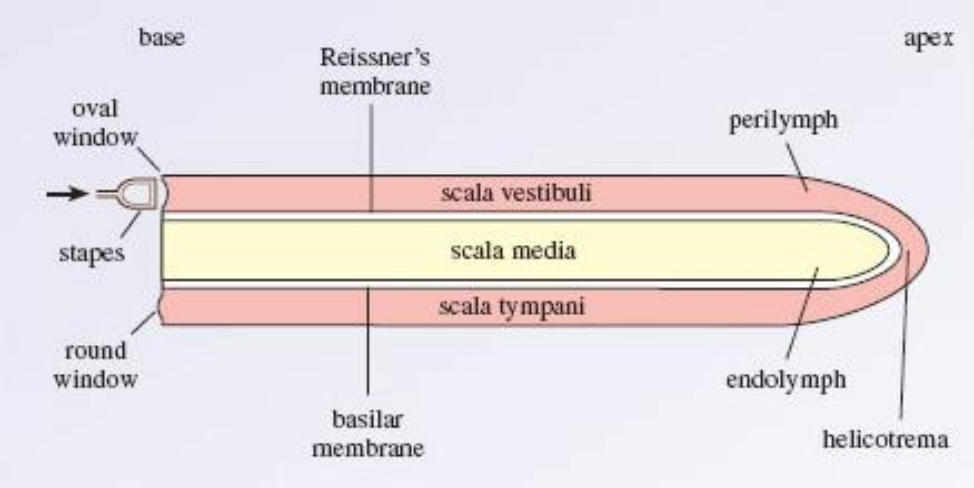


Figure 2-2: A diagrammatic representation of an uncoiled cochlea. Reproduced unchanged under the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 Licence from The Open University, available at https://www.open.edu/openlearn/science-maths-technology/biology/hearing/content-section-3.2 (308).

The spiral cavity is divided into three chambers by two membranes (see Figure 2-2 and

Figure 2-3). Reissner's membrane sits superiorly and the basilar membrane inferiorly. The scala vestibuli and scala tympani contain perilymph and are connected apically at the helicotrema. The basal end of the scala tympani ends at the round window, the membrane covered opening into the middle ear cavity. They form a closed system such that when pressure is applied to the oval window through vibration of the stapes, there is simultaneous deformation of Reissner's membrane and the basilar membrane, and bulging of the round window into the middle ear cavity.

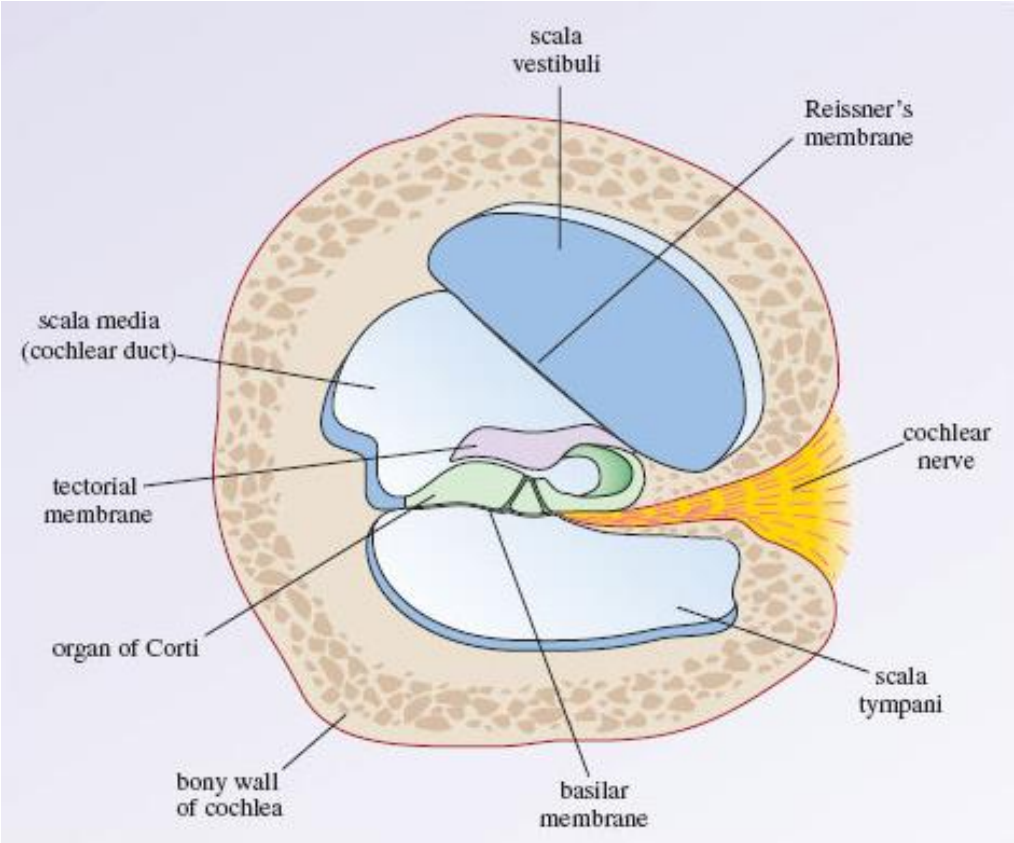


Figure 2-3: A diagrammatic representation the cochlea in cross-section. Reproduced unchanged under the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 Licence from The Open University, available at https://www.open.edu/openlearn/science-maths-technology/biology/hearing/content-section-3.2 (308).

The scala media contains endolymph, a fluid with a high concentration of potassium ions relative to other bodily fluids. This concentration gradient is actively maintained by cells of the striae vascularis in the lateral wall of the scala media.

2.3.3.1 The organ of Corti

The organ of Corti, which sits on the basilar membrane, is the body containing the auditory mechanoreceptors which convert sound waves into electrical impulses. These mechanoreceptors are called hair cells,

due to the presence of approximately 100 stereocilia on the apical surface of each cell. The tips of the stereocilia are embedded in another membrane, the tectorial membrane. This is anchored medially along the modiolus but the lateral edge floats freely in the endolymph (see Figure 2-4). Inner hair cells (IHCs) are the primary auditory response cells, while outer hair cells (OHCs) provide active amplification to improve hearing sensitivity and frequency selectivity (306).

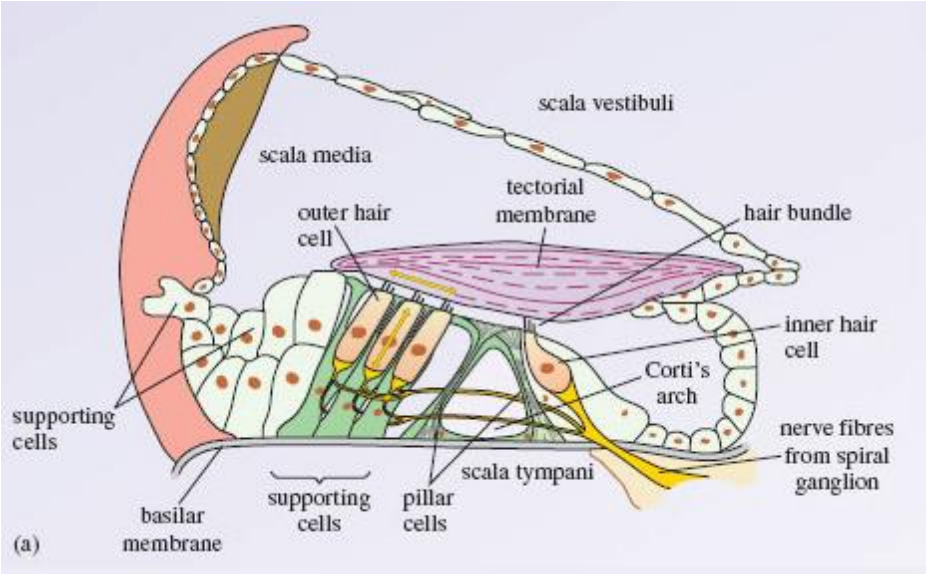


Figure 2-4: A diagrammatic representation the detail of the organ of Corti. Reproduced unchanged under the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 Licence from The Open University, available at https://www.open.edu/openlearn/science-maths-technology/biology/hearing/content-section-3.2 (308).

2.3.3.2 Tonotopic arrangement of the basilar membrane.

The basilar membrane becomes wider and less rigid as it progresses from the base to the apex such that it vibrates at different frequencies along its length. Higher frequency sounds are detected preferentially in

the basilar region and low frequency sounds are detected in the apical region (see Figure 2-5). Deformation of the basilar membrane in response to a sound wave causes a travelling wave to pass from the base to the apex of the membrane. The amplitude of this wave is maximal at the point on the membrane which is most sensitive to the frequency of the sound. Following the point of maximal amplitude the wave rapidly diminishes so that low frequency sounds stimulate most of the membrane, though are maximal apically, while high frequency sounds stimulate only the basilar part of the membrane.

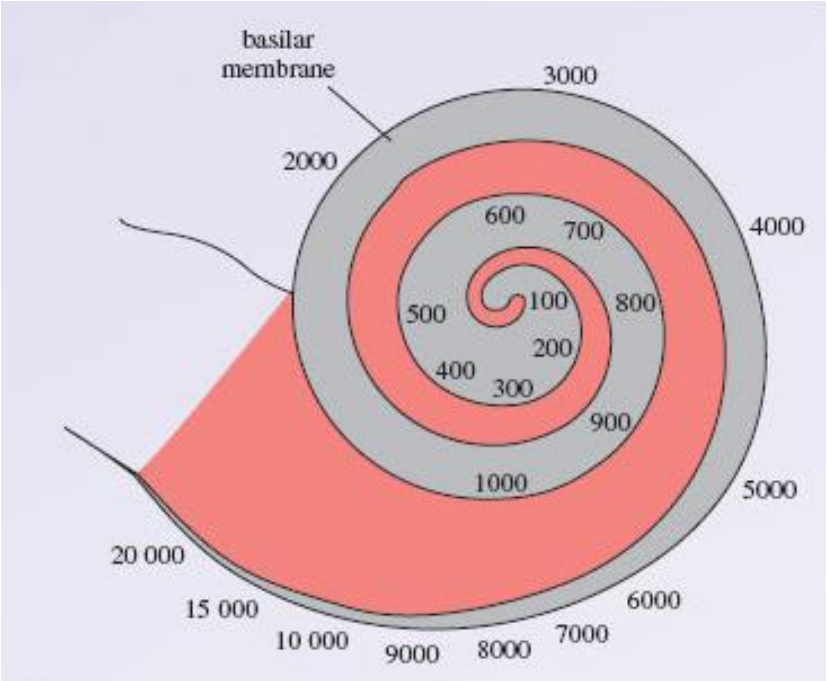


Figure 2-5: Tonotopic arrangement of the basilar membrane. Basally it is thin and rigid, and maximally stimulated by high frequency sounds. Apically it becomes wider and floppier and is maximally stimulated by lower frequency sounds. Reproduced unchanged under the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 Licence from The Open University, available at https://www.open.edu/openlearn/science-maths-technology/biology/hearing/content-section-3.3. (309).

Where complex sounds are detected, the basilar membrane breaks these down into the constituent pure tones and is simultaneously stimulated at each constituent frequency.

2.3.3.3 Signal transduction

Deformation of the basilar membrane relative to the tectorial membrane causes shearing forces across the stereocilia. Ion channels are opened in response to the mechanical deformation resulting in an influx of potassium ions from the potassium rich endolymph, with subsequent depolarisation of the cell. At the basal end of the cell is a pre-synaptic cleft. Depolarisation leads to the opening of voltage-gated calcium channels in the cell wall and release of an excitatory neurotransmitter, probably glutamate (310), into the synaptic cleft. The afferent fibres of the auditory nerve are depolarised leading to the generation of action potentials. These afferent fibres have their cell bodies in the spiral ganglia, located within the modiolus.

The intensity of the noise stimulus is proportional to the amplitude of the vibration of the basilar membrane. Louder noises lead to greater shear forces, increased electrical response and increased release of neurotransmitter at the synaptic cleft. This response is sigmoidal; very little deformation is required to elicit most of the response, with additional deformation having a cumulatively lesser effect up to a point of saturation.

Each IHC synapses with approximately 10 afferent auditory neurons (306, 311). Sequential recruitment of neurons with increasing thresholds of activation gives information to the higher auditory pathways about the intensity of the signal. This process is supported by firing of neurons synapsing to IHCs in adjacent areas of the basilar membrane, which occurs as sound intensity increases.

The IHCs are also tonotopically arranged, sharing the same maximal frequency stimulation with the adjacent basilar membrane. This arrangement continues into the cochlear nerve. The lowest frequency fibres, running from the apex of the cochlea are positioned centrally within the nerve, with higher frequency fibres joining circumferentially. The outer fibres of the cochlear nerve therefore carry the highest frequency sounds.

2.3.3.4 Higher auditory pathways

This tonotopic arrangement continues throughout the higher auditory pathways. The cochlear nerve runs to the ipsilateral cochlear nucleus within the brain stem. Information about the frequency, intensity and timing of the stimulus is coded by different cells. From here the majority of the fibres cross to the contralateral superior olivary complex where sound localisation occurs. Differences in the intensity of the stimulus between both ears and differences in timing between the arrival of the stimulus are used to localise the source of a sound.

In the inferior colliculus information about what the stimulus represents and its location are co-ordinated. This information is sent to the medial geniculate nucleus and on to the auditory cortex where interpretation of the sound stimulus is undertaken.

2.3.3.5 The role of the outer hair cells

As previously described, each area of the basilar membrane responds maximally to a given frequency. As the intensity of the stimulus increases a wider area of the basilar membrane is excited leading to reduced frequency selectivity with louder noises.

OHCs actively stretch and contract at the same frequency as the basilar membrane leading to active amplification of the signal. This effect has most impact at low sound intensities, thus the action of the OHCs improves hearing sensitivity (306).

However, the frequency causing maximal stimulation of a given OHC is up to half an octave higher (i.e., more basal on the basilar membrane) than the tuning frequency of the corresponding IHC. Thus, OHC activity improves the frequency selectivity of the response by narrowing the area of basilar membrane stimulated but moves this point of maximal stimulation basally (306).

2.4 Hearing loss

2.4.1 Types of hearing loss

2.4.1.1 Conductive hearing loss

Conductive hearing loss is caused by mechanical obstruction preventing the sound wave from reaching the inner ear. In children it is commonly caused by fluid within the middle ear cavity, “glue-ear”. In view of the prevalence of upper respiratory tract symptoms in people with CF it could be supposed that middle ear symptoms would be more prevalent than in the general population. For a long time no difference in the rate of conductive hearing loss between people with CF and the general population has been found (312, 313) however more recently this has been disputed (314).

2.4.1.2 Sensorineural hearing loss

Sensorineural hearing loss (SNHL) is due to damage to the cochlea, auditory nerve, or higher auditory centres (315). There are a number of causes of SNHL, of which ototoxicity is one. Other causes of SNHL include age-related deterioration (presbycusis), noise-induced damage, genetic causes, infection, head trauma and Ménière’s disease (316).

SNHL tends to be irreversible, though in some cases of ototoxicity cessation of the causative agent may allow recovery of hearing (303, 317). However, since the hair cells and the cells of the spiral ganglion are terminally differentiated (318) once lost they are irreplaceable.

2.4.1.2.1 Symptoms of sensorineural hearing loss

The symptoms of SNHL may be insidious, particularly with gradual onset, such that people may not notice hearing loss until significant damage has occurred (319).

Common symptoms include a loss of hearing acuity; sounds may be unclear and speakers perceived to be mumbling. People with SNHL may struggle in group situations, particularly with background noise (320). Higher frequency sounds are lost first with loss of speech clarity and difficulty communicating with people in background noise (321, 322). Hearing loss may also be associated with tinnitus.

2.4.1.2.2 Treatment of sensorineural hearing loss

Very few causes of sensorineural hearing loss have any specific treatment. Avoidance of preventable causes, such as exposure to ototoxic medication and noise, is therefore the most effective strategy.

Where prevention is impossible, for example in a patient in whom the clinical benefit of aminoglycosides outweighs the risk of hearing damage, the only option is hearing assistance (321). Even with current technology hearing aids are unable to selectively amplify sounds (323). Many users struggle to adapt as background noise is amplified in conjunction with useful sounds. Using an aid earlier in the course of SNHL increases the chance of adaptation to the device with better future adherence (324).

2.5 Sound perception

2.5.1 What is sound?

Sound waves are distortions of pressure in a medium, such as air or fluid. The pressure wave causes increased density of the medium (condensation) followed by a reduction in density (rarefaction). These waves may be visualised by considering a spring compressing and stretching. The frequency of a wave is the number of complete compression/rarefaction cycles in one second and is measured in Hertz (Hz). For a pure tone this may be a simple, sinusoidal waveform, while complex sounds may have multiple peaks and troughs within a single cycle.

The intensity of a sound equates to the amount of sound energy passing through a unit area in one second. It is measured on a logarithmic scale, decibels (dB) sound pressure level (SPL), where 0dB SPL equates to a pressure of $2x10^{-5}N/m^2$. Since the scale is logarithmic both an increase from 0 to 10 dB SPL and from 80 to 90 dB SPL equate to a 10-fold increase in intensity.

However, human perception of loudness is dependent on the frequency of the sound, since human ears are attuned to those in the range produced by human vocalisations. Sounds outside of this range need to be orders of magnitude more intense to be perceived (325). For the purposes of measuring hearing therefore a transformed scale, dB

Hearing Level (HL) is used. At each tested frequency a correction is made to convert the quietest sound perceived by a reference population in dB SPL to 0 dB HL (325).

2.5.1.1 What is noise?

Noise is a sound whose pressure waves vary randomly with time (306). Masking noise is any noise that may potentially cover up another noise (304). This noise may be fluctuant, varying by frequency and/or intensity, for example traffic noise, or steady state, for example a fan whirring.

White band noise is distributed across the frequency spectrum, commonly from 20 - 20,000 Hz; as masking noise it will affect all speech frequencies. Masking noise can be adapted to preferentially cover specific frequencies. Low-pass filtered noise contains sounds only below a certain frequency; for example, masking noise low-pass filtered at 1500 Hz will only contain sound below this frequency. In contrast band-pass noise is filtered between two frequencies (306). Speech-shaped noise covers the standard speech frequencies (0.25 - 8 kHz) and is weighted so that the level of the lower frequencies is higher than that of the higher frequencies, mimicking the distribution of speech sounds (see Figure 2-6).

2.5.2 Speech perception

Speech is generated by the passage of air over the vocal cords, causing them to vibrate. The frequency of vibration equates to the fundamental frequency of the sound. The vocal cords will also resonate at harmonics of the fundamental frequency; these are frequencies which are integer multiples of the fundamental frequency, i.e. twice the fundamental frequency, three-times and so on (326).

For vowel sounds changing the configuration of the pharynx and oral cavity will affect which of the harmonics are preferentially amplified by the resonance of the vocal tract. These preferentially amplified frequencies are called formants (326, 327).

Whilst the fundamental frequency of a vowel sound may change depending on the speaker, the spectral pattern (the relative frequencies of the formants) of each sound remains approximately constant. The relative frequencies of the first and second formants (the two closest to the fundamental frequency) give information about which vowel is being formed (328). Vowel sounds tend to be lower pitched, between 500-2000 Hz (329) (see Figure 2-6).

2.5.2.1 Consonants

Consonant sounds are vocalised through a partially or completely occluded vocal tract. Some are vocalised (i.e., require vibration of the vocal cords) while others are unvoiced. They are higher pitched

sounds, most commonly between 2000-5000 Hz (329) (see Figure 2-6). Consonants are important in speech definition (321, 330).

Fricatives are a class of consonants which are produced during very close apposition of the vocal tract, for example the tongue and teeth or tongue and hard palate. Fricative consonants are typically high frequency sounds, particularly /f, s ,θ, ð/ (see Table 2-1, Figure 2-6).

Phoneme	Examples of sound described
θ	think; bath
ð	the; mother
f	fish; if
v	vision; hive
s	sand; floss
z	zoo; buzz
ʃ	shell; splash
ʒ	vision; pleasure
h	hard; heavy

Table 2-1. Fricative consonants in English and the sounds they produce. Adapted from http://www.antimoon.com/how/pronunc-soundsipa.htm. (331).

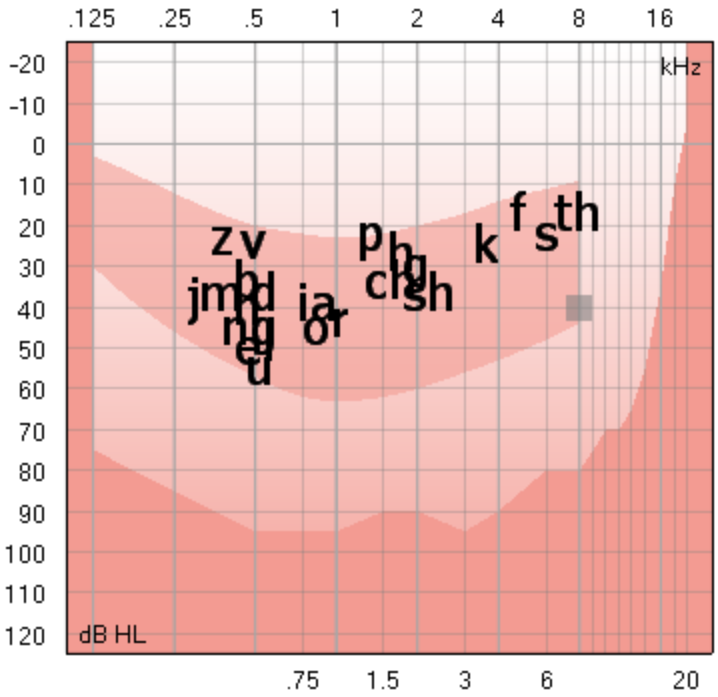


Figure 2-6: The Speech Banana. A representation of the frequencies of speech phonemes and their intensities. OtoAccess database v 1.5, InterAcoustics, Lanarkshire, UK.

Fricatives are highly prevalent in English speech; /s/ and /z/ make up 8% of consonants in spoken English (322). Loss of ability to perceive these sounds is therefore highly detrimental in oral communication (332).

The identification of speech sounds (phonemes) is made more difficult by the dynamic nature of speech. Each individual phoneme takes on slightly different characteristics depending on those adjacent, leading to subtly different spectral patterns (330). Understanding what is said is therefore dependent on context as well as the exact words.

2.5.3 Challenges of signal within noise

Signal to noise ratio (SNR) is a measure of the intensity of the target (speech) signal to the background noise. A ratio of 1:1 indicates that the intensity of the speech and the noise (in dB) are equal (i.e., the difference between the two is 0 dB). SNR loss is a measure of the necessary increase in signal to overcome by the background noise compared to someone with normal hearing; a more positive number indicates worse hearing (333). The SNR may be negative in people with above average hearing.

Speech recognition requires decoding of the auditory signal. In noisy environments the cognitive load required to correctly perceive the speech signal is increased; this is disproportionately true for people with hearing loss (334). Where the background noise is of similar frequency to the speech this may be particularly challenging (304).

Whilst loss of speech perception in noisy environments is a common concern for patients with hearing loss (320), patients with normal performance on conventional audiogram may also self-report difficulty hearing in background noise (335). Hearing loss as little as 10-15 dB HL above 4000 Hz is sufficient to impact on speech perception (336), though this deficit may be considered normal on conventional testing.

The clinical significance of hearing loss in the extended high frequencies is becoming more widely recognised. Hearing loss in frequencies commonly believed to be above those necessary for

speech intelligibility (above 8000 Hz) has been shown to correlate with self-reported difficulty hearing speech in background noise (337, 338). Consonant recognition, in particular, is improved by the perception of high frequency components of speech (322, 339). Spectral information in these frequencies has been shown to contribute to comprehension of speech in noise (335, 338). It may be that in noisy environments the additional information provided by the extended high frequency components of speech is more important due to the masking effect of lower frequency background noise (339).

2.6 Consequences of hearing loss

Hearing loss has a negative impact on education, work and social functions. Uncorrected it is associated with cognitive decline (340) whilst earlier identification and correction can reduce the impact (305).

Loss of hearing may negatively affect quality of life (341, 342). The impact may be felt in many activities of daily living through reduced communication and a loss of pleasure sounds, such as listening to music or watching television (343). Simple acts such as using the telephone become a challenge (344). Hearing is important for safety since high frequency tones and sirens are commonly used to warn of danger (321).

Acquired hearing loss is associated with psychological distress (345). People with CF are already more prone to social isolation than the

general population (346); this may be compounded by the negative impact on social functioning which has been associated with acquired hearing loss in the general population (347).

2.6.1 Children

Hearing loss which occurs pre-lingually has a greater effect on communication than where it develops later. Impaired hearing negatively affects children's ability to engage in age-appropriate activities (348). Children with hearing loss have reduced quality of life compared with normal-hearing peers. Socialisation and school are particularly affected (342). Severe hearing loss is associated with reduced self-perception of social ability (347) and an increased chance of low educational achievement (349).

2.6.2 Work and earnings

Unemployment is higher in people with hearing loss (344, 347). People with hearing loss have a lower income (349) and are less likely to be promoted (344). In the workplace noise is a major barrier for communication (305).

2.6.3 Social situations

Acquired hearing loss is associated with isolation and social withdrawal (305). Communication becomes more effortful, particularly in noisy or group settings (350) such that the amount attempted may be reduced

(343). Hearing loss leads to reduced time spent outside the home and an increased risk of social withdrawal (351).

The impact is therefore felt not only by the person with hearing loss but also their social contacts (352), particularly their significant other (350). Repetition and reliance on a partner for communication can lead to frustration (344). Hearing loss in one partner can be detrimental to the health of the normal hearing partner (353).

2.7 Ototoxicity

2.7.1 Epidemiology of ototoxicity in CF

Ototoxicity is increasingly recognised as a common complication in CF. In 1979 Forman-Franco *et al.* reported the prevalence of hearing loss in a CF clinic as only 1% (312). Estimates of the prevalence are now much higher; the prevalence of SNHL in paediatric CF populations ranges from 4-24% (354-357). In adult patients the prevalence may be as high as 50% (358, 359). The estimated prevalence depends on the definition of hearing loss used in each study, and whether this is based on standard testing, EHF audiometry, or a combination of these modalities.

The prevalence of vestibulotoxicity has been less extensively studied. Scheenstra *et al.* reported a prevalence of 30.4%, in 23 adult patients recruited from a Dutch CF clinic (360). Handelsman *et al*. found a

prevalence of 79% in 71 people with CF undergoing aminoglycoside therapy (361).

The risk of non-CF patients developing ototoxicity following a course of aminoglycosides has been variably estimated at 5-10% [95]. Non-CF patients tend to have single, short-lived aminoglycoside courses. Since people with CF have longer courses with higher doses, it could be expected that the per course risk would be higher. Mulheran *et al*. looked at the per course risk of ototoxicity in CF patients treated with one- or thrice-daily tobramycin and estimated the risk of ototoxicity associated with a single course of aminoglycoside treatment to be 1.7% (362). However, a later study by Harruff *et al.* found evidence of cochleotoxicity in 89% of patients two weeks after a course of IV tobramycin and in 93% of patients four weeks after the course finished (363).

A person with CF undergoes a far greater lifetime aminoglycoside exposure than the general population. An increase in exposure to aminoglycosides correlates with worsening hearing loss (145, 364, 365). However, this is not the case in all patients (365, 366), therefore other mechanisms, be these genetic, environmental or drug-induced, must interact with aminoglycoside exposure, leaving some patients at greater risk than others.

2.7.2 Risk factors for ototoxicity

2.7.2.1 Mitochondrial DNA mutations

One known risk factor is the presence of mutations in mitochondrial 12S rRNA which have been shown to significantly increase the risk of aminoglycoside-related ototoxicity. The most studied of these, m.1555A>G, has a prevalence of 0.19% in European children (367) and 0.21% in adults of European descent (368). In China 12S rRNA variants are responsible for approximately 30% of aminoglycoside-induced hearing loss (369). Previous reports suggested that the development of deafness in people with the m.1555A>G variant undergoing aminoglycoside therapy was 100% (370). It is now known that the penetrance is lower; a child with CF, carrying this variant, was found to have normal hearing despite multiple aminoglycoside courses (357). However, for CF patients who also carry a mutation of this kind special consideration should be given prior to the prescription of aminoglycosides (371).

2.7.2.2 Other risk factors

Other patient factors are also likely to contribute to the risk of developing ototoxicity. In a retrospective analysis of an audiology database, patients with CF and CF-diabetes had more decline in their hearing over time than patients without diabetes (372).

Co-administration of other ototoxic medications may produce a synergistic effect on hair cell damage; loop diuretics have been show to

potentiate the ototoxic effect of aminoglycosides (373). Azithromycin, commonly used in CF as a prophylactic antibiotic, has been reported to cause hearing damage (374), though a Cochrane review of azithromycin in people with CF found no difference in the rate of hearing impairment or tinnitus compared with placebo (81).

Noise exposure in combination with aminoglycoside administration has a synergistic effect on hearing loss (375). Concomitant exposure to both noise and aminoglycosides, and aminoglycoside administration in someone with prior sub-clinical noise damage, can lead to the development of hearing loss (375). Equally exposure to noise in the aftermath of aminoglycoside treatment can potentiate the ototoxic damage (375).

2.7.3 Mechanism of aminoglycoside ototoxicity

After systemic administration aminoglycosides can first be identified in the striae vascularis and then within hair cells, with increasing hair cell concentrations over time (376). Aminoglycosides enter hair cells by apical endocytosis (377) and through the ion channels opened by deformation of the stereocilia (378). The exact mechanisms leading to cell death are not fully understood (379) however hair cells exposed to aminoglycosides demonstrate increased concentrations of intracellular calcium and reactive oxygen species (ROS) (380) with release of cytochrome c from mitochondria and activation of caspases (381). These processes ultimately result in cellular apoptosis.

Aminoglycoside-mediated damage has been shown in cochlear spiral ganglion cells in the absence of hair cell loss (382-384). This process may also contribute to hearing loss.

Ongoing cell death occurs for up to four weeks after aminoglycoside administration (375). This may be due to persistence of aminoglycoside in hair cells. Two phases of elimination have been described. The first phase occurs with a half-life of two days; the second has a half-life of up to six months (385). Accumulation of aminoglycoside through repeated antibiotic courses may potentiate hair cell loss (386).

2.7.3.1 Aminoglycoside ototoxicity initially causes high frequency hearing loss

High frequency hearing loss associated with ototoxicity is due to the varying susceptibility of hair cells along the basilar membrane to aminoglycoside. Ototoxicity secondary to cisplatin treatment also occurs initially at high frequencies (387).

In animal studies aminoglycoside preferentially target OHCs over IHCs (376, 388, 389). They also target basal OHCs prior to more apical cells (376, 380). Progressive loss of OHCs from the cochlea base to the apex has also been found in humans exposed to aminoglycosides (390), including people with CF (391).

Basal OHCs have a higher density of endocytic vesicles compared to apical cells (392) which may lead to increased uptake of aminoglycoside and explain their greater susceptibility.

2.8 Strategies to investigate and monitor ototoxicity

2.8.1 Strategies to prevent ototoxicity

A number of strategies have been trialled in an attempt to reduce aminoglycoside ototoxicity. Therapies, including antioxidants, glutathione and dexamethasone, have some in vitro or in vivo evidence (393). A Cochrane Systematic Review found no effect of once- vs three-times daily dosing on the development of hearing loss in patients with cystic fibrosis (394).

Antioxidants, such as vitamin E and D-methionine, have been used in animal studies in an attempt to reduce ROS formation and subsequent hair cell damage (395). In humans, however, there is no benefit of vitamin E supplementation (396). The iron chelator desferoxamine may reduce hair cell death in guinea pigs (397), but in humans this agent is itself known to be ototoxic (398).

There is better evidence for N-acetyl cysteine (NAC). A systematic review found a relative risk of 0.14 for ototoxicity in patients treated with aminoglycosides, concomitantly treated with NAC compared to placebo (399). Care must be taken with extrapolating this data to a CF

population as all the patients in the review had end-stage renal failure and were on dialysis. Additionally, the aminoglycosides reviewed were amikacin and gentamicin, both used less commonly in CF than tobramycin. A later study of NAC in continuous ambulatory peritoneal dialysis (CAPD) patients treated with gentamicin for peritonitis found benefit of NAC in reducing hearing loss at one month post treatment but no long-term effect (400).

High dose aspirin also appears to have some benefit in the prevention of gentamicin indued ototoxicity. In an RCT, 195 patients undergoing gentamicin treatment were randomised to receive 3g aspirin per day or placebo. In the placebo group 13% of patients had hearing loss of ≥15dB HL at 6 or 8 kHz at six weeks, compared to 3% of those treated with aspirin (401). Promising evidence has also been found for the use of sodium thiosulphate in preventing cisplatin mediated ototoxicity, but this has yet to be examined in the case of aminoglycosides (402).

Further work is ongoing to look at modification of treatment agents to prevent their penetrating the inner ear or to locally deliver otoprotective agents to the cochlear and currently formal approval is lacking for any therapeutic agent (403).

2.8.2 Hearing testing

2.8.2.1 Pure-tone audiogram

The current gold standard test for hearing is the pure tone audiogram (PTA) (404). The test aims to determine the quietest level of sound, the auditory threshold, an individual can hear at set frequencies between 125 Hz and 8000 Hz and is measured in dB HL.

A PTA should be carried out in a sound-proof room. The patient should be sat comfortably with their face visible to the examiner, though they must not be able to see the audiometer controls. They will wear appropriate earphones and have a response button to indicate when they hear a tone. Starting at 1000 Hz a tone is presented to the patient at 40 dB HL. If they respond appropriately the intensity is reduced in 10 dB HL steps until they can no longer hear the tone. Increases of 5 dB HL are then presented until the patient is once again responds. To determine the threshold the patient must correctly identify a given intensity for at least 2 out of three ascending trials. The test then moves on to examine 2000, 4000, 8000, 500 and 250 Hz. Additional steps at 3000 and 6000 Hz can be measured if required. The process is then repeated with the opposite ear.

2.8.2.2 Extended high-frequency audiogram

An extended high frequency (EHF) audiogram is carried out using the same process as a standard audiogram but tests frequencies above

8000 Hz, commonly 9, 10, 11.2, 12.5, 14, 16 kHz but may also include 18 and 20 kHz.

EHF audiometry is more sensitive at identifying early ototoxic damage than standard PTA (145, 364, 405). In view of the base to apex pattern of cochlear damage previously discussed this conclusion is unsurprising. Al-Malky *et al*. in 2015 recommended yearly EHF audiometry as the test of choice for monitoring CF patients undergoing regular courses of aminoglycosides, having found that conventional PTA alone would have missed two patients with ototoxicity (145).

2.8.2.3 Speech-in-noise testing

Audiometry testing does not however provide a truly comprehensive picture of hearing health (406). The ability to identify tones in a contained environment does not always equate to real-life hearing. Additionally, significant processing occurs in the primary auditory cortex to translate sounds to identifiable speech. Speech-in-noise testing is a more, “real-world,” assessment of auditory function, requiring participants to identify words, phrases or digits within a background noise (333).

Digit triplet tests (DTT) present three randomly selected digit words (e.g., six-three-eight). Tests using digits are well-validated since the words are familiar and are less reliant on linguistic skills (320). DTTs are adaptive; a correct response increases the background noise relative to the speech intensity making the test harder, while following

an incorrect response the background noise gets quieter. The signal (speech)-to-noise ratio (SNR) is then calculated, representing the ratio of speech to masking noise at which 50% of the digits are correctly identified. This is termed the speech reception threshold (SRT). SRT is lower, and may be negative, where an individual has better hearing (333). DTTs have been developed which can be administered by phone (406) or via the internet (407).

2.8.3 Current recommendations

The American Academy of Audiology (AAA) has made recommendations for the monitoring of general patients undergoing aminoglycoside therapy. They suggest a protocol comprising a baseline audiogram (or within 72 hours of initial administration), then weekly or biweekly testing during treatment and follow-up testing a few months after cessation (303).

There are currently however no clear guidelines for hearing monitoring in people with CF treated with aminoglycoside antibiotics. The UK CF Trust recommends an annual pure tone audiogram for patients undergoing frequent courses of intravenous aminoglycosides (146). The definition of “frequent” is not specified. The CF Foundation recommends, “periodic,” audiograms to monitor for toxicity (224). The European Cystic Fibrosis Society guidelines on treatment of lung infection do not mention ototoxicity or the need for monitoring (408).

Understanding of the impact and prevalence of ototoxicity in CF has increased dramatically in the last decade therefore this issue may be rectified in updated guidelines.

2.8.4 Definitions of hearing loss

Multiple definitions of hearing loss exist in the literature (145, 409). They can be divided into two groups; those that report absolute threshold values and those based on a change from baseline. Change from baseline definitions may be a more sensitive measure in the individual patient and are commonly advocated for ototoxicity monitoring (303). However, when using PTA as a gold standard for comparing other tests a standalone definition is required. The British Society of Audiology (BSA) definition of hearing loss (404) (see Table 2-2) is commonly used in the UK.

Descriptor	**Average hearing thresholds (dB HL at 250, 500, 1000, 2000 and 4000 Hz).**
Mild hearing loss	20-40
Moderate hearing loss	41-70
Severe hearing loss	71-95
Profound hearing loss	In excess of 95

Table 2-2. The British Society of Audiology definition of grades of hearing loss (404).

This definition is useful for standard audiometry but does not consider higher speech frequency hearing loss, nor the extended high frequencies.

Mulheran *et al.*, when looking for evidence of cochleotoxicity in CF patients enrolled in a study comparing the safety and efficacy of once versus three-times-daily tobramycin, defined hearing loss as: “A persistent increase in the PTA threshold above 20 dB HL in either ear at any two frequencies in the standard audiogram between 2 and 8 kHz.” (362). This definition has the advantage of considering the higher speech frequencies which are the first part of the standard PTA to be affected in ototoxicity.

2.8.5 Challenges of hearing monitoring

Self-reported hearing loss is an insensitive method of assessing ototoxicity due to the often-insidious onset of hearing loss (359, 410).

Audiometry testing also has limitations. The sensitivity of standard PTA testing is limited by its upper frequencies; significant damage to the basal turns of the cochlea may have occurred before any abnormality is identified (405). As discussed in section 2.8.2.2 EHF audiometry is more sensitive to early cochlear damage but the equipment required to carry out this testing is less widely available (145).

There are multiple logistical challenges to hearing monitoring in CF (411). PTA and EHF-audiometry require a specialist audiologist to

conduct the tests, specialist equipment and, ideally, sound-proofed testing rooms. Hearing testing can therefore rarely be accommodated in the CF clinic and requires additional appointments. For people with CF who already attend clinic approximately every eight weeks (412) additional appointments may be challenging to attend and can interfere with work and school attendance. If the AAA protocol for aminoglycoside monitoring is followed, then multiple additional appointments are necessary. In patients who have regular courses of IV aminoglycosides this requirement would rapidly multiply. These factors all increase the cost of providing appropriate hearing monitoring.

2.9 The High Frequency Digit Triplet Test

Speech-in-noise tests have a number of audiological benefits, particularly as a measure of functional hearing (see section 2.8.2.3). They also have logistical benefits which may be important in developing a screening program for hearing loss in CF care. They do not need any specialist equipment or special training to administer and can even be self-administered (319, 320). These characteristics make them potentially ideal candidates to facilitate high-volume ototoxicity screening in people with CF.

Much speech sound is in the lower frequencies (see Figure 2-6). Vlaming *et al.* adapted a DTT for the investigation of noise-induced hearing loss, termed the High Frequency Digit Triplet (HFDT) test (319).

Noise induced hearing loss typically shows on a PTA as loss of hearing in the range 4000-6000 Hz. In order to increase the sensitivity of the test for high frequency hearing loss the masking noise was low-pass filtered to a maximum frequency of 1500 Hz. Background noise at this frequency masks the low-frequency phonemes in the presented speech without affecting high frequency sounds. People with normal high frequency hearing can use the unmasked higher frequency information to discriminate the digits. In the case of high frequency hearing loss neither low nor high frequency information is available leading to an inability to identify the digits presented. In this test better hearing equates to a more negative SRT.

2.10 Summary, aims and objectives

P. aeruginosa is known to cause harm in cystic fibrosis. Most work has been done examining the physical harm caused, particularly in chronic infection. This thesis will examine other harms caused by *P. aeruginosa* in CF and examine primary and secondary measures to mitigate harm caused in specific circumstances.

2.10.1 Chapter 3

Chapter 3 will explore the psychological harms associated with fear of infection.

2.10.1.1 Hypothesis

People with CF, their families and friends have strong reactions to first infection with *P. aeruginosa.*

2.10.1.2 Aims

To examine the reactions of a sample of people with CF, their families and friends to first infection with *P. aeruginosa.*

2.10.1.3 Objectives

To undertake a qualitative review of the responses to a survey, conducted by a CF patient, into how the CF community feels about first infection with *P. aeruginosa.*

2.10.2 Chapter 4

Chapter 4 will examine what is known about sources of *P. aeruginosa* infection.

2.10.2.1 Hypothesis

Infection control measures used in CF to prevent infection with *P. aeruginosa* are evidence-based.

2.10.2.2 Aims

To identify infection control measures which are proven to successfully prevent *P. aeruginosa* infection in people with CF.

To generate recommendations for avoidance strategies for patients and their families.

To identify gaps in the evidence which need to be addressed to provide these recommendations.

2.10.2.3 Objectives

To carry out a systematic review into infection control practices to prevent *P. aeruginosa* infection in CF and assess the quality of the evidence for each measure.

2.10.3 Chapter 5

Chapter 5 will examine evidence for secondary prevention of *P. aeruginosa* infection in patients who have undergone successful eradication.

2.10.3.1 Hypothesis

Treatment with additional therapy after successful eradication will prolong time to next infection.

2.10.3.2 Aims

To identify treatments given after successful eradication therapy which delay recurrence of infection with *P. aeruginosa* in people with CF.

2.10.3.3 Objectives

To carry out a Cochrane Systematic Review of Interventions into the evidence for treatments which successfully delay recurrence.

2.10.4 Chapters 6 and 7

Chapters 6 and 7 will focus on a novel method for screening patients for hearing loss.

2.10.4.1 Hypothesis

The High Frequency Digit Triplet (HFDT) test will be an effective screening tool for the identification of early hearing loss in people with CF.

2.10.4.2 Aims

To test this hypothesis in a sample of CF patients.

2.10.4.3 Objectives

Workstream 1: To compare the HFDT test to formal pure tone audiogram in a sample of adults with CF and adolescents aged 11 and over at a time of health stability. This will be described in chapter 6.

Workstream 2: To compare the HFDT test to formal pure tone audiogram in a sample of adults with CF and adolescents aged 11 and over at a time of pulmonary exacerbation to assess whether factors such as ill-health and increased coughing affect performance of the test.

Workstream 3: To compare the HFDT test to formal pure tone audiogram in a sample of children with CF aged 5-10, at a time of health stability to assess the youngest age at which the test can reliably be performed.

Workstreams 2 and 3 will be described in chapter 7.

2.10.5 Chapter 8

Chapter 8 will summarise the work presented and present conclusions regarding the harm caused by *P. aeruginosa* in CF and potential mechanisms to reduce this.

Chapter 3: How people with CF, their close family and friends, feel about first infection with *Pseudomonas aeruginosa.*

In this chapter I will argue that *Pseudomonas aeruginosa* causes harm to people with CF even prior to first infection and that the harm caused by *P. aeruginosa* may be psychological as much as physical.

The work presented in this chapter builds upon work submitted for a research paper entitled, “Perception of first respiratory infection with *P. aeruginosa* by people with cystic fibrosis and those close to them: an online qualitative study.” (220). The contributions of the co-authors to this study are shown in Table 3-1.

The project was conceived by Oliver Rayner (OCR), a UK Cystic Fibrosis Trust Patient Representative. He became aware of wide-ranging suggestions about strategies to avoid infection with *P. aeruginosa* proliferating on social media and was concerned about the potential harm caused to families trying to follow these practices. He set up an on-line survey to better understand how the CF community felt about first infection with *P. aeruginosa.* The response rate was unanticipated and, realising the thoughts of so many members of the CF community required further analysis, OCR presented the data to Prof. Alan Smyth (AS), with whom he had previously worked. AS, as my PhD. supervisor, asked me to analyse the data. Paul Leighton (PL),

a specialist qualitative researcher at the University of Nottingham was asked to assist in developing the qualitative analysis. PL designed a novel analysis approach, detailed in section 3.2.2, which I carried out with PL as a second reviewer. We presented our initial analyses to AS and OCR at each stage to agree the final choices of keywords, keywords-in-context and themes. I wrote the first draft of the paper, with revisions by AS and contributions by the whole team. I presented the work at the European Cystic Fibrosis Society Conference in Brussels, 2015 (413). We used video technology to allow OCR to attend the poster session since, for infection control reasons, he was unable to attend the conference in person.

Study Stage	**Contributors**
Conceiving, designing and conducting the SurveyMonkey® survey	Mr Oliver Rayner
Designing the analysis plan	Dr Paul Leighton
Survey primary analysis	Dr Sally Palser Dr Paul Leighton
Survey additional analysis	Prof. Alan Smyth Mr Oliver Rayner
First draft of the paper	Dr Sally Palser Dr Paul Leighton (methods)
Revision of the paper	Prof. Alan Smyth

Table 3-1: Contributions of co-authors to the study, "How do people with CF, their close families and friends feel about first infection with P. aeruginosa."

This work was undertaken between October 2014 and June 2016.

3.1 Introduction

As discussed in chapter 1, chronic infection with *P. aeruginosa* causes an increase in morbidity and mortality in people with CF; indeed the development of chronic *P. aeruginosa* infection has been described as, “Arguably the most significant event in the life of a person with cystic fibrosis…” (212).

In recent years eradication therapy has been shown to delay the development of chronic *P. aeruginosa* infection after the first isolation (203). A number of regimens have been proposed but no single eradication regimen has been shown to be superior (208). Suggested strategies range from fourteen days to fifteen months in length and may include oral, nebulised or inhaled therapy (203, 205, 206) see Table 3-2.

Study	Participants	Intervention	Comparator	Findings
Valerius 1991 (203)	26 children aged 2-9 years	3/52 PO ciprofloxacin plus inhaled colistin	No treatment	Significantly fewer participants in the treatment group became chronically colonised and significantly less recurrence of infection.
Wiesemann 1998 (414)	22 children aged 4-18 years	Nebulised TIS BD for 12 months	Placebo nebuliser solution	Time to conversion to negative PA sputum cultures significantly shorter in treated participants.
Gibson 2003 (415)	21 children aged 6months to 6 years	Nebulised TIS BD for 28 days	Placebo	Stopped early due to significantly fewer exacerbations in treatment group. Treated group 100% PA free on BAL at D28 vs 7.7% of placebo group.
Ratjen 2010 (205) (ELITE)	132 children consented, 88 randomised	Nebulised TIS BD for 28 days	Nebulised TIS BD for 56 days	Median time to recurrence and proportion free of PA at end of treatment and end of study similar between groups. No benefit for 56 days of treatment over 28 days.

Treggiari 2011 (204) (EPIC) – 1st comparison	304 children aged 1-12 years	Nebulised TIS cycled every three months for 15 months	Nebulised TIS given for PA positive culture for 15 months	No difference in time to exacerbation of OR of PA positive culture between groups (but see Chapter 5:)
Treggiari 2011 (204) (EPIC) – 2nd comparison	304 children aged 1-12 years	Nebulised TIS plus placebo when indicated	Nebulised TIS plus oral ciprofloxacin when indicated.	No difference in time to exacerbation of OR of PA positive culture between groups.
Taccetti 2012 (416)	233 participants	Nebulised TIS BD plus PO ciprofloxacin for 28 days	Nebulised colistin BD plus PO ciprofloxacin for 28 days	No difference in eradication rates between the two regimens.
Proesmans 2013 (206)	58 children	Nebulised TIS BD for 28 days	Nebulised colistin BD plus PO ciprofloxacin for 3 months	No difference in eradication at end of therapy and no difference in time to PA recurrence.
Gilchrist 2023 (417) (ALPINE2)	149 children under 18 years	Nebulised AZLI for 28 days	Nebulised AZLI for 14 days followed by 14 days of placebo	14 days of AZLI inferior to 28 days at achieving eradication at 4 weeks.

Mayer-Hamblett 2018 (418) (OPTIMIZE)	221 children	Nebulised TIS for 28-56 days plus PO azithromycin three times a week for 15 months	Nebulised TIS for 28-56 days	Trial stopped early due to significant reduction in pulmonary exacerbations in intervention group. No significant difference in percentage of patients in each group PA negative at the end of the first quarter or in the time to PA recurrence.
Ratjen 2019 (419) (EARLY)	51 children aged 3 months to <7 years	Nebulised TIS for 28 days (plus further 28 days if still positive). If negative option of further 28 days of placebo	Nebulised placebo for 28 days, Followed by 28 days of nebulised TIS if positive at D29. If negative option of 28 days of TIS.	At D29 significantly more participants in the placebo group PA positive. After crossover (optional) participants treated in first 28 days significantly more likely to be PA negative than those treated later.
Langton-Hewer 2020 (420) (TORPEDO-CF)	286 children and adults	14 days IV ceftazidime and IV tobramycin plus 3 months nebulised colistin	3 months PO ciprofloxacin plus 3 months nebulised colistin	No significant difference between the groups but trend towards better chance of eradication in the oral group.

Table 3-2: Randomised controlled trials of strategies to eradicate P. aeruginosa in cystic fibrosis and the findings thereof, in date order.

Treatment burden in cystic fibrosis is already high (421); additional treatment for the eradication of *P. aeruginosa* requires a significant commitment from patients and their families. Nebulised treatment in particular adds to the burden; each nebuliser may take up to 20 minutes (421) and maintenance and cleaning of the equipment is also time consuming (421).

The source of *P. aeruginosa* acquisition is unclear in most cases. There is evidence that cross-infection can occur, both directly from contact with other people with CF already infected with *P. aeruginosa* (176, 422) and indirectly, from a contaminated environment, most commonly within a healthcare setting (159, 180, 185, 423). However it is likely that most *P. aeruginosa* infections are acquired from the environment (424).

Parents of children with CF are stressed by the prospect of *P. aeruginosa* acquisition and undertake a range of measures in an attempt to prevent infection (187, 188), however less is known about how the CF community as a whole feels about first infection with *P. aeruginosa*. The perspectives of a population on a given topic are best understood using qualitative research which can improve understanding of the day-to-day complexities inherent in people's lives (425).

Qualitative research aims to understand a research question from the point of view of the population it affects (426). In healthcare this is

commonly done through in-depth, semi-structured interviews or focus groups. The latter are rarely appropriate in CF research due to infection control concerns, but semi-structured individual interviews have been widely used to gather qualitative data in CF.

In a semi-structured interview the interviewer can concentrate on subjects important to the individual interviewee and expand on interesting points raised, providing in-depth information about the topic under discussion (427). The responses are then transcribed and can be analysed using a number of qualitative methodologies, depending on the desired balance between description and interpretation of the data. Applying themes to data is a generic skill across qualitative methodologies (428), thus thematic analysis is, “a foundational method for qualitative analysis,” (429), allowing the recognition and categorisation of patterns within a dataset.

Thematic analysis allows an important depth of insight. The dataset for this type of analysis tends to be smaller and is based on longer accounts from fewer participants. Interviews can be continued until saturation is achieved; either additional interviews reveal no new themes (inductive thematic analysis) or no new examples are found to represent the themes previously agreed upon (deductive thematic analysis) (430). However this has led to criticism that qualitative research is not representative of the whole population under investigation (431).

An alternative approach to analysis is content analysis, a semi-quantitative approach, in which the frequency of words used in the study dataset is compared to the frequency of words used in a reference dataset, or corpus (body of words representative of the language used) (432). Words used more frequently in the dataset under investigation are deemed more interesting. This is particularly useful in the analysis of large datasets with thousands of responses and millions of words, such as those gleaned from social media posts and healthcare blogs and forums which would be cumbersome and time-consuming to analyse with a thematic approach (433).

Seale *et al*., analysing online support forums for breast and prostate cancer, developed this further (434), comparing the most frequently used words between the two forums and then analysing the context in which the keywords were used. These keywords-in-context were then coded in a manner more typical of qualitative research. The approach is good for providing an overview of the data with greater insights obtained from areas suggested by the keywords. However there is a danger that this approach can be over-simplistic without, "…a deeper contextual analysis." (435).

In the present study we used a mixed approach of both content and thematic analysis to better understand how people with CF and those close to them feel about first infection with *P. aeruginosa*.

3.2 Methods

The original version of this methods section, found in the published paper (220), was written by PL who developed the analysis approach. This version was adapted for the present thesis but remains based on that found in the published work.

3.2.1 Data collection

The five-question questionnaire detailed below was conceived and designed by OCR using the SurveyMonkey online survey tool (http://www.surveymonkey.com), (see Table 3-3). This was disseminated using personal social media accounts, the UK CF Trust (http://www.cysticfibrosis.org.uk), the CF Aware social media channel (#cfaware), and through CF groups on Facebook. Participants were also invited to share the link with other interested parties. The survey was open for responses between 6th and 18th May 2014.

There were no limits on the number of times a participant could complete the survey. No other data was collected about participant demographics. Parents/carers could complete the survey on behalf of a dependent and there were no limits on the free-text answers, which could be as brief or as detailed as the participant wished.

Survey Question		Response options
Q1:	What is your relationship with CF?	I have CF I have a child with CF I have a grandchild with CF I have a brother/sister with CF I have a friend with CF Other (please specify)
Q2:	What does the first infection by *Pseudomonas aeruginosa* mean to you?	Free text
Q3:	Would earlier infection with *P. aeruginosa* be a concern to you?	Free text
Q4:	What is the impact of first infection by *P. aeruginosa* on quality of life?	Free text
Q5:	How important do you think it is to develop more effective ways to deal with P. aeruginosa?	Top priority/Urgent A priority/Not urgent No strong feelings Relatively unimportant Totally unimportant Not answered

Table 3-3: Questions from a SurveyMonkey® survey sent out to the CF community in May 2014 entitled, "A short survey to help leading CF researchers better understand how people with CF and parents of children with CF feel about Pseudomonas aeruginosa."

The data were subsequently shared with AS at the University of Nottingham for further analysis. The Research Ethics Committee of the Faculty of Medicine and Health Sciences, University of Nottingham, was consulted but indicated that the research did not require formal ethical approval.

3.2.2 Data analysis

The nature of the present survey meant that it did not easily fit into a conventional qualitative analysis approach. It had neither the depth of information, attained through semi-structured interviews, nor the breadth of data that would be available in a larger data corpus to be reliably representative of the whole CF community. For this reason, a novel, structured, iterative approach was devised by PL, drawing on elements of both keyword analysis and thematic analysis to bring a rounded overview of the data.

Data were exported for analysis from SurveyMonkey into N'Vivo qualitative data software package (QSR International Pty, 2014) and Microsoft Excel (MS Excel 2016) (436).

3.2.2.1 Keywords

For each free-text question the whole body of responses was analysed using the N'Vivo "word frequency" function. Short words of three or fewer characters were excluded to remove common words, such as "and" and "the". Words of a similar derivation, for example "scare,"

“scary,” and “scared,” were linked. The top 25 most frequently used words in response to each question were then identified.

3.2.2.2 Keywords-in-context

The lists of the 25 most commonly used words for each question were examined independently by two authors (SP and PL) to identify the ten most interesting words used in response to each question.

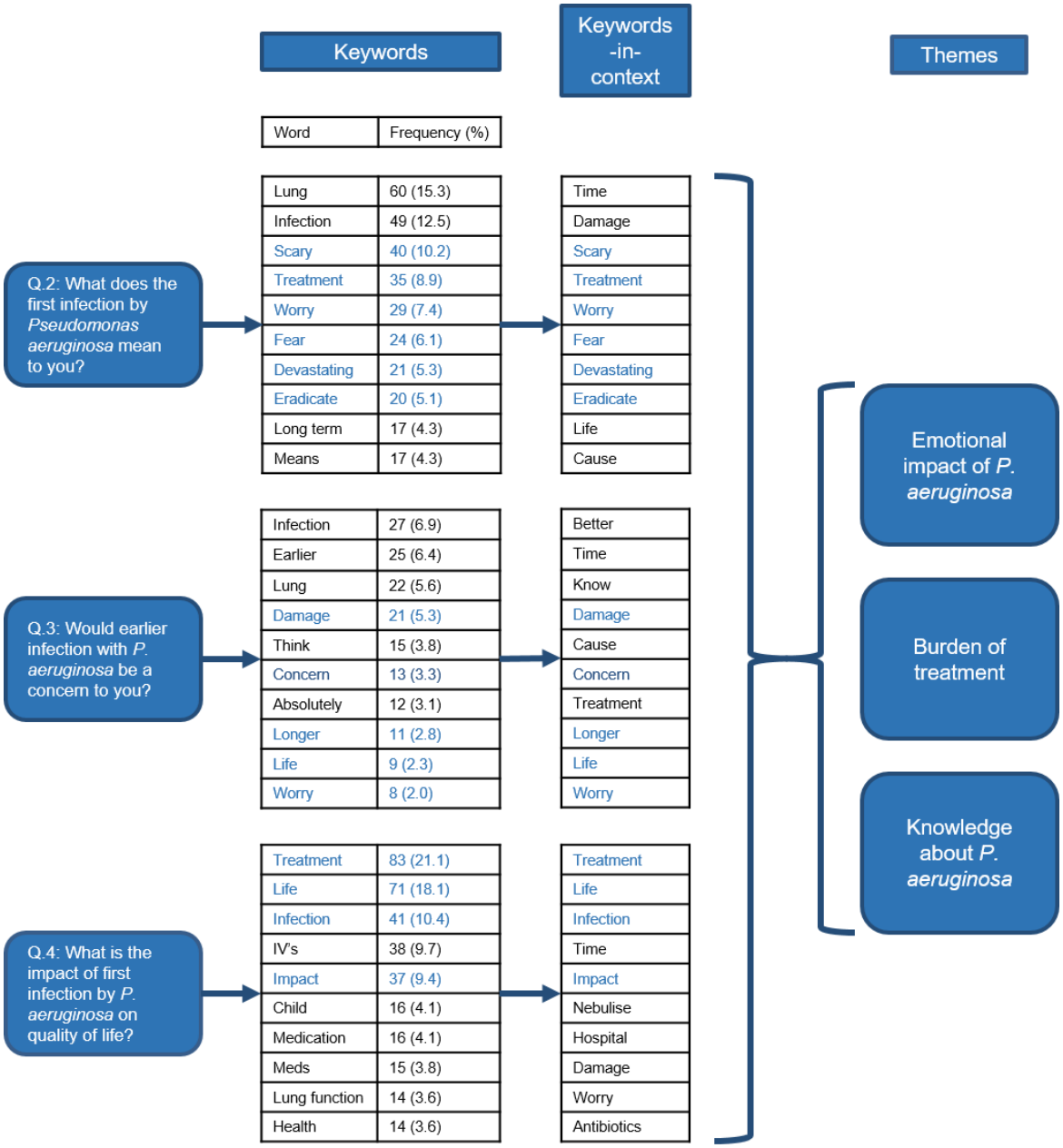


Figure 3-1: Flow diagram for the qualitative analysis approach to questions 2-4. For each free text question, the ten most commonly used words were identified. The ten most important words were then determined using a combination of frequency and significance; words carried over from the keywords to the keywords-in-context are highlighted in blue. The words in context informed the three overarching themes. This figure is adapted from that designed by Professor Alan Smyth published in the original paper (220).

This selection was based on a combination of the frequency of word use, and a subjective assessment of the interest generated. For example, structural words, such as lung, think and feel were excluded since they offered little substantive insight and may have merely reflected the question asked or linked topics. By contrast emotive words, which gave greater insight into the feelings of respondents, were prioritised. Arbitration by a third author (AS) was applied when necessary and all authors agreed the final lists.

The N'Vivo "text search" and "word tree" functions were used to demonstrate instances where each of the top ten words was used in the text. The different contexts in which each word was used were summarised and coded, with attention given to the number of different uses and any particularly interesting uses. Modifiers used in the context of emotive words (for example "I know" versus "I don't know") were noted.

3.2.2.3 Thematic analysis

The lists of keywords and keywords-in-context, along with the contexts in which the words were used, provided an initial overview of the whole dataset to identify areas which were likely to be of further interest (437). Agreement between all the authors on the important keywords and the most important contexts in which they were used facilitated the identification of three overarching themes across the responses to all the free-text questions posed in the survey.

The entire dataset was then analysed independently by SP and PL, independent of the results of the keyword and keyword-in-context analysis. Each response was considered individually and extracts from the responses were categorised by codes linked to each theme in Microsoft Excel (SP) or using the N'Vivo coding function (PL); some responses were applicable to more than one code. These were discussed by both authors and further corroborated by all authors to ensure validity.

3.3 Results

3.3.1 Demographics

Responses were received from 393 people. Of these 267 were parents of one or more people with CF and 97 were people with CF. Other respondents identified as siblings, friends or partners of a person with CF and members of the extended family (see Table 3-4). Three respondents identified in more than one category (for example "I have CF" and "I have a child with CF"). Of the two 'Other' respondents, one had bronchiectasis and one was a healthcare professional caring for people with CF.

Data on age, gender and country of residence were not collected. The *P. aeruginosa* status of the person with CF was also not formally collected but in 164/393 (42%) responses the participant made a direct reference to their experience of first infection with *P. aeruginosa* within

their free text answers. The median length of time to complete the survey was 2 minutes, 26 seconds with most participants completing it within 5 minutes, however some took over half an hour, with one participant taking over an hour to complete their responses. The total dataset included 1955 separate entries, comprising more than 15,000 words.

Relationship to person with CF	**Frequency (%)**
I have CF	97 (24.7)
I have a child/children with CF	267 (67.9)
Extended family member has CF	15 (3.8)
I have a sibling with CF	2 (0.5)
I have a friend with CF	2 (0.5)
My partner/spouse has CF	6 (1.5)
Skipped question	5 (1.3)
Other	2 (0.5)

Table 3-4. Frequency of respondents to the survey about how people in the CF community feel about first infection with CF by their relationship to a person with CF. Three participants identified in more than one category.

3.3.2 Keywords and Keywords-in-context

The ten most commonly used words in the responses to the free-text questions (2-4) are summarised in the keywords column of Figure 3-1. This figure is adapted from that designed for the published paper by Professor Alan Smyth. In response to question 2 (What does the first

infection by *Pseudomonas aeruginosa* mean to you?) there was a high proportion of emotive words, including scary, worry, fear and devastating. Question 3 (Would earlier infection with *P. aeruginosa* be a concern to you?) elicited words that represent time (earlier, longer, life) while in response to question 4 (What is the impact of first infection by *P. aeruginosa* on quality of life?) the most common words included those related to therapy such as treatment, IV's (meaning intravenous antibiotics), medications and "meds".

The ten most important words selected, on the basis of a combination of frequency of use and significance are presented in the keywords-in-context column (see Figure 3-1). The words retained from the keyword frequency analysis to the keywords in context are highlighted in blue.

The context in which these ten most important words were used was explored further. Words with an obviously emotive connotation were preferred to simple descriptive terms, or those which repeated back the question, such as lung, think and pseudomonas. Equally words which were less commonly used but which were used in a variety of contexts were explored further.

The word "time," was used in a number of situations including:

A single time point: *"They've never cultured PA at the same time"* (4P4)

Multiple time points: *"We have had it 4 times"* (3P3)

Time consumed by having/managing *P. aeruginosa*: *"Taking more time out of my life"*

Time and emotion: *"Horrible sad times"*

Time as in the future: *"However as time goes on"*

Other words used in a wide variety of meanings included "know" and "life".

In contrast other words had very specific meanings. "Scare," and "scary," were used to denote things that respondents were afraid of; either a non-specific, single-word response such as, "Scary", or detailing specific fears. Other examples of words with specific meanings were "damage," (used in the context of lung damage) and "devastating".

A small number of words had two opposing meanings, for example, "longer," which was used either to state that the longer a patient remained free of *P. aeruginosa* was better or that the longer one had *P. aeruginosa* the worse the effect on health. Similarly, "better," was used to denote either that the longer a person remained *P. aeruginosa* free was better versus those who felt that earlier treatment was "better".

3.3.3 Themes

3.3.3.1 Emotional Impact

The most commonly expressed emotion in relation to first infection with *P. aeruginosa* was fear. 152/393 (38.7%) of respondents reported either fear or worry. For some this was a simple statement such as, "scared," or, "it scares me." Others however expressed a complex range of fears relating to *P. aeruginosa* infection.

Fear about the impact of *P. aeruginosa* infection on future health was frequently expressed. Participants reflected on the impact of *P. aeruginosa* infection on quality of life and on the effect on longevity. There was a concern that infection would lead to increased symptoms which would reduce the quality of life:

> *"Not experienced it yet but I would assume, feeling a lot worse, stubborn cough, more productive and lung damage." Participant 241, parent.*

The role of *P. aeruginosa* in reducing lung function and thereby impacting on life expectancy was a concern for both parents and people with CF:

> *"As I was growing up I was scared of it because we were told that once you got it, you couldn't get rid of it and that it would bring frequent hospitalisations and declining lung function." Participant 367, patient.*

In addition, parents commented on the fear of their children dying sooner:

> *"Terrifying! I thought it was a death sentence for my daughter." Participant 265, parent*.

Fear about the impact of infection on practical matters were raised. The impact of ill-health and treatment on school attendance and their ability to work was raised by parents:

> *"…he spent less time in school than ever." Participant 346, parent.*

Adults with CF were concerned about time off work and their subsequent financial security:

> *"…if my condition deteriorates would I be able to continue working to pay bills, mortgage etc." Participant 227, patient.*

There was also worry about how *P. aeruginosa* infection would affect relationships, with one CF patient voicing a concern about, "letting people down" (*Participant 367, patient)* since *P. aeruginosa* infection had made their health and need for additional treatment unpredictable.

The stigma of *P. aeruginosa* infection within the CF community also caused fear about the future of relationships and,

> *"…marks you out as being different from peers." Participant 376, patient.*

This may increase feelings of isolation and contribute to poor mental health.

Combined, these fears led first infection with *P. aeruginosa* to be a cause of “devastation,” with a sense that infection with *P. aeruginosa* was associated with a loss; of health, of life expectancy and of normality.

P. aeruginosa infection marks a turning point in the CF journey. For some it is the start of, “the end.” Others felt that it was the beginning of a new set of symptoms or treatments. It also represented a change in how they saw themselves in the context of CF:

> *“… you think your child is invincible with mild cf until the first pseudonomas call.” Participant 212, parent.*

Fear of *P. aeruginosa* was not ameliorated with successful eradication therapy. Respondents acknowledged that in future they dreaded the result of every microbiological specimen in case the result was once again positive for *P. aeruginosa*.

Neither was fear of *P. aeruginosa* infection limited to those who had experienced *P. aeruginosa* positive cultures. CF patients and their families who were *P. aeruginosa* naïve still expressed fear and terror in anticipation of the infection. Lack of knowledge as to the source of infection magnified this fear. For parents in particular their child’s

acquisition of *P. aeruginosa* was associated with guilt and a sense that they had failed in some way to protect their child:

> *"… makes you feel as though you haven't been doing a good job taking care of things." Participant 204, parent.*

Fear in anticipation of infection, uncertainty regarding the source and guilt over "allowing" infection led people to take a wide variety of measures to attempt to avoid *P. aeruginosa*. Respondents described restricting their children's activities, having their kitchens and bathrooms refitted and even moving house in an attempt to reduce the risk. There was acknowledgement that these measures caused limitations to normal life for both the person with CF, their family and even the wider community:

> *"We don't go near stagnant or still water, hay, taps, mud, anything in the garden, we don't allow animals in our house, we don't visit friends' houses with pets. We don't do horse riding. We are incredibly picky where she uses swimming pools, especially private ones and test levels and try and avoid changing rooms. Her school have removed all their water butts, they don't have tadpoles in the classrooms anymore." Participant 3, parent.*

Respondents reported these measures had negative consequences on stress levels and mental health. Parents became, "paranoid" about avoidance strategies, yet despite undertaking so many measures to

reduce the risk there was a sense that *P. aeruginosa* acquisition is somehow inevitable:

> *"Terrifying bacteria we spend our lives trying to avoid, yet accept it is probably inevitable in the course of our child's life."*
> *Participant 165, parent.*

The energy consumed by some families to avoid a situation that they do not believe is truly avoidable has a negative impact on all aspects of their lives;

> *"…the majority of all our decisions to do with cf are actually because of pseudomonas." Participant 3, parent.*

The psychological effect of living with fear was, for some, at least as debilitating than the infection itself:

> *"I suspect the fear of PsA (for pwCF and the people close to them) may be almost as serious as the reality." Participant 367, patient.*

3.3.3.2 Treatment Burden

It was widely perceived that treatment of *P. aeruginosa* infection requires an increase both in the amount of therapy required and the time required to complete this treatment. Fear of the increased treatment burden was frequently expressed and, for some, equal to the fear of the health consequences of infection. This change in treatment

is another example of the turning point that first *P. aeruginosa* infection marks in the life of the CF patient.

> *"…it terrifies me...not just the affects it could have on her lung but also the treatments she would have to have introduced…" Participant 177, parent.*

Some treatments were specifically identified as a source of difficulty, in particular nebulised therapy, physiotherapy, intravenous antibiotics (IVs) and hospitalisations were felt to have a negative impact.

The major concern amongst respondents about all these treatments was in their impact on the quality of life of both the person with CF and their immediate family. Nebulised therapy and increased need for physiotherapy caused a day-to-day increase in the time taken to perform treatments, impacting on the ability to undertake normal day-to-day activities:

> *"I now have an extra nebuliser or inhaler to do twice a day for the rest of my life - which means another hour of every day lost to treatments." Participant 163, patient.*

In addition to the time taken to do nebulised therapy, the time taken to prepare the solution and cleaning and maintenance of the nebuliser was a problem. Many children did not want to undertake these therapies, leading to familial strain as parents tried to enforce the new treatment regimen:

“Now Jack needs nebs our day is filled with resistance, arguing and tension because it's another thing he doesn't want to do…” Participant 113, parent.

P. aeruginosa infection was perceived to herald an increase in hospitalisations and the need for IV antibiotics. In contrast to nebulisers and physiotherapy these did not have such impact day-to-day but had much more significant impact at the time required. Time in hospital required time off school and work (for both parents and adult patients). The uncertain trajectory of illness meant that these interventions were sometimes required, “at the drop of a hat,” making planning for day-today life more difficult. These difficulties were compounded in families with other children:

“Regular hospitals stays become the "norm" and affect the lives of all the family.” Participant 174, parent.

Again, *P. aeruginosa* infection was associated with a loss, this time of the ability to lead a normal life.

Another turning point was the advent of more invasive treatment than may previously have been necessary. For some, treatment of the infection required novel therapy, such as intravenous antibiotics, with the associated need for intravenous access. For some patients this mandated a general anaesthetic for the insertion of an intravenous line.

For others the effect of worsening health necessitated additional invasive treatment such as parenteral feeding.

The increase in treatment made the development of side effects more likely. Oral ciprofloxacin was mentioned by many respondents, causing change in bowel habit and an increase in sun sensitivity. Respondents also worried that the increased frequency of antibiotics led to an increased chance of developing antibiotic sensitivities which would limit future treatment options and contribute to the development of antibiotic resistance.

3.3.3.3 Knowledge

Knowledge about *P. aeruginosa* in the CF community can be both beneficial and detrimental. Much of the fear of *P. aeruginosa* comes from prior understanding that infection in CF is associated with declining health:

> *"It's scary as you know it's not good." Participant 7, parent.*

Equally, not knowing what the identification of *P. aeruginosa* infection would mean for the future caused distress. This distress was both related to the uncertain health impact as discussed above but also the practical difficulties of treatment:

> *"Scary time as not sure what it all meant." Participant 333, patient.*

In the surveyed population knowledge about *P. aeruginosa* was very variable. Many respondents acknowledged very little knowledge about *P. aeruginosa*, both before and after infection had been identified:

> *"Not sure, no experience yet." Participant 237, parent.*

> *"I have to attend a different day to clinic, apart from that I don't know much about it." Participant 142, patient.*

Misinformation about the inevitability of health decline following first infection with *P. aeruginosa* also contributed to anxiety:

> *"It scared me because I had only heard negative info about the bug. I thought I was going to die very quickly after getting it*." *Participant 16, patient.*

Several respondents were also under the impression that once *P. aeruginosa* infection had occurred, even after successful eradication, recurrence is inevitable. This incorrect knowledge contributed to their fear of first infection. Correcting misinformation helped:

> *"Was worried at first but I know it's treatable". Participant 242, parent.*

Investigating whether *P. aeruginosa* infection had occurred was a mixed blessing; some dreaded the tests which caused high levels of anxiety. For others knowing about the presence of infection early was a positive to enable early treatment.

Lived knowledge of *P. aeruginosa* infection was also mixed and depended upon the clinical outcome; for respondents where the CF sufferer's health was reasonable the experience of living with *P. aeruginosa* infection tempered their fears:

> *"…10 years on I can look back and say it didn't cause the problems I thought it would." Participant 387, parent.*

For patients and their families not knowing where the infection may come from and therefore being unable to prevent it left them feeling out of control. A variety of avoidance strategies were described, many of which have unclear benefit. However, there was acknowledgement that the practice of these measures could lead to harm:

> *"…this has made me pretty paranoid about everything as pseudo is everywhere and whilst I know it shouldn't as life is for living etc, it does make me stop my little boy from doing a few things due to the risks." Participant 151, parent.*

3.3.4 Importance of future research into *P. aeruginosa*

In question 5 participants were asked to rate on an ordinal scale how important it is to develop novel ways to deal with *P. aeruginosa*. 314/393 (79.9%) of respondents ranked it as Top priority/Urgent. The other responses are shown in Table 3-5.

How important do you think it is to develop more effective ways to deal with P. aeruginosa?	**Frequency (%)**
Top priority/Urgent	314 (79.9)
A priority/Not urgent	61 (15.6)
No strong feelings	3 (0.8)
Relatively unimportant	2 (0.5)
Totally unimportant	10 (2.5)
Not answered	3 (0.8)

Table 3-5: Frequency of each response to the question, "How important do you think it is to develop more effective ways to deal with P. aeruginosa". Question 5 from a survey of how people with CF, their close family and friends feel about first infection with Pseudomonas aeruginosa.

3.4 Discussion

3.4.1 How people with CF and those close to them feel about first infection with *P. aeruginosa*

First infection with *P. aeruginosa* has a significant impact on people with CF and those close to them. There is an emotional impact, predominantly fear, a perceived increase in treatment burden and problems with knowledge around first infection with *P. aeruginosa*.

Fear associated with *P. aeruginosa* infection occurs in people who are yet to experience infection and continues after successful eradication and once chronic colonisation has occurred. This fear primarily relates to negative health consequences associated with *P. aeruginosa*

infection. Fear of the health consequences of *P. aeruginosa* infection is linked to a fear of disease progression which is a common feature of chronic illness (438). The unpredictable nature of CF lung disease increases worry about the future (439); young adults with CF reported a fear of death and dying, compounded by uncertainly over their future clinical course (440).

P. aeruginosa infection is felt as a turning point in the course of CF disease (441); this new chapter is met with feelings of devastation and loss, linked to both guilt that acquisition has occurred and the fear of disease progression described above. Similar feelings of devastation, fear and guilt have previously been identified as occurring in families at the time of CF diagnosis (442) and in child and teenage patients at the time of a major health change (443). An event such as new *P. aeruginosa* acquisition can bring a family which has adapted successfully to living with a child with CF back to the stage of, “falling apart,” (442).

Caring for a child with a chronic illness places a responsibility on parents to do their best to prevent progression of the disease. Acquisition of *P. aeruginosa*, associated as it is with a new stage in illness, leaves parents feeling guilty that they have not done enough to protect their child (443, 444). In a survey of parents of children with *P. aeruginosa* 88% of participants reported that fear of *P. aeruginosa* acquisition markedly or somewhat increased their levels of stress (187).

The desire to prevent acquisition may promote unrealistic avoidance measures which can in themselves be damaging (187, 188).

Living in fear is detrimental for the mental health of patients and their families. It is important to note that in some circumstances fear may be productive, promoting adherence to therapy. However, as fear becomes more extreme this benefit is lost since engaging in treatment triggers fear and is therefore avoided (444).

Increased burden of treatment has a negative impact on the lives of people with CF and their families. The main burden was perceived to be related to inhaled therapies and the increased need for physiotherapy, hospital treatment and IV antibiotics. In a prior survey of adults with CF the use of two or more nebulised medications per day and spending more than 30 minutes carrying out airway clearance techniques was associated with a significantly increased burden of treatment (421). A study examining factors affecting adherence to nebulised medicine in people with CF found that the time-consuming nature of treatment was a big barrier to adherence (445).

This investment of time in treatment was sometimes found to be impossible at the same time as managing a relatively normal quality of life. As such patients have reported that rather than forgetting to take treatment, non-adherence was a conscious decision in order to prioritise normal life (439). These decisions were not straightforward and led to guilt.

Inpatient treatment creates a further, different, burden for patients and their families. In our survey parents reported the difficulties a sudden, two-week, admission had on work and on ensuring care for the rest of the family. People with CF reported the difficulty that it produced for school, university and work. The difficulties presented by recurrent, long-term hospital admissions have also been found to affect relationships between partners (440). In addition, spending time in hospital has the potential to expose patients and their families to a possible view of the future in the form of other, sicker, patients; something which many do not wish to face (440).

Knowledge about *P. aeruginosa* infection is variable, with some respondents describing little knowledge and others being acutely aware of the infection and its possible consequences. Lack of understanding as to the source of infection has led to the adoption of potentially harmful avoidance strategies; where these (unproven) measures fail, the guilt caused can lead to a redoubling of efforts which have a further negative impact on quality of life. People with CF have variable knowledge about risky environmental scenarios with respect to infection control and decisions to engage in potentially risky activities are balanced between the perceived risk and the impact of the activity on daily life (446). However better knowledge about *P. aeruginosa* has been associated with less stress and the undertaking of fewer preventative measures (187).

A study by Ullrich *et al*., interviewing families of 21 children with CF from a CF centre in Germany, found similar misconceptions about *P. aeruginosa* to those found in this study, including a belief that initial *P. aeruginosa* infection would lead to rapid health decline. They also identified a cohort of families who undertook a wide range of avoidance practices that expanded with every new perception of risk (188).

There remains uncertainty as to sources of *P. aeruginosa* infection and the day-to-day factors affecting how acquisition occurs. Current infection control guidelines tend to concentrate on strategies to prevent direct and indirect cross-infection, primarily within healthcare settings. In the home environment the CF Foundation's "Infection prevention and control (IP&C) guideline for cystic fibrosis: 2013 update" recommend disinfecting nebulisers and avoiding the use of hot-tubs and whirlpool spas (174) and the UK CF Trust recommends avoiding, "spas and other forms of aerated baths," (175). The European Cystic Fibrosis Society Standards of Care do not give any advice about infection control precautions in the home (447). This lack of understanding as to potential environmental sources of *P. aeruginosa* infection has led to physicians providing a wide range of advice (186) which is conceivably confusing to families.

Providing information of an appropriate level is challenging for healthcare professionals. Patients and their families generally want

accurate information (443) and rely most heavily on their healthcare team to provide this (446).

Themes similar to those we have identified have been recognised previously in studies using semi-structured interviews to explore the experiences of CF patients and their families. Themes comparable to Treatment Burden were common (439, 441). Higham *et al*. describe, "Living with Unpredictable Health and the Fear of Death and Dying," and, "Hopes for Normality," as important themes identified in interviews with young adults with CF discussing their hopes and fears for the future (440). "Fear" and "Fright" were identified as themes when Jessup and Parkinson examined the experience of parent, children and young adults living with CF (443). The importance of knowledge was acknowledged by parent participants in a study by Gjengedal *et al*. in the theme, "From uncertainty to certainty" – clarification of the diagnosis gave them an explanation for their child's symptoms, allowing them to move on (441).

3.4.2 Novel methodology

Our study highlights the possibilities inherent in the social media age in facilitating understanding of how the CF community feels about important issues, such as first infection with *P. aeruginosa*. Novel methods to gain these insights are particularly important in a condition such as CF where infection control practices limit group participation. We were able to elicit responses from nearly 400 people in only two

weeks and gained perspectives from both people with CF and those close to them.

Our survey was designed and implemented by a lay member of the CF community; involvement of the whole CF community in the design of research was called for by the UK CF Trust in its Research Strategy 2013-2018 (448). The value of research engagement by patients and the public is increasingly acknowledged (449).

However, this new form of data collection presents challenges for traditional qualitative analysis. Data from social media platforms and online surveys is of variable quality and quantity – participants are free to give as much or as little detail as they choose. In the present survey the free text questions were optional leading to widely differing levels of detail between respondents. Hence the data we gathered did not lend itself to either a traditional thematic analysis or a semi-quantitative approach; the dataset was too large and lacking in specificity for a purely thematic analysis and too small and non-representative to rely solely on content analysis and therefore necessitated a novel approach.

The “best bits” of both keyword analysis and thematic analysis were therefore combined. Examination of the keywords and their placement in context sensitised us to the themes inherent in the data; we were then able to scrutinise the answers to understand the feelings of our respondents. This may be a useful strategy for researchers in future analysing similar datasets, such as blog posts and healthcare forums in

which there is no formal structure to the data. Despite the large volume of data, thematic analysis provided a richer understanding of the topic than the keywords and keywords-in-context was able to do.

3.4.3 Strengths and limitations

We gained responses about how the CF community feel about *P. aeruginosa* from nearly 400 people. This is a much larger sample than previous studies looking at the question have managed (186-188). Additionally, whilst these previous studies have concentrated on parents of children with CF or physicians, our study was open to anyone with a connection to CF. We were able to understand the feelings of adult patients, parents of adult children, and possibly children with CF, as well as people with other connections, including spouses and friends.

The anonymity of the SurveyMonkey format means respondents may have felt more able to be honest than if they were talking to a member of the CF team or a researcher, particularly if they were critical of care. Honesty in on-line surveys, compared to face-to-face questionnaires, can vary with the questions asked, for example people may be more honest about mental health concerns online (450). Participants could provide as much information in their answers as they wished, allowing us to get some very detailed descriptions of the distress and burden associated with a first isolation of *P. aeruginosa*. Understanding these

lived experiences has helped direct research funding to ensure the most urgent concerns of those living with CF are prioritised (451).

Anonymous surveys also have limitations. There was no provision in the survey to prevent repeat responses therefore data could be skewed by people who wished to promote a particular point of view. It was also impossible to be certain that the participant had the stated relationship to CF; for example, parents could have answered on behalf of children. We have no data, for example, on the differences between adult and child responses to *P. aeruginosa.* Participation in the survey was limited to those with access to online devices, therefore we could, for example, have missed younger children who may have been differently affected by the avoidance measures practiced by their parents.

By definition, participation in an online survey is voluntary. We do not know how many people had access to the survey and chose not to respond, therefore the sample cannot be considered representative (452). Our self-selecting population may be biased towards with a stronger reaction to *P. aeruginosa*. This reduces the generalizability of these results – we can only state that the themes identified here are reported by some of the CF community. Nonetheless and awareness that a proportion of the CF community have these reactions to first infection with *P. aeruginosa* allows conversations to be opened by clinicians and support given to those who need it.

The survey was designed by a knowledgeable lay person, to give a rapid insight into the feelings of the community with no initial expectation of formal analysis. Had the survey been designed for publication at conception we would have gathered additional information from participants, for example age of participants, the *P. aeruginosa* status of the index person with CF and the country of residence, all of which may have affected the responses. However, these additional questions would have increased the length of the survey and potentially reduced participation.

3.4.4 The need for further research

Respondents in our survey overwhelmingly supported the need for additional research into more effective ways to deal with *P. aeruginosa*. Further work with the CF community supports this need. In a recent James Lind Alliance (JLA) Priority Setting Partnership (PSP) in CF the most important questions requiring further research were identified by a group of CF professionals, people with CF and their families. The best way to eradicate *P. aeruginosa* infection in people with CF was the tenth question identified. The top-ranked question was how to reduce the burden of treatment in CF care (453).

Two further topics of research are suggested by this survey. Firstly, respondents identified a need to gain a better understanding of how *P. aeruginosa* acquisition occurs, the sources of *P. aeruginosa* infection beyond cross-infection and factors affecting susceptibility to infection.

Recent work from a group in Belfast has looked at strategies to reduce *P. aeruginosa* acquisition in the home. Toothbrushes, household utensils and crockery were postulated as potential sources of *P. aeruginosa* infection and the group advocates the use of baby bottle sterilisers to disinfect these items before use by people with CF, particularly in households with more than one CF patient (454, 455). These studies, whilst interesting, cannot prove the efficacy of these methods in preventing infection. Promotion of unproven prevention strategies should be avoided as they increase the daily burden of living with CF. Parents may already perform more measures than are recommended (187) thus any recommendations must have a robust evidence base. In the home this evidence is unlikely to be forthcoming since studies of acquisition would either put patients at unacceptable levels of risk and therefore be unethical (157, 456, 457) or be prohibitively long and expensive.

Secondly there needs to be a better understanding of the information needs of parents and their families, in order that information is given at an appropriate level and pace. According to a survey of CF Centres in Germany 89% of Centres discuss infection prevention and control measures with families but only 6% of Centres include patients and families in the development of educational material (458). Improved knowledge may reduce the fear experienced by the CF community surrounding first infection with *P. aeruginosa*. It is however interesting

that parents who remembered physicians advising them not to undertake arduous preventative measures did not undertake fewer measures than other families (187).

3.5 Conclusions

First infection with *P. aeruginosa* causes fear in the CF community and is associated with an increased burden of treatment. Knowledge about the topic is limited in both the lay community and to some extent in healthcare providers. A better understanding, particularly about the likely success of eradication therapy, may allay fear but care must be taken that information is pitched at an appropriate level. Improved awareness of the role of the environment in the acquisition of *P. aeruginosa* is important for the CF community as a whole.

The psychological harm caused by *P. aeruginosa* infection is not limited to those who have chronic infection, and are therefore subject to long-term, detrimental, effects on their health. Nor is this harm confined to people with CF but also affects their parents, relatives and close friends.

Fear of the unknown is a potent source of anxiety (459). In this case there are two unknowns for patients and their families. The first is the long-term effects of *P. aeruginosa* infection which may not be as bad as initially feared. The second is the source of infection and whether it can be prevented.

In the next chapter I will examine evidence for prevention of infection with *P. aeruginosa*, both at home and within healthcare settings, in an attempt to understand what can reasonably be advised by healthcare professionals.

Chapter 4: A systematic review of interventions to prevent acquisition of *Pseudomonas aeruginosa* in Cystic Fibrosis

Details of the contributions made by colleagues, to assist in this chapter, are given in Table 4-1. This work was undertaken between October 2021 and July 2023.

Study stage	**Contributors**
Writing the protocol	Dr Sally Palser Dr Adil Khan Prof Alan Smyth
Study searches	Dr Sally Palser Mrs Sherie Smith
Title and abstract screening	Dr Sally Palser Mr Darren Sills Miss Hannah King
Full text screening	Dr Sally Palser Dr Sophie Dawson
Data extraction	Dr Sally Palser Dr Alex Yule

Table 4-1: A summary of the contributions made by colleagues to assist in the production of this systematic review.

I conceived this systematic review to look at primary prevention of *P. aeruginosa* infection. It was important in the story of this thesis to bridge the gap between the perception of harm caused by *P. aeruginosa* to patients and their families, and the strategies to reduce harm once infection has occurred. Additionally, after the work on chapter 3, and realising the lengths some patients and families will go to, in an attempt to prevent infection, I was keen to understand what prevention strategies have any evidence base, so that I could advise my own patients appropriately.

I was involved in each step of this process; writing the protocol, performing the searches, screening all the title and abstracts and full texts, data extraction and analysis. The size of this review meant that I was unable to find a single second author. I am very grateful to the many colleagues who have given their time to perform second reviewer checks at each stage. This difficulty prolonged the time for both this chapter and my thesis to be completed but I believe it was necessary to the flow of my argument.

4.1 Introduction

4.1.1 Why is it important to prevent infection with *P. aeruginosa*?

As discussed in chapters 1 and 3, infection with *Pseudomonas aeruginosa* has a detrimental impact on both physical and mental

health. It is clear from the responses detailed in chapter 3 (see section 3.3.3.3) that the CF population is keen to have a better understanding of strategies to prevent infection. The impact on mental health is not confined to the person with CF and may predate acquisition of the organism. Acquisition of *P. aeruginosa* is also associated with an increase in the burden of treatment, reduction of which was the top-ranked priority in the James Lind Alliance Priority Setting Partnership in CF (453).

4.1.2 Why do people with CF acquire *P. aeruginosa*?

Acquisition of *P. aeruginosa* requires exposure of a susceptible individual to the organism. Much is unknown about what factors affect both individual susceptibility, which may change with time, and the infectious capacity of individual strains. The impact of the infectious dose, the site of inoculum (for example nose vs mouth vs lung), the health-status of the exposed individual (recent exacerbation vs stable health) and different *P. aeruginosa* strains (epidemic vs sporadic) are all unknown. All these factors likely play a part in the balance of acquisition, rather than clearance of the organism, at a given time point. Studies to understand these factors are unethical as it is impossible to study them directly without exposing people with CF to serious risk of harm (157, 457).

A large body of work has developed examining risk factors, both environmental and personal, for *P. aeruginosa* acquisition.

4.1.3 Factors which affect individual susceptibility to *P. aeruginosa* infection

That not all episodes of contact between susceptible patients and those with a potentially transmissible strain end in novel acquisition suggests there may be more to acquisition than contact with *P. aeruginosa* (198).

4.1.3.1 Demographics

No demographic factors have been consistently found to affect acquisition of *P. aeruginosa*. Female sex has been identified as an independent risk factor in some studies (460, 461), while other studies have found no impact of sex (462-464).

Hispanic patients are more likely to acquire *P. aeruginosa* (461), and acquire it earlier, than people of non-Hispanic white ethnicities (465).

Family income is not associated with *P. aeruginosa* acquisition (462) but the effect of maternal education is less clear (460, 462).

4.1.3.2 CF genotype

CFTR functions as a pattern-recognition molecule for *P. aeruginosa*, with wild-type CFTR important in the innate immune response to infection (53, 466). Patients with minimal CFTR function are more likely than those with residual function to acquire *P. aeruginosa* infection (461, 467). Patients homozygous for the deltaF508 variant are particularly at risk (460).

4.1.3.3 Immunological response to *P. aeruginosa*

The immune response to *P. aeruginosa* infection may impact acquisition. Patients with high levels of Th-17 cytokines in bronchoalveolar lavage (BAL) fluid have greater chance of isolating *P. aeruginosa* in the following two years (468).

Alterations in components of the innate immune system may increase susceptibility to *P. aeruginosa* infection. Some genetic variants of surfactant protein D are associated with a greater chance of acquiring *P. aeruginosa* infection and CF patients with mannose-binding lectin deficiency are more likely to progress to chronic *P. aeruginosa* infection (469).

4.1.3.4 Pulmonary exacerbations

Pulmonary infections appear to increase patients' susceptibility to *P. aeruginosa* acquisition. Five of six children newly acquiring *P. aeruginosa* developed infection during, or in the subsequent three weeks after, a viral pulmonary exacerbation (470). Of 31 children with CF admitted to hospital with a pulmonary exacerbation in their first year, ten developed *P. aeruginosa* infection, compared with only 3/49 infants who were not admitted (471). Facilitation of *P. aeruginosa* infection by another pathogen is a possible cause of this difference, though nosocomial exposure or intrinsically worse clinical state may also contribute (471).

Respiratory syncytial virus (RSV) infections are particularly associated with new onset *P. aeruginosa* infection. Patients developing chronic *P. aeruginosa* infection were more likely to have had recent RSV infection, which commonly preceded a rise in anti-*P. aeruginosa* antibodies (472). RSV has been shown to facilitate acute *P. aeruginosa* infection in a mouse model of cystic fibrosis (473) and to increase *P. aeruginosa* binding to CF respiratory epithelial cells in cell culture (233).

4.1.3.5 Other illnesses and medical treatment

CF diabetes and CF liver disease have been identified as risk factors for *P. aeruginosa* infection (463), though whether this is intrinsically due to these conditions or rather as a marker for more severe disease is unclear.

Gastro-oesophageal reflux is more common in people with CF and is associated with poorer lung function (474). *P. aeruginosa* can live in CF stomach juices (475), thus reflux may facilitate infection and/or re-infection. Patients with *P. aeruginosa* have a higher reflux burden, particularly of non-acid reflux, than those without infection, however the association is no longer significant once account is taken of age and FEV_1 (476).

P. aeruginosa infection of the paranasal sinuses has been shown to precede infection in the lower airways (477). In a case series of five patients undergoing dental and ear, nose and throat (ENT) treatment all developed novel *P. aeruginosa* infection in the subsequent six months

(478). Incident infection in the paranasal sinuses may have spread to the lower airway during the upper airways procedures though, equally, *P. aeruginosa* infection is common in non-CF ENT patients and may have spread through inadequate infection control (478).

4.1.4 Factors which affect exposure to *P. aeruginosa*

4.1.4.1 Environmental sources of P. aeruginosa exposure

For the majority of people with CF the source of *P. aeruginosa* infection is likely to be environmental (479).

4.1.4.1.1 Sources of P. aeruginosa in the home

In the home environment *P. aeruginosa* is most commonly found in the drains of sinks, baths and taps (163-166, 480, 481). *P. aeruginosa* has also been found in kitchen taps (163, 166), bathroom taps (163, 166, 482), sinks (163, 166, 480) and bottled water (424). Household environmental *P. aeruginosa* strains do not necessarily match resident patient's strains; where genotypically matching strains have been found the direction of organism transfer cannot be determined (163). A study in Victoria, Australia, found the only home environmental risk factor associated with earlier acquisition of *P. aeruginosa* was living in a household where a water sprinkler system was used (479).

Toilets are more likely to be contaminated with *P. aeruginosa* in the homes of people with CF than those without (167). Bacteria in the toilet

bowl can be aerosolised during flushing, contaminating nearby surfaces and leaving organisms detectable in the air an hour later (483).

P. aeruginosa has been identified on toothbrushes from people with CF (166, 484, 485). Contamination of toothbrushes may occur from a patient's mouth or via deposition of bioaerosols, for example from a sink drain or flushed toilet.

Home healthcare devices, including nebulisers, airway clearance devices and mist tents, have been suggested as a source of infection. In a large epidemiological study examining risk factors for *P. aeruginosa* acquisition use of aerosolised treatment was found to increase the risk (462). *P. aeruginosa* identical to the clinical strain was identified on the nebuliser in one patient with new infection however again the direction of transfer could not be determined (163).

4.1.4.1.2 Water sources

Novel *P. aeruginosa* infection was described in CF patients who had recently spent time in a hydrotherapy pool. Subsequent sampling of the water found an identical *P. aeruginosa* strain using pyocin typing and a DNA probe (173). Hydrotherapy pools, spa pools and hot tubs are commonly contaminated with *P. aeruginosa* (424, 482, 486). Swimming pools may also be contaminated (486) but swimming has been found to be protective against *P. aeruginosa* infection (461). Both the UK CF Trust and US CF Foundation advise against use of

hydrotherapy pools, spa pools and hot tubs (174, 175) due to the risk of *P. aeruginosa* infection.

P. aeruginosa has been found in rivers (170, 487), lakes (487) and sea water (488, 489). Living closer to an open water source (both as the crow flies and by walking distance) was associated with an increased risk of chronic *P. aeruginosa* infection (172). However, the study was limited by a number of confounding factors and a subsequent registry study in the US found no effect of living close to an open body of water on age at *P. aeruginosa* acquisition (490).

4.1.4.1.3 Dental care

P. aeruginosa contamination of water from dental units has been widely reported (491-494). Water sampling from dental apparatus found *P. aeruginosa* more commonly during CF patient visits (18/327) than for people without CF (3/103). In one sample there was a concordant strain between the patient and the water sample (492).

4.1.4.1.4 Climate and geography

The impact of geography on *P. aeruginosa* acquisition is complex and in many cases contradictory. Earlier acquisition of *P. aeruginosa* is associated with living in hotter climates (490, 495) with a higher dewpoint (humidity) and average rainfall (490). However, living at higher latitude (i.e., further from the equator) is also associated with increased risk (490). This last point neatly summarises the complexity

of assessing the risk; in a purely geographical or purely meteorological model, living at lower latitude was associated with increased risk, once the models were combined living closer to the equator was protective (490).

Living in a rural area has been associated with earlier *P. aeruginosa* acquisition (462, 479). This may be related to greater exposure to environmental sources of *P. aeruginosa*. However, it contrasts with data suggesting that increased exposure to airborne fine particulate matter (pollution) early in life, typical of an urban area, is associated with earlier incidence of *P. aeruginosa* (496).

The effect of season on the acquisition of *P. aeruginosa* is equally unclear. Early reports from Denmark suggested an increase in the incidence of infection in October to March, with the authors postulating a role for respiratory viruses in predisposing patients to acquire *P. aeruginosa* (497). A study in the US using registry data from the CF Foundation found an increase in *P. aeruginosa* in summer and autumn compared to winter, with reduced incidence in the spring (498).

4.1.4.2 Shared strains

The emergence of *P. aeruginosa* as a significant CF pathogen coincided with the commencement of care within dedicated CF Centres in the 1960s (499). Genotypically proven strain sharing was identified in the 1990s (176, 195, 500). Since the identification of the Liverpool Epidemic Strain (LES) in 1996, shared strains have been described

worldwide. In a review of *P. aeruginosa* samples from 31 CF Centres in the UK six clustered strains were identified, including LES (present in 15 clinics), Manchester Epidemic Strain (MA, present in 3 clinics) and Clone C. Of three newly described clustered strains, one, Midlands-1 was found in 9 clinics (501). In Australia AUST-01 has been found in five geographically distinct clinics in Eastern Australia (502). LES has been identified in Canada (503) and Australia (albeit in a UK patient) (504). Other shared strains include AUST-02 (505) and AUST-03 (506) from Australia; DK-1 and DK-2 from Denmark (507); Clone C, found widely in Europe (487); Prairie Epidemic strain (PES) from Canada (503) and Dutch Epidemic Strain from The Netherlands (503).

Individuals infected with a unique *P. aeruginosa* strain may acquire infection with an additional, epidemic, strain in a process known as superinfection. Superinfection has been shown for a number of epidemic *P. aeruginosa* strains including LES (176, 456, 503, 508, 509), PES (503, 510) and the MA strain (456).

4.1.4.2.1 Mechanism of transmission

Transmission of *P. aeruginosa* between patients may be through direct contact or indirectly, through contact with a contaminated intermediary (fomite transmission, such as respiratory equipment, furniture and the hands of care staff), droplet spread or airborne dissemination (see section 1.2.1).

Coughing, sneezing, singing and to a lesser extent talking, generate a turbulent jet of air containing droplets of moisture from the airways; *P. aeruginosa* present within the respiratory tract may be expelled within the respiratory droplets. Deposition of these droplets on mucosal surfaces can lead to direct transmission of the infective organism. Alternatively, these droplets settle rapidly under the effect of gravity, potentially contaminating the immediate vicinity, with indirect infection following contact with contaminated surfaces. Viable *P. aeruginosa* has been found settling on sterile drapes up to two metres away from an infected patient (511).

Evaporated respiratory droplets form droplet nuclei which can remain airborne for prolonged periods, spreading on air currents far from the original source. Particles of this size are within the respirable range and can penetrate the lower airways. Airborne transmission via droplet nuclei has been demonstrated for respiratory pathogens including measles (512), influenza (513) and tuberculosis (514). Experimentally *P. aeruginosa* can be aerosolised into respirable particles < 2.0µm in diameter (183).

4.1.4.2.2 Fomite transmission

Fomites are objects which, when contaminated with an infectious organism, enable transmission of said organism to another object or individual through direct contact (515).

Acquisition of *P. aeruginosa* within a healthcare setting may be related to contact with a common, environmental source. Such a source could be endemic to the healthcare setting or introduced by an index patient(s). Multiple attempts to identify *P. aeruginosa* from hospital environments have been made. Transient environmental reservoirs of LES have been found in sinks, bed linen, clothes and on respiratory equipment (180). LES may also contaminate the hands of those infected (180). Despite extensive sampling no environmental reservoirs of AUST-01 (181), AUST-02 (516), AUST-03 (506), the MA strain (157) or PES (503) have been found.

P. aeruginosa strains matching patient samples have been identified in the hospital environment (159, 160, 180, 517) however the direction of transmission cannot be ascertained. As with household sampling, drains are a common source of *P. aeruginosa* in healthcare settings (159, 160, 180, 517, 518). *P. aeruginosa* has been cultured from sinks, showers and baths (157, 181, 509). Dry environmental surfaces have not been found to harbour *P. aeruginosa* (157, 180, 181, 519) though experimentally contaminated surfaces were positive up to 48 hours after inoculation (180).

Experimentally contaminated hands can transmit *P. aeruginosa* through hand shaking; where the contaminant is CF patient sputum viable transmission persists much longer (159). Patients with *P. aeruginosa* infection may have contaminated hands (520). A study in the early

1990s found *P. aeruginosa* contamination of staff hands during shifts, though not at the start of the shift (517). More recent studies have found no evidence of *P. aeruginosa* on the hands of healthcare staff (157, 180, 197, 521, 522).

Hand hygiene is important in the prevention of fomite transmission of infection. The 2013 IP&C guidelines emphasise the need for good hand hygiene, for both patients, their families and healthcare professionals (174). Healthcare professionals are recommended to perform hand hygiene in accordance with the World Health Organisation's, "5 moments for hand hygiene" (523). For patients and visitors hand hygiene is recommended on entering and leaving hospital rooms, and after contact with respiratory secretions (174). The importance of hand hygiene in reducing the spread of respiratory pathogens has been highlighted by the COVID-19 pandemic (524).

4.1.4.2.3 Airborne transmission

Viable *P. aeruginosa* may be present in the air in the vicinity of infected patients (180, 519). After forced expiratory manoeuvres, including physiotherapy and spirometry, the proportion of positive samples increases (423, 519). LES was found in up to 80% of air samples from the inpatient rooms of patients harbouring this strain and remained detectable up to three hours after the patient had vacated the room. More worryingly *P. aeruginosa*, including LES, has been found in the air

of communal areas including corridors (180), clinic rooms (180) and waiting rooms (157).

Cough aerosols generated by people with CF release *P. aeruginosa* into droplet nuclei (525, 526). These droplet nuclei may contain viable *P. aeruginosa* at a distance of up to four metres and up to 45 minutes after the patient departs (185). Patients with higher sputum counts of *P. aeruginosa* tend to produce higher aerosol counts (185, 526), as do those with a higher FEV_1 (who may have a stronger cough) (526) though considerable variability between individuals suggests some people are more prone to causing air contamination than others.

At the standard rate of air changes in a patient room (approximately two changes per hour (527)) it would take at least 50 minutes after a patient has left for 90% of the viable *P. aeruginosa* to be gone (185).

Routine wearing of face masks in hospital settings, particularly communal areas, was recommended in the 2013 IP&C guidelines (174). The necessity of mask wearing was initially debated, particularly in individual examination rooms (528, 529). However, mask-wearing has become more acceptable in the light of the COVID-19 pandemic (524) and prior experience of using face masks may have reduced the incidence of SARS-CoV-2 infection in people with CF (530).

4.1.4.2.4 Degree of contact required for transmission

CF siblings commonly share strains (161, 177, 181). Close, prolonged contact was assumed necessary for transmission to occur (462). This assumption was used to dispute wider strain sharing; where common strains were found in unrelated patients with little or limited contact the possibility of transmission was discounted (531).

Participation in summer camps or training courses was found to be a risk factor for acquisition of a clustered *P. aeruginosa* strain in Norway (532). Novel acquisition of a *P. aeruginosa* strain identical to that harboured by another camp participant has been described (533-536). Other studies have failed to find evidence of transmission (537-539) though one study did not follow participants up after the end of camp (537) and the other two are ambiguous as to the *P. aeruginosa* status of all their participants at commencement (538, 539).

Potential transmission of shared strains during in- or out-patient care has been described in a number of centres (157, 540, 541). Related *P. aeruginosa* strains have been found to cluster in groups of patients with increased in-patient, or social, contact (178, 542). Conversely, cross-colonisation was only found in 3/7 pairs of CF patients sharing a hospital room and the shared strains were only identified transiently and not re-isolated during a two-year follow-up. The use of phenotypic typing only in this study may have underestimated the extent of the problem (543).

In fact, attendance at clinic on the same day may be sufficient contact to facilitate transmission in some cases (179, 544). Stapleton *et al.* found shared strains in 29/70 children with early *P. aeruginosa* infection. Shared strains were defined as those differing by four or fewer single nucleotide polymorphisms (SNPs). There was epidemiological evidence to support patient-to-patient transmission in a third of these cases, with more contact found in the clinic than on the ward (160). Another study described five cases of putative patient-to-patient transmission of strains matched using whole genome sequencing (WGS). Contact was in the clinic in two instances and with multiple overlapping clinic and ward admissions in the other three cases (161).

4.1.5 Aim

Prevention of *P. aeruginosa* infection, therefore, may be via strategies to reduce host susceptibility or to reduce exposure to *P. aeruginosa*. The aim of this chapter is to systematically examine the available evidence for methods which successfully prevent *P. aeruginosa* acquisition in people with CF.

4.2 Methods

This study was conducted according to the rapid review recommendations outlined by Garrity *et al*. 2021 (545).

The full study protocol was registered on the online registration database PROSPERO (CRD42022289086), https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42022289086.

4.2.1 Inclusion criteria

4.2.1.1 Population

Studies examining people with CF of any age were eligible for inclusion. Studies which included both people with and without CF were eligible if the data for the people with CF could be separated from those without.

4.2.1.2 Intervention(s) and Comparator(s)

We considered any intervention strategy which had the potential to prevent infection with *P. aeruginosa*.

Comparison could be made with no intervention, or any other strategy to prevent *P. aeruginosa* infection.

Any type of study in which an intervention occurred which had potential to prevent *P. aeruginosa* infection was eligible. Epidemiological studies, with no active intervention, were excluded. Studies of patients in simulated clinical settings were eligible, but purely laboratory studies with no patient involvement were excluded (for example studies examining cleaning of participants equipment were included but cleaning of experimentally contaminated equipment was not).

4.2.1.3 Outcome measures

4.2.1.3.1 Primary outcome

The primary outcome measure was acquisition of new *P. aeruginosa* in respiratory tract samples (sputum cultures, BAL washings, cough swabs etc.) in patients with no previous infection or free of infection. Freedom of infection could be defined by each individual study but there had to be some criteria to ensure that novel infection, rather than further isolation of intermittently occurring *P. aeruginosa*, was reported. Acquisition of *P. aeruginosa* could also be reported as time to, or age at, novel infection. Studies reporting new chronic *P. aeruginosa* infection were excluded where data about initial infection could not be extracted since these studies may also represent success (or not) of eradication methods, rather than simple prevention of acquisition.

4.2.1.3.2 Secondary outcome measures

The secondary outcome measures considered were:

- Impact of the intervention on quality of life, measured by any validated quality of life tool.
- Adverse effects of the intervention.
- Adherence to the intervention strategy, measured by any method.
- Change in FEV_1-percent predicted ($ppFEV_1$) as a consequence of the intervention.

- Environmental dissemination of *P. aeruginosa*

4.2.2 Search strategy

In November 2022 searches were carried out in the following databases: MEDLINE, EMBASE, CINAHL, and PubMed.

The search strategy was updated from that developed by SS for an earlier systematic review of infection prevention and control methods in cystic fibrosis (546). Search terms were deliberately kept broad to increase sensitivity. Only studies in English were included and unpublished studies and grey literature were not sought. The full search terms can be found in Appendix A: Chapter 4 search strategies.

4.2.3 Study selection

Titles and abstracts were imported into the Rayyan software (547). A pilot exercise using 30 abstracts was performed by two reviewers (SP and AK) to test the standardised title and abstract form. One reviewer (SP) screened all the returned titles and abstracts. A second reviewer screened twenty percent of the returned titles and abstracts and all the remaining excluded abstracts (DS or HK). Conflict resolution was by discussion.

Studies which appeared to meet the inclusion criteria were retrieved in full. One reviewer (SP) screened all the remaining full-text articles with excluded articles screened by a second reviewer (SD). At this stage

reasons for exclusion were recorded and any disagreements were resolved by discussion.

4.2.4 Data extraction and synthesis

One reviewer (SP) extracted data from all included reviews using a pre-defined and agreed data extraction form. A second reviewer (AY) checked data completeness and correctness. Extracted data were collated in Microsoft Excel (548).

Based on the previously conducted review of infection prevention and control in CF (546) it was felt unlikely that sufficient comparable studies would be available to perform a meta-analysis. A narrative synthesis was therefore planned, using the principles outlined in the SWiM guidelines (549). Interventions were grouped into Therapeutics; Vaccines; Neonatal care; Environmental (Healthcare settings); and Environmental (Non-healthcare settings).

The direction of effect for each outcome was reported as showing either a beneficial effect, a negative effect or no significant effect. The definition of beneficial and harmful effect for each outcome was as follows:

- For the primary outcome, acquisition of *P. aeruginosa*, a beneficial effect is defined as fewer participants in the intervention group acquiring *P. aeruginosa* than in the control

group, or later time to/age at acquisition in the intervention group.

- For quality of life a beneficial effect is defined as a higher/better quality of life score in the intervention group compared to the control group after application of the intervention.
- For adverse effects a beneficial effect is defined as fewer adverse effects in the intervention group than the control group. Should a study report fewer effects in one group but more serious in the other this will be acknowledged in the analysis.
- For adherence to the intervention a beneficial effect is defined as a higher proportion of participants adhering to the study protocol than in the comparator group.
- For change in ppFEV$_1$ a beneficial effect is defined as either a greater increase in the ppFEV$_1$ in the intervention group or a less negative decrease in the ppFEV$_1$ in the intervention group as compared to the comparator group.
- For all the above outcomes a negative impact is defined as where the above definition is reversed, i.e., the outcome is better in the comparator group than the intervention group.

Where multiple studies with different effect directions for a given intervention were identified, harvest plots were used to examine whether different study characteristics had any impact on the direction of effect.

For each intervention identified, the certainty of the body of evidence for the primary outcome was assessed using the GRADE approach. This was done by a single reviewer (SP) with verification of judgements by a second reviewer (AY).

4.2.5 Risk of bias assessment

Risk of bias was ascertained for the primary outcome for all studies. For randomised controlled trials (RCTs) the Cochrane Risk of Bias tool (RoB 2) (550) was used while for non-RCT studies the ROBINS-I tool was used (551). The risk of bias assessment was carried out by a single reviewer (SP) with decisions verified by a second reviewer (AY). Conflict was resolved by discussion.

All studies meeting inclusion criteria were included regardless of the risk of bias, though this was considered in the analysis. Where multiple studies were available for a given intervention, priority was given to reporting those with the lowest levels of bias.

Confounding factors considered included: age of participants (increased age leads to increased chance of exposure to *P. aeruginosa*); sex; baseline ppFEV$_1$ (lower ppFEV$_1$ may increase chance of infection); co-morbidities including acute illnesses and chronic conditions such as CF-diabetes and site (CF-Centre vs. Local hospital). Important co-interventions considered included: regular intravenous antibiotic therapy (may reduce *P. aeruginosa* burden and

therefore reduce chance of identifying the organism in samples); inhaled antibiotics and combination of modalities of intervention. Frequency of sputum sampling was noted as a possible source of measurement bias.

4.3 Results

The database searches returned 8596 articles with an additional seven studies identified through additional reading (see Figure 4-1). Duplicate searching was done using the EndNote duplicate search tool and then hand searching of the remaining articles. After completion of duplicate searching 5862 articles remained.

Following title and abstract screening 128 articles were included for full text review. Of these, 52 references to 49 studies met the inclusion criteria and were included in the review. Reasons for exclusion are described in Figure 4-1 and Appendix B: Chapter 4 excluded studies.

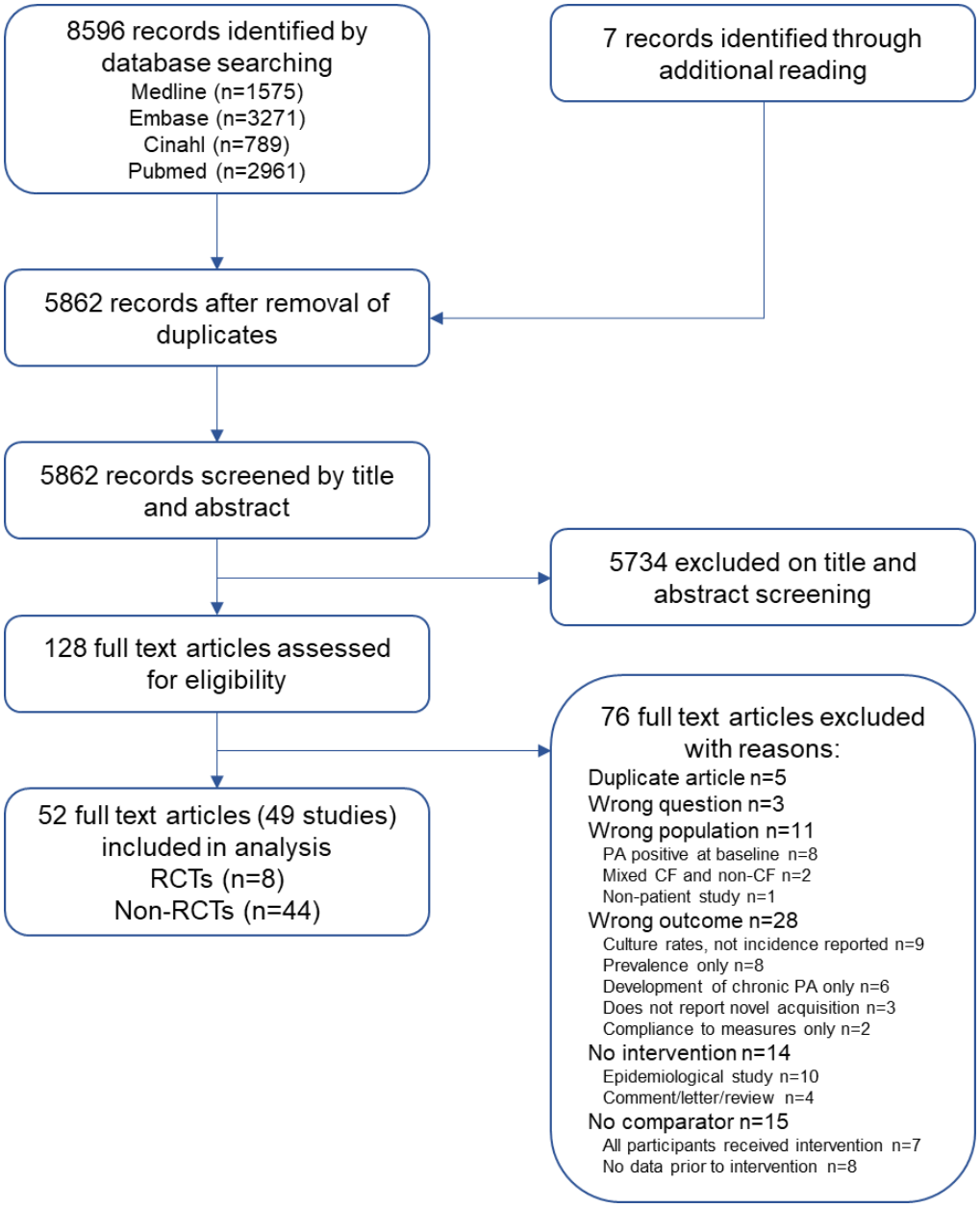


Figure 4-1: PRISMA diagram of studies identified, screened and reasons for exclusion for the systematic review of interventions to prevent P. aeruginosa acquisition in cystic fibrosis.

We initially planned to analyse the interventions in four categories: therapeutics, vaccines; environmental strategies (clinical) and environmental strategies (home). After review of the studies identified and the high frequency of studies examining the effect of newborn

screening (NBS), we included a fifth category, neonatal care. The numbers of studies in each category, the study types, participant numbers, risk of bias and outcomes measured (with effect direction) are shown in Table 4-2.

The GRADE assessment of the level of certainty of the evidence for each intervention for the primary outcome is shown in Table 4-3.

Intervention	Study design	N	Risk of Bias	Reported outcomes and direction of effect						
				PA acquisition	Time/age to PA	Quality of Life	Adverse events	Adherence	Change in FEV_1	Environmental dissemination
Therapeutics n=13										
Antibiotic prophylaxis n=6										
Anti-pseudomonal prophylaxis n=2										
Tramper-Stranders 2010 (552)*	RCT	65 (34/31)	Some concerns	<>	<>		<>		<>	
Heinzl 2002 (301)*	Retrospective cohort	28 (12/16)	Serious	+			-	-	†	

Intervention	Study design	N	Risk of Bias	Reported outcomes and direction of effect						
				PA acquisition	Time/age to PA	Quality of Life	Adverse events	Adherence	Change in FEV_1	Environmental dissemination
Anti-staphylococcal prophylaxis n=3										
Weaver 1994 (553)*	RCT	38 (18/20)	High	<>						
Ratjen 2001 (554)*	Retrospective registry	639 (308/258/73)	Serious	-					†	
Hurley 2018 (555)*	Retrospective registry	604 (278/326)	Serious		-					
Azithromycin n=1										
Saiman 2010 (83)	RCT	263 (131/132)	Low	<>			+	<>	<>	

Intervention	Study design	N	Risk of Bias	Reported outcomes and direction of effect						
				PA acquisition	Time/age to PA	Quality of Life	Adverse events	Adherence	Change in FEV_1	Environmental dissemination
BAL-directed therapy n=1										
Wainwright 2011 (556)	RCT	170 (84/86)	Low	<>	<>		<>		†	
Modulators n=2										
Frost 2019 (100)	Retrospective registry	5572 (276/5296)	Serious	+						
Singh 2019 (101)*	Retrospective cohort	193 (88/105)	Serious	<>	+					

Intervention	Study design	N	Risk of Bias	Reported outcomes and direction of effect						
				PA acquisition	Time/age to PA	Quality of Life	Adverse events	Adherence	Change in FEV_1	Environmental dissemination
Palivizumab n=4										
Fink 2019 (557)*	Retrospective registry	4267 (1588/2679)	Moderate		<>				†	
Groves 2016 (558)	Retrospective cohort	92 (45/47)	Moderate	-	-				†	
Buchs 2017 (559)*	Retrospective case-control	180 (40/140)	Serious	<>	<>					
Linnane 2015 (560)	Retrospective cohort	49 (19/30)	Serious	<>						

Intervention	Study design	N	Risk of Bias	Reported outcomes and direction of effect						
				PA acquisition	Time/age to PA	Quality of Life	Adverse events	Adherence	Change in FEV_1	Environmental dissemination
Vaccination n=4										
Döring 2007 (561)*	Multicentre RCT	483 (239/244)	Some concerns	+			-	<>	<>	
Lang 2004 (562)*	Retrospective case-control	52 (25/25 analysed)	Serious	+	+		<>		†	
Cryz 1997 (563)*	Retrospective case-control	52 (26/26)	Serious	<>			-			
Lang 1995 (564)*	Retrospective case-control	52 (26/26)	Serious	+			-			

Intervention	Study design	N	Risk of Bias	Reported outcomes and direction of effect						
				PA acquisition	Time/age to PA	Quality of Life	Adverse events	Adherence	Change in FEV_1	Environmental dissemination
Neonatal care n=10										
Newborn screening n=7										
Rosenfeld 2022 (565)	Retrospective registry	9571 (4634/4421)	Low		<>					
Coffey 2017 (566)	Retrospective case-control	135 (45/90)	Low	+	<>				†	
Dijk 2011 (567)	Retrospective cohort	117 (60/57)	Moderate		+				†	
Farrell 2003 (568)	RCT	103 (56/47)	High	-	-				†	

Intervention	Study design	N	Risk of Bias	Reported outcomes and direction of effect						
				PA acquisition	Time/age to PA	Quality of Life	Adverse events	Adherence	Change in FEV_1	Environmental dissemination
Lim 2014 (569)	Prospective (NBS) and retrospective (pre-NBS) cohort	283 (180/103)	Serious	+	-					
Baussano 2006 (570)*	Retrospective cohort	71 (44/27)	Serious	<>	<>					
Mak 2016 (571)	Retrospective registry	303 (201/102)	Serious	+						

Intervention	Study design	N	Risk of Bias	Reported outcomes and direction of effect						
				PA acquisition	Time/age to PA	Quality of Life	Adverse events	Adherence	Change in FEV_1	Environmental dissemination
Breastfeeding n=2										
Munck 2018 (572)	Prospective multi-centre cohort	105 (69/30 analysed)	Serious	<>	<>					
Jadin 2011 (573)	Prospective multicentre cohort study	103 (53/50)	Serious	+						
NICU admission n=1										
Faniyi 2021 (574)	Retrospective cohort	40 (13/27)	Serious	<>	<>					

	Study design	N	Risk of Bias	Reported outcomes and direction of effect						
				PA acquisition	Time/age to PA	Quality of Life	Adverse events	Adherence	Change in FEV_1	Environmental dissemination
Environmental strategies (healthcare) n=22										
Adherence to IP&C guidelines n=2										
Muhlebach 2022 (575)	Pre-/post-registry study	Adult Centres 82/54; Paediatric Centres 108/102	Serious	<>						
Kim 2020 (576, 577)*#	Pre-/post-observational study	146-155 (varies with time)	Serious	<>						

Intervention	Study design	N	Risk of Bias	Reported outcomes and direction of effect						
				PA acquisition	Time/age to PA	Quality of Life	Adverse events	Adherence	Change in FEV_1	Environmental dissemination
Cohort segregation n=3										
Hayes 2010 (578)*	RCT	39 (21/18)	High	+	<>			<>		
Farrell 1997 (499)	Retrospective cohort (using data from an RCT)	123 (64/59)	Moderate	+	+					
Frederiksen 1999 (579)*	Pre-/post-observational study	122-286 (varies with time)	Serious	+	<>					

Intervention	Study design	N	Risk of Bias	Reported outcomes and direction of effect						
				PA acquisition	Time/age to PA	Quality of Life	Adverse events	Adherence	Change in FEV_1	Environmental dissemination
Cohort segregation plus n=8										
Lee 2004 (580)*	Pre-/post-observational study	232 (varies with time)	Moderate	<>	<>					
Griffiths 2012 (581)*	Pre-/post-observational study	277-325 (varies with time)	Moderate	+		+				

Intervention	Study design	N	Risk of Bias	Reported outcomes and direction of effect						
				PA acquisition	Time/age to PA	Quality of Life	Adverse events	Adherence	Change in FEV_1	Environmental dissemination
Kevat 2018 (582)*	Pre-/post-observational study	244-344 (varies with time)	Moderate	+		+				
Jones 2005 (456)*	Pre-/post-observational study	216-250 (varies with time)	Moderate	+						
Pedersen 1986 (422)*	Pre-/post-observational study	119	Moderate	+						<>

Intervention	Study design	N	Risk of Bias	Reported outcomes and direction of effect						
				PA acquisition	Time/age to PA	Quality of Life	Adverse events	Adherence	Change in FEV_1	Environmental dissemination
Gilchrist 2017 (583)*	Retrospective cohort	8 CF Centres	Serious	<>						
Tümmler 1991 (584)*	Pre-/post-observational study	(varies with time)	Serious	+						
McKay 2009 (585)*	Pre-/post-observational study	72-90 (varies with time)	Serious	+				+		

	Study design	N	Risk of Bias	Reported outcomes and direction of effect						
				PA acquisition	Time/age to PA	Quality of Life	Adverse events	Adherence	Change in FEV_1	Environmental dissemination
Cleaning n=4										
Disinfection n=2										
Fawdon 2017 (586)*	Pre-/post-observational study	13 (13/13)	Serious							<>
Ferroni 2008 (519)	Pre-/post-observational study	211 samples (9/121/81)	Critical							+

Intervention	Study design	N	Risk of Bias	Reported outcomes and direction of effect						
				PA acquisition	Time/age to PA	Quality of Life	Adverse events	Adherence	Change in FEV_1	Environmental dissemination
UV cleaning n=2										
Allen 2019 (587)*	Pre-/post-observational study	10 (10/10)	Moderate							+
Allenby 2020 (588)*	Pre-/post-observational study	4(4/4)	Serious							+

	Study design	N	Risk of Bias	Reported outcomes and direction of effect						
				PA acquisition	Time/age to PA	Quality of Life	Adverse events	Adherence	Change in FEV_1	Environmental dissemination
Facemasks n=4										
Zuckerman 2015 (423)*	RCT	303 (149/154)	Some concerns							<>
Driessche 2015 (589)*	Pre-/post-observational study	11 (11/11)	Moderate							+
Wood 2018 (590, 591)*#	Pre-/post-observational study	25 (25/24/24/25/24/25)	Moderate			<>	-			+

Intervention	Study design	N	Risk of Bias	Reported outcomes and direction of effect						
				PA acquisition	Time/age to PA	Quality of Life	Adverse events	Adherence	Change in FEV_1	Environmental dissemination
Stockwell 2018 (592, 593)*#	Pre-/post-observational study	25 (25/25/25/23/25)	Moderate			-				+
Hand hygiene n=1										
Zuckerman 2009 (520)*	Multicentre pre-/post-observational study	100 (100/100)	Serious							<>

*Table 4-2: Included studies grouped by intervention, with study characteristics, risk of bias, outcomes and direction of effect. *Denotes study in which prevention of P. aeruginosa acquisition is a primary outcome. # associated abstract. + beneficial effect; - harmful effect; <> no significant effect; †FEV_1 difference at end of study but not measured at baseline; red symbols denote statistical significance; black symbols denote no significance testing reported. Greyed out cells, outcome not measured in this study. PA, P. aeruginosa. FEV_1, Forced expiratory volume in 1s. RCT, randomised controlled trial. BAL, bronchoalveolar lavage. NBS, Newborn screening. NICU, neonatal intensive care unit. IP&C, infection prevention and control.*

Intervention	Studies	Study design	Direction of evidence	GRADE rating	GRADE description
Therapeutics					
Antibiotic prophylaxis					
Anti-pseudomonal prophylaxis	2 (301, 552)	1 RCT and 1 retrospective cohort	The RCT found no significant effect on *P. aeruginosa* acquisition while the smaller retrospective cohort study found a significant effect.	Very low	Evidence downgraded once for risk of bias (no true control group for cohort study), once for imprecision (few participants) and once for indirectness (only children).
Anti-staphylococcal prophylaxis	3 (553-555)	1 RCT and 2 retrospective registry studies	The RCT found no significant difference in *P. aeruginosa* acquisition between groups, whilst both (larger) retrospective studies found anti-staphylococcal prophylaxis significantly increased risk of *P. aeruginosa* acquisition.	Low	Evidence downgraded twice for risk of bias (all high/serious).

Intervention	Studies	Study design	Direction of evidence	GRADE rating	GRADE description
Azithromycin	1 (83)	1 RCT	No effect of azithromycin on *P. aeruginosa* acquisition	Low	Evidence downgraded once for consistency (only one study) and once for indirectness (not the study question and short follow-up).
BAL-directed therapy	1 (556)	1 RCT	No effect of BAL-directed therapy on *P. aeruginosa* acquisition	Low	Evidence downgraded once for consistency (only one study) and once of indirectness (only children).
Modulators	2 (100, 101)	1 retrospective cohort and 1 retrospective registry study	The cohort study found no effect on *P. aeruginosa* rates but later time to acquisition, the registry study found lower *P. aeruginosa* acquisition	Very low	Evidence downgraded twice for risk of bias (both serious), and once for indirectness (only relates to a subset of pwCF)

Intervention	Studies	Study design	Direction of evidence	GRADE rating	GRADE description
Palivizumab	4 (557-560)	2 retrospective cohort studies, 1 retrospective registry study and one retrospective case-control study	The case-control and one cohort study found no effect on *P. aeruginosa* acquisition, while one cohort study found higher rates with palivizumab. The registry study and the case-control found no effect of palivizumab on time to *P. aeruginosa* while one cohort study found it was reduced.	Very low	Evidence downgraded once for risk of bias (two moderate, two serious) and once for indirectness (small studies, except registry study which only looked at time to *P. aeruginosa* and only two had prevention of *P. aeruginosa* acquisition as primary question).
Vaccination	4 (561-564)	1 RCT, three retrospective case-control	The RCT and two of the case-control studies found significant benefit of *P. aeruginosa* vaccination, the other found no significant difference.	Low	Evidence downgraded twice for risk of bias (one RCT had some concerns due to 20% of patients not receiving all vaccinations, all others serious)

Intervention	Studies	Study design	Direction of evidence	GRADE rating	GRADE description
Neonatal care					
Newborn screening	7 (565-571)	1 RCT, 2 retrospective registry studies, two retrospective cohort studies, one pro- and retrospective cohort and one retrospective case control.	Three studies found positive impact of NBS on *P. aeruginosa* acquisition, 1 found no effect and 1 found a negative effect. For time to *P. aeruginosa* acquisition three found no effect, two found a negative effect and 1 found a positive effect.	Very low	Evidence was downgraded once for risk of bias (the only RCT was at high risk), once for consistency (heterogeneity of results) and once for indirectness (*P. aeruginosa* acquisition the primary outcome in only one study).

Intervention	Studies	Study design	Direction of evidence	GRADE rating	GRADE description
Breastfeeding	2 (572, 573)	2 prospective cohort studies	One found breastfeeding delayed *P. aeruginosa* acquisition and one found no significant difference.	Very low	Downgraded twice for risk of bias (both studies had serious flaws with a lot of overlap between breast and formula feeding) and once for indirectness (in neither study was *P. aeruginosa* prevention the primary outcome).
NICU admission	1 (574)	1 retrospective cohort	No effect of NICU admission on *P. aeruginosa* acquisition	Very Low	Evidence downgraded twice for risk of bias (serious risk) and once for consistency (only one study).

Intervention	Studies	Study design	Direction of evidence	GRADE rating	GRADE description
Environmental strategies (healthcare)					
Adherence to IP&C guidelines	2 (575-577)	1 pre-/post-registry study and 1 pre-/post-observational study	No effect of adherence to IP&C guidelines on *P. aeruginosa* acquisition	Very low	Evidence downgraded twice for risk of bias (both studies serious) and once for indirectness (follow-up may have been insufficient to see an effect, no breakdown by studies for transmissible strains which would be the only ones likely to be impacted by these measures and the registry study did not evaluate the degree of implementation at each site).

Intervention	Studies	Study design	Direction of evidence	GRADE rating	GRADE description
Cohort segregation	3 (499, 578, 579)	1 RCT, 1 retrospective cohort using RCT data and 1 pre-/post observational study	All studies found lower rates of *P. aeruginosa* acquisition in segregated cohorts, though the time to *P. aeruginosa* was not significantly different in two studies.	Low	Evidence downgraded twice for risk of bias (although one study was an RCT it had high risk of bias due to very low recruitment). Despite this, all three studies found a positive effect of cohort segregation.
Cohort segregation plus	8 (422, 456, 580-585)	7 pre-/post-observation al studies and 1 retrospective cohort.	6/8 studies report reduced *P. aeruginosa* acquisition with cohort segregation, two found no significant effect. The two studies which found no effect did not break down acquisition by strain; only transmissible strains would be expected to be affected by these measures.	Low	Evidence was downgraded once for study design (most moderate risk of bias) and once for indirectness (different additional measures used).

Table 4-3: GRADE table for level of certainty of the evidence for the systematic review of interventions to prevent acquisition of P. aeruginosa in cystic fibrosis.

4.3.1 Primary outcome – acquisition of *P. aeruginosa*

Thirty-six studies (37 articles) reported data on acquisition of novel *P. aeruginosa* infection and 20 studies (20 articles) reported data on time to novel *P. aeruginosa*.

4.3.1.1 Therapeutics

4.3.1.1.1 Antibiotic prophylaxis

Six studies (83, 301, 552-555) examined the effect of antibiotic prophylaxis on the acquisition of *P. aeruginosa* infection. Two considered anti-pseudomonal prophylaxis (301, 552), three considered anti-staphylococcal prophylaxis (553-555) and one considered the use of azithromycin (83).

Anti-pseudomonal prophylaxis was found to have a significant beneficial effect in one study (301) and no significant effect in the other (552). Tramper-Stranders *et al*. conducted an RCT comparing three weeks of oral ciprofloxacin and inhaled colistin every three months with placebo (552). The study only recruited small numbers (64 participants) but found that active treatment did not prevent *P. aeruginosa* acquisition at three years. An earlier observational study by Heinzl *et al*. used ongoing nebulised gentamicin as prophylaxis for children with CF exposed to a pre-determined risk factor for *P. aeruginosa* acquisition (301). Prophylaxis was continued for a minimum of three years after a potential exposure. The authors

compared children who continued on long-term prophylaxis (who had ongoing risk factors) with those who stopped treatment. No participants with ongoing prophylaxis had acquired *P. aeruginosa* by the end of the study (median follow-up approximately seven years), while 7/16 participants who discontinued treatment met the study definition of acquisition (two cultures positive for *P. aeruginosa*). The study was limited by small numbers (n=28) and by lack of a true comparator group.

One RCT found no significant effect of anti-staphylococcal prophylaxis on *P. aeruginosa* acquisition (553). This was a small study which compared 18 children treated with continuous flucloxacillin prophylaxis with 20 who had episodic antibiotics only. This study had a high risk of bias due to lack of blinding. In comparison two large retrospective registry studies found anti-staphylococcal antibiotic prophylaxis had a significant harmful effect on *P. aeruginosa* acquisition (554, 555). Ratjen *et al*., using data from the CF Foundation Patient Registry (CFFPR) compared children treated with continuous anti-staphylococcal prophylaxis with those with intermittent or no prophylaxis. They found that children treated with continuous anti-staphylococcal antibiotics were significantly more likely to acquire *P. aeruginosa* than those on intermittent or no treatment (554). This finding was supported by Hurley *et al*. who found the chance of acquiring *P. aeruginosa* was significantly higher in children treated with

anti-staphylococcal prophylaxis (555). Both these studies have a serious risk of bias because adherence to therapy was not measured, and confounding is likely in registry studies.

The impact of azithromycin treatment on *P. aeruginosa* prevention was examined by Saiman *et al*. in a multicentre RCT comparing the effect of the drug to placebo in patients free of *P. aeruginosa* infection (83). No impact of therapy was found but follow-up was only 168 days which may be insufficient for any difference to be identified.

4.3.1.1.2 BAL-directed therapy

One study, by Wainwright *et al.*, examined the impact of BAL-directed therapy on acquisition of *P. aeruginosa* in young children with CF (556). Children diagnosed by newborn screening were randomised to either BAL-directed care or standard, culture-based care. BAL was performed prior to age six months, at hospitalisation for a pulmonary exacerbation, if *P. aeruginosa* was isolated from standard culture and at the end of eradication. The authors found similar numbers of children in each group were treated for *P. aeruginosa* infection at least once in the study and there was no significant difference in the age at acquisition between the two groups.

4.3.1.1.3 Modulators

The effect of CFTR modulators on *P. aeruginosa* acquisition was considered in two studies (100, 101), one of which found significant

beneficial effect on *P. aeruginosa* acquisition (100) and one on time to *P. aeruginosa* (101). Frost *et al*. used data from the UK CF registry to compare the incidence of novel *P. aeruginosa* acquisition between patients treated with ivacaftor and those untreated (100). In the subgroup with no evidence of *P. aeruginosa* infection in the two years preceding the study they found significantly fewer episodes of *P. aeruginosa* acquisition in the group treated with ivacaftor than in the unmodulated cohort. The study was limited by the inherent differences in genotype between the two groups and the limitations of registry data.

Singh *et al*. compared a group of patients treated with either ivacaftor or lumacaftor/ivacaftor with the same group prior to commencement of treatment and with a non-treated group (101). Acquisition of *P. aeruginosa* was lower in the modulator group than the untreated group both pre- and post-treatment, though no direct statistical comparisons of the rates of acquisition were made between the two groups. The trend towards declining acquisition rates in both groups over time was non-significant though the time to *P. aeruginosa* acquisition was significantly longer in the modulator group after commencement of treatment; no difference was found for the non-treatment cohort. Some subjects in the modulator group only had treatment for one of the four years of the study; these patients may not have been comparable to other non-modulated patients who would also have become eligible for

this treatment. Small numbers (particularly of *P. aeruginosa* free participants) also limit the conclusions which can be drawn.

4.3.1.1.4 Palivizumab

Four studies examined the effect of palivizumab prophylaxis for respiratory syncytial virus (RSV) infection on subsequent *P. aeruginosa* acquisition (557-560). Three studies were retrospective cohort studies (557, 558, 560), and one was a retrospective case-control (559). One study found a significant harmful effect of palivizumab, i.e., treatment increased the chance of *P. aeruginosa* acquisition (558), while the other three found no significant effect (557, 559, 560).

Groves *et al*. compared the rate of, and time to, *P. aeruginosa* acquisition in two cohorts of paediatric patients in a single CF Centre, the later cohort underwent palivizumab prophylaxis as standard whilst the earlier cohort did not (558). They found significantly higher rates and earlier infection in the cohort treated with palivizumab. In contrast, a large retrospective registry study found no significant effect on time to *P. aeruginosa* acquisition once adjustments were made for the likelihood of a child receiving palivizumab (557). These findings were supported by two smaller studies, both with serious risk of bias. A retrospective case-control study matched infants given palivizumab with at least three age- (year and month of birth), sex- and genotype-matched untreated patients and found no significant difference in the rates of *P. aeruginosa* acquisition or time to infection (559). Another

retrospective cohort study comparing a cohort in which all infants with CF received palivizumab with an earlier cohort which did not receive prophylaxis found no effect on the rate of *P. aeruginosa* acquisition between the two groups (560).

4.3.1.2 Vaccination

Four studies reported on the impact of anti-pseudomonal vaccination on subsequent acquisition of infection (561-564). An RCT comparing vaccination with a bivalent *P. aeruginosa* flagella vaccine with placebo found a reduction in *P. aeruginosa* acquisition in vaccinated patients, which was significant in the subgroup who had received the entire course of immunisations (561). Where infection occurred, the majority were with *P. aeruginosa* isolates expressing flagella types not included in the vaccine.

Three studies analysed the same retrospective case-control data at different time points (562-564), considering a cohort of 30 CF patients who received an octavalent O-PS-toxin A conjugate vaccine. Four patients were excluded from analysis after they were later discovered to have had intermittent *P. aeruginosa* infection prior to vaccination. The 26 remaining patients were matched with 26 controls attending the same Centre who had not taken part in the initial vaccine safety trial. Age and sex-matching was only possible for 21 case-subjects. At one and two years significantly fewer immunised patients had acquired *P. aeruginosa* than in the non-immunised group (564). This difference

was lost after three and four years of follow-up and the authors noted that subjects who failed to maintain an antibody response were significantly more likely to acquire *P. aeruginosa* than those with ongoing immunity (563, 564). At 10 years however, significantly fewer vaccinated patients were infected than control patients (562). All three reports of this study had serious risk of bias through patient selection and matching. Also, all three defined infection as two positive *P. aeruginosa* cultures, so the numbers acquiring a first infection may be higher than those reported (562-564).

4.3.1.3 Neonatal care

4.3.1.3.1 Newborn screening

Seven studies were identified which explored the effect of NBS (565-571), though in only one study was the effect on *P. aeruginosa* acquisition the primary outcome (570). One study was an RCT (568), two were registry studies (565, 571), two were retrospective cohort studies (567, 570), one was a retrospective case-control (566), and one was a prospective study for the NBS cohort, compared with retrospective analysis of the pre-NBS cohort (569).

Three studies found a significant beneficial effect of NBS on *P. aeruginosa* acquisition (566, 567, 571), with another reporting a positive effect but without any statistical testing (569). In a well-conducted study children with a late diagnosis of CF (missed on NBS or NBS positive but sweat test negative) were matched 1:2 with children identified

through NBS for age, sex, pancreatic status and CF clinic, though not for genotype (566). They found significantly higher rates of *P. aeruginosa* acquisition in the late diagnosis cohort but no difference in age at acquisition. A registry study, comparing two Canadian provinces which had implemented NBS with one province which had not, found significantly higher rates of *P. aeruginosa* acquisition in the unscreened population but did not control for any effect of differing care between provinces (571). In a smaller, retrospective cohort study, Dijk *et al.* compared a screened cohort with a prior, unscreened cohort and followed them up until transition to adult services. They found significantly later *P. aeruginosa* acquisition in the screened patients (567). A cohort study, which collected prospective data on a NBS cohort and compared it to the previous, non-screened, birth cohort, reported a trend towards lower *P. aeruginosa* rates in the screened population, but a trend towards younger age at first infection in those who did acquire *P. aeruginosa* in the screened cohort (569).

Two studies found no significant effect, one on the rate of acquisition and one on time to *P. aeruginosa* (565, 570). A large, well-conducted, registry study using data from the CFFPR compared a period prior to screening, a period during roll-out of screening (which was introduced in each state at a different time point) and a period when all included states used NBS (565). This allowed them to adjust for the effect of birth cohort as well as the effect of individual state-wide care. The

authors found no significant difference in the time to first *P. aeruginosa* acquisition between screened children and those diagnosed clinically. In the only study directly examining the effect of NBS on *P. aeruginosa* acquisition no difference was found in the proportion of patients infected in the pre- and post-NBS cohorts (570). The median age of *P. aeruginosa* acquisition was also not significantly different when the whole of each cohort was compared, though in the subgroup who cultured *P. aeruginosa,* acquisition was earlier in the screened children. This finding was negated if children with a first *P. aeruginosa* isolation within 60 days of diagnosis were included in the analysis.

One study found a harmful effect of NBS on *P. aeruginosa* acquisition. An RCT comparing children randomised to NBS or clinical diagnosis found a significantly higher rate of, and significantly earlier time to, *P. aeruginosa* acquisition in the screened group (568). Subgroup analysis revealed that this difference was related to earlier *P. aeruginosa* acquisition in the screened group at one of the two CF centres involved in the study. The site with earlier acquisition brought all patients to a single clinic with a small waiting area, while the other site had a dedicated clinic for screened patients. The authors postulated that the difference in *P. aeruginosa* acquisition was due to the exposure of young, potentially more vulnerable, screened patients to older patients already infected with *P. aeruginosa* in the small, mixed clinic setting.

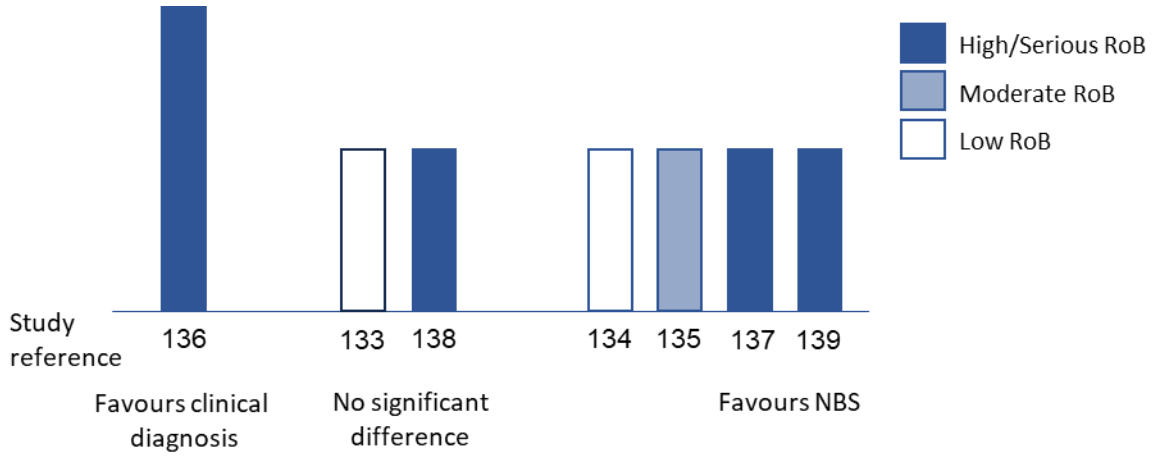


Figure 4-2: Harvest plot of effect directions for the seven identified studies examining effect of Newborn screening (NBS) on P. aeruginosa acquisition, stratified by risk of bias. Tall bar represents randomised controlled trial (RCT), shorter bars represent observational studies.

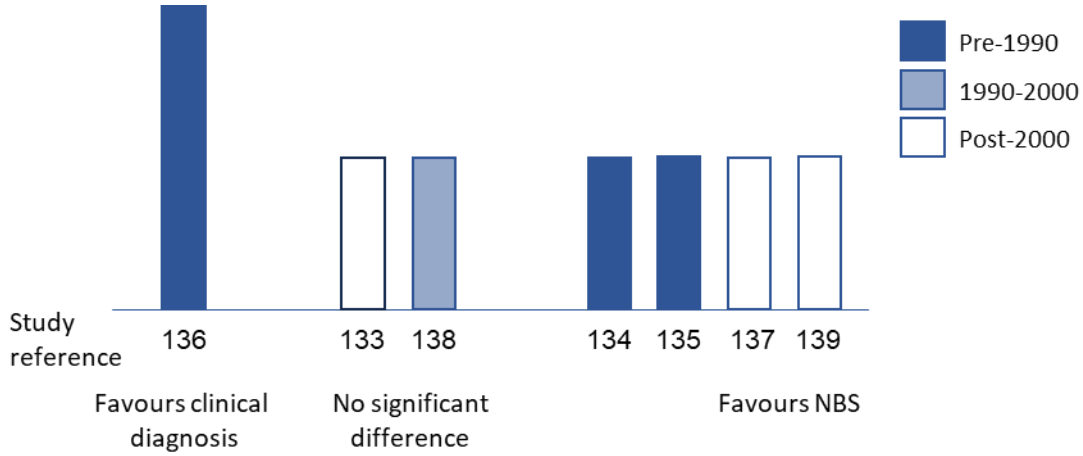


Figure 4-3: Harvest plot of effect directions for the seven identified studies examining effect of Newborn screening (NBS) on P. aeruginosa acquisition, stratified by first year of follow-up. Tall bar represents randomised controlled trial (RCT), shorter bars represent observational studies.

In view of the differing effect directions and the number of studies of NBS, harvest plots were constructed to assess the effect of risk of bias (see Figure 4-2) and first year of follow-up (see Figure 4-3) on the results. First year of follow-up was examined to see whether there was

any impact of longitudinal improvements in CF care on the association between NBS and *P. aeruginosa* acquisition, however no such link was found.

4.3.1.3.2 Breastfeeding

Two studies report the effect of breastfeeding on *P. aeruginosa* acquisition, though in neither study was this the primary outcome (572, 573). A prospective, multi-centre cohort, devised to examine the effect of pancreatic status on long-term nutritional outcomes, found no significant effect of breastfeeding on *P. aeruginosa* acquisition, though there was a trend towards later infection in infants who had received any breastmilk, compared to exclusively formula-fed babies (572).

Jadin *et al.* compared the impact of breastfeeding (varying durations) with formula feeding on nutritional and pulmonary outcomes (573). When they combined all durations of exclusive breastfeeding and compared this group with formula-fed infants they found a significant reduction in the number of infants with one or more *P. aeruginosa* positive cultures in the breastfed cohort. Both studies are at serious risk of confounding as the type of feeding may have been influenced by the health status of the child which may have independently affected the risk of acquiring *P. aeruginosa*.

4.3.1.3.3 NICU admission

The impact of neonatal intensive care unit (NICU) admission on *P. aeruginosa* acquisition in children with CF was reported by one study (574) which found no significant difference in the rate of *P. aeruginosa* acquisition, or time to infection, between children admitted to NICU or not. There was a trend towards less, and later, *P. aeruginosa* in those admitted to NICU which was contrary to expectations, however the numbers are small and the study is confounded by the indications for NICU admission and additional treatments received.

4.3.1.4 Environmental strategies (healthcare settings)

4.3.1.4.1 Adherence to IP&C guidelines

Three reports of two studies (575-577) considered the impact of implementation of recommendations from the 2013 IP&C guidelines (174).

Muhlebach *et al*. compared incidence of *P. aeruginosa* infection in adult and paediatric CF Centres before and after the publication of the guideline, using data from the CFFPR (575). They found a significant decline in incident infection between the pre- and post-guideline study periods. No data were however collected as to what (if any) recommendations were implemented at each site therefore it is not possible to conclude with certainty that the effect was due to the guideline.

Kim *et al.* compared *P. aeruginosa* incidence rates pre- and post-guideline implementation in a single Centre where all in- and out-patient recommendations were implemented (576, 577). They found no change in incidence between the two time periods however they did not analyse acquisition by transmissible or unique strains, using multi-resistant *P. aeruginosa* as the only marker.

4.3.1.4.2 Cohort segregation

Three studies reported the effect of isolated cohort segregation (499, 578, 579). One study was an RCT (578), one was a retrospective analysis of data from an RCT (499) and one a pre-post-segregation observational study (579).

All three studies found a beneficial effect of cohort segregation on *P. aeruginosa* acquisition, which was significant in two studies (499, 579). The RCT randomised CF infants diagnosed by newborn screening to either a segregated, “screening,” clinic or a mixed age and pathogen CF clinic (578). They found a trend towards less acquisition in the children attending the screened clinic though there was no difference in age at first *P. aeruginosa* infection. The authors did note that the time to first *P. aeruginosa* infection was significantly longer at the time of the study than in a prior cohort. Unfortunately, the study was underpowered, recruiting only 26.5% of the required numbers.

Farrell *et al.* used data from the Wisconsin Neonatal Screening study to assess the impact of segregated and mixed clinics on *P. aeruginosa*

acquisition (499). Neonates were randomised to NBS or not on the basis of the final digit of their Guthrie cards. Screened and clinically diagnosed patients were seen at one of two CF Centres. *P. aeruginosa* acquisition was significantly higher at Centre B, but when this was broken down by diagnosis method only the screened children were affected. Time to *P. aeruginosa* acquisition was also shorter for screened children at Centre B compared to all other groups. The difference was attributed to the small, old clinic building at Centre B which ran a single clinic serving all patients and utilised a small waiting room. In contrast Centre A set up a separate clinic to see the screened patients and time in the waiting room was limited.

Frederiksen *et al*. reviewed a microbiology database and compared the incidence of *P. aeruginosa* infection before and after implementation of cohort screening (579). They found a significant reduction in the median incidence between the two time periods but found no difference in the age at first infection.

4.3.1.4.3 Cohort segregation plus other IP&C measures

Eight studies reported the effect of cohort segregation, plus a variety of other IP&C measures, introduced either at the same time or sequentially, on *P. aeruginosa* acquisition (422, 456, 580-585). Seven of the studies were pre-/post-implementation observational studies (422, 456, 580-582, 584, 585) and one (583) was a comparison of measures between CF Centres.

Five studies report a beneficial effect of cohort segregation plus other IP&C measures, without reporting any significance testing (422, 456, 581, 582, 584). Griffiths *et al*. compared the incidence of *P. aeruginosa* infection in a cohort including the year prior to segregation (both in- and out-patient) and another, two-five years post segregation (581). Multiple cohorts including epidemic strain *P. aeruginosa*, other *P. aeruginosa*, MRSA, *Burkholderia cepacia* complex (Bcc) and other pathogens, were implemented. The incidence of novel infection fell between the two time periods, though segregation was introduced part-way through the first (2000). In addition to segregation the clinic practice changed to include *P. aeruginosa* eradication of novel infection and cohorts were segregated for lung function, physiotherapy and in communal spaces. Mixing between cohort members was discouraged and standard infection control procedures were reinforced. Changes to hand-washing practice were introduced towards the end of the second time period. This group reported further findings after additional measures were introduced (582). A newly built facility, opened in 2011 enabled single rooms for all in-patients, single rooms for clinic to prevent congregation in a waiting area and in 2016 mask-wearing was introduced for all communal spaces. Ongoing decline in incidence of the original transmissible strain was noted, with no new infections after 2011 but acquisition of other transmissible strains, though also declining, was not entirely prevented.

Tummler *et al.* also found a reduction in the incidence of closely related *P. aeruginosa* strains after implementing hygienic precautions, including separate wards for *P. aeruginosa* positive and negative patients, with those found to have multi-resistant *P. aeruginosa* isolated, and room disinfection on discharge (584). In the out-patient clinic patients were separated for physiotherapy and lung function, but no clinic cohorts were introduced.

Jones *et al.* compared the rates of *P. aeruginosa* acquisition before and after the introduction of segregation of *P. aeruginosa* positive and negative patients (456). Following segregation all patients admitted had their own rooms on the ward; *P. aeruginosa* negative patients were admitted to en-suite rooms and all aerosol-generating procedures were done in individual rooms with the doors shut. In the out-patient clinic patients infected with transmissible strains were segregated from those with unique *P. aeruginosa* but on the ward communal spaces and bathrooms were open to all *P. aeruginosa* positive individuals, regardless of strain. No further cases of transmissible *P. aeruginosa* were found in the segregated patients, although acquisition of unique isolates continued. Ongoing transmission of epidemic *P. aeruginosa* to patients with a previously unique strain (superinfection) was noted and was suggested to be related to contact during in-patient admissions. The authors noted that no patients infected with Bcc were infected with

the epidemic strain, and suggested this was due to long term segregation of this cohort from the rest of the CF clinic.

The need to separate patients with potentially transmissible *P. aeruginosa* from those with unique strains was also highlighted in Denmark in the 1980s. Pedersen *et al.* reported a significant reduction in superinfection with multi-resistant *P. aeruginosa* after further refining their cohort segregation strategy (422). Prior to this, patients with *P. aeruginosa* infection had simply been separated from those without. The authors noticed an increase in infection with the multi-resistant strain in patients who previously had unique *P. aeruginosa*. Incidence of infection with the transmissible strain fell after the *P. aeruginosa* positive cohort was split into those with the multi-resistant strain and those without, in both the in-patient and out-patient setting. In addition, hygiene precautions were reinforced, in-patient rooms were disinfected on discharge and social contact outside of the hospital was discouraged, with CF camps cancelled.

One study found a beneficial impact of cohort segregation, with reduction in the acquisition of mucoid, though not non-mucoid, *P. aeruginosa*. McKay *et al.* compared *P. aeruginosa* acquisition before and after segregation was implemented (585). Prior to segregation a single clinic for all patients was held weekly, with free mixing in the waiting room. After segregation clinics were divided into pre-school, primary school and secondary school. Toys were removed from the

waiting room, hand hygiene was reinforced and, on the ward, patients were treated in single rooms, or if shared, with non-CF patients. Unfortunately, this study did not report on the *P. aeruginosa* strains acquired.

A study by Lee *et al*. did not find a significant impact of segregation on *P. aeruginosa* acquisition though this study also failed to report the strains acquired (580). This single centre study compared the annual incidence of *P. aeruginosa* in the year prior to segregation and with the following ten years in which separate clinics were established for chronically infected and uninfected patients, a new, purpose-built CF Centre was opened, and hygienic measures were intensified. The incidence of *P. aeruginosa* acquisition in patients either free from- or with no previous infection, was unchanged.

Finally, Gilchrist *et al*. surveyed the infection control practices of eight CF Centres, five tertiary and three shared-care (583). They found variation in practice but no difference in incidence of *P. aeruginosa* for any strategy, including tertiary Centre versus Shared care Centre or segregated vs mixed clinics. All Centres promoted the avoidance of communal areas, a single patient room for the entirety of a clinic appointment and cleaning of rooms between patients. One Centre used different wards for *P. aeruginosa* positive and negative patients, six admitted all patients to en-suite side rooms, two used negative

pressure side rooms and four segregated clinics according to *P. aeruginosa* status.

4.3.1.4.4 Cleaning

No studies examined the effect of cleaning methods on *P. aeruginosa* acquisition.

4.3.1.4.5 Facemasks

No studies reported the effect of facemasks on *P. aeruginosa* acquisition. Experimental studies do suggest that face masks may reduce the release of *P. aeruginosa* in cough aerosols. These studies are considered below under the outcome measure: “Environmental dissemination”.

4.3.1.4.6 Hand hygiene

No studies reported the effect of hand hygiene in isolation on *P. aeruginosa* acquisition.

4.3.1.5 Environmental strategies (non-healthcare settings)

No studies were identified in this category for any outcome.

4.3.2 Secondary outcomes

4.3.2.1 Quality of life

Four studies (six articles) reported qualitative observations on the acceptability of interventions, which may in turn have an impact on quality of life (581, 582, 590-593).

4.3.2.1.1 Environmental strategies (healthcare settings)

Griffiths *et al.* reported that the newly introduced IP&C measures were, "met positively by CF families." p50 (581). This was supported by a later study from the same group who reported that the new IP&C measures were, "generally well accepted by staff, patients and families." p1500 (582).

Stockwell *et al*. reported that people with CF found all durations of mask wearing less comfortable than healthy controls. Those with higher lung function found masks more comfortable for 10 and 20 minutes but not at 40 minutes. The surgical masks were rated more comfortable than the N95 mask (592, 593). Wood *et al*. found surgical masks were rated more comfortable by significantly more people than N95 masks (590, 591)

4.3.2.2 Adverse events

Nine studies reported adverse events (83, 301, 552, 556, 561-564, 591).

4.3.2.2.1 Therapeutics

Three studies of antibiotic prophylaxis provided data on adverse events (83, 301, 552). Tramper-Stranders *et al*. found no difference in adverse events between the treatment and placebo groups (552). Heinzl *et al.* reported three participants stopping inhaled gentamicin due to feeling tight chested (301). Saiman *et al*. found significantly less cough, productive cough and sore throat in the treatment group with no

significant difference between the two groups for all other adverse events (83). Adverse events following bronchoscopies reported by Wainwright *et al*. included fever, cough and clinical deterioration but no difference was reported between groups (556).

4.3.2.2.2 Vaccination

Three studies of vaccination found a higher rate of adverse events in the treatment group (561, 563, 564). Döring *et al*. reported more adverse events in the treatment group but most of these were mild or moderate. Only one of five severe events was definitely related to the study medication (561). Mild adverse events were reported in half of participants by both Lang *et al.* (1995) and Cryz *et al*. (563, 564), with no adverse events in the control subjects. Lang *et al*. (2004) reported only mild local and systemic effects in the vaccinated group and two deaths each in the vaccinated and control groups, all unrelated to the vaccine (562).

4.3.2.2.3 Environmental strategies (healthcare settings)

Wood *et al.* reported one patient, with an FEV_1 of 32.5% predicted, who had to remove an N95 mask due to shortness of breath and claustrophobia (591).

4.3.2.3 Adherence

Five studies reported data on adherence (83, 301, 561, 578, 585).

4.3.2.3.1 Therapeutics

Heinzl *et al*. reported five of the 16 participants who stopped nebulised gentamicin did so due to due to non-compliance or wanting to stop (301). Saiman *et al*. found no significant difference in adherence between the treatment (azithromycin) and placebo groups (83).

4.3.2.3.2 Vaccination

One study reported adherence to vaccination; Döring *et al*. found a similar proportion of participants missed one or more doses of both vaccine and placebo (561).

4.3.2.3.3 Environmental strategies (healthcare settings)

Two studies looking at cohort segregation techniques reported good adherence to the measures. McKay *et al.* reported good adherence to the clinic cohort strategy which was maintained over time (585). Hayes *et al*. reported 100% adherence to both the segregated and mixed clinic groups (578).

4.3.2.4 Change in FEV_1

Change in FEV_1 from baseline was reported by four studies (83, 552, 561, 577). None of these found a significant difference in FEV_1. In addition, a further ten studies reported FEV_1 between groups at the end of the study but did not compare to baseline data (301, 554, 556-558, 562, 565-568).

4.3.2.4.1 Therapeutics

Two studies reported change in FEV_1 from baseline (83, 552) and a further five reported differences in FEV_1 at study end (301, 554, 556-558).

Neither Tramper-Stranders *et al*., examining anti-pseudomonal prophylaxis, nor Saiman *et al*., assessing azithromycin, found any significant difference in the decline in $ppFEV_1$ between the treatment and placebo groups (83, 552).

No significant difference in FEV_1 at study end was reported for prophylactic gentamicin nebulisation (301) or anti-staphylococcal prophylaxis, compared to intermittent or no prophylaxis (554). Similarly, FEV_1 at study end was not significantly different for BAL-directed therapy versus culture-based therapy (556), or palivizumab compared to no palivizumab (557, 558).

4.3.2.4.2 Vaccination

One study reported change in FEV_1 over the course of the study (561) and one study reported on FEV_1 at study end (562). Döring *et al.* showed no data but reported no difference in the rate of FEV_1 decline between the vaccinated group and the placebo group (561). A trend towards improved FEV_1 in vaccinated patients, which was significant in the over-18s group, was found at study by Lang *et al*. (562).

4.3.2.4.3 Neonatal care

Four studies reported FEV_1 at study end but without any comparison to baseline (565-568). Such a comparison is not feasible in this group of patients as reliable pulmonary function testing is only possible in children over at least three years of age (594). Two studies found a beneficial impact of NBS on FEV_1 at study end (566, 567), one reported a trend towards improved FEV_1 in the NBS cohort (565) and one found no difference (568).

4.3.2.4.4 Environmental strategies (healthcare settings)

Kim *et al.* found no difference in the median $ppFEV_1$ between the year prior to IP&C guideline implementation and the subsequent three years (577).

4.3.2.5 Environmental dissemination

Ten studies reported environmental dissemination (422, 423, 519, 520, 586-593).

4.3.2.5.1 Cohort segregation plus other IP&C measures

Pedersen *et al*. reported no environmental source of the epidemic strain was found but no details were provided (422).

4.3.2.5.2 Cleaning

Four studies reported the effect of cleaning on environmental *P. aeruginosa* dissemination (519, 586-588). Two examined disinfectant

solutions (519, 586) and two examined ultraviolet (UV) light cleaning (587, 588).

Fawdon *et al*. compared cleaning of a CF outpatient clinic with Clinell wipes or Actichlor solution (586). They sampled the room prior to patient entry, after the patient left, one- and four-hours post cleaning with Clinell wipes and the next morning (with Actichlor solution used after the four-hour Clinell sampling). Only one patient was colonised with *P. aeruginosa*. They found Clinell wipes achieved 99.99% kill of *P. aeruginosa*, with Actichlor solution achieving total kill, though this sampling was performed much later. Ferroni *et al*. performed air and surface sampling of in-patient bedrooms on waking, after physiotherapy and after cleaning (519). Of twenty-nine patients whose rooms were sampled, 22 had chronic or intermittent *P. aeruginosa* infection. They found significantly fewer *P. aeruginosa* positive samples after cleaning but took only nine post-cleaning samples, compared to 121 on waking and 81 post-physiotherapy.

Allen *et al*. sampled outpatient rooms (spirometer, tap, sink, door, door handle, desk and chair) after the patient left the room, after manual cleaning with chlorine-based disinfectant and after UV disinfection (587). Seven of ten patients whose rooms were sampled were colonised with *P. aeruginosa*. They found a significant reduction in CFUs on the tap, sink, desk and chair after chlorine disinfection, but contamination remained on the door, door handle and spirometer. After

subsequent UV light disinfection there was further significant reduction in the CFUs found at all sites, with only negligible contamination once this process was complete. Allenby *et al*. also compared UV light decontamination to cleaning with Clinell wipes and air filtration (588). Four rooms were cleaned by each method over two separate days. They found a significant reduction in the total viable surface colonies identified after UV light disinfection compared to control measures, but no effect on air contamination.

4.3.2.5.3 Facemasks

Six articles reported the results of four studies examining the effect of mask wearing on *P. aeruginosa* dissemination (423, 589-593). One study was conducted within a CF clinic setting (423) and the other three within an experimental cough rig (589-593). An RCT, comparing the rates of air contamination in clinic rooms of patients randomised to wearing surgical masks for the duration of their clinic appointment or no mask, found no difference (423). They did find significantly higher rates of air contamination in the rooms in which spirometry was performed than the clinic rooms, though this cleared within 30 minutes. The authors suggest that spirometry is a risk factor for air contamination.

In contrast a much smaller observational study found a significant reduction in airborne *P. aeruginosa* load in patients while wearing a mask (589). Two further experimental studies supported this finding. Wood *et al*. compared the dissemination of *P. aeruginosa* in subjects

coughing uncovered, with talking, talking with a surgical face mask, coughing with a surgical facemask, coughing with an N95 mask and coughing while performing cough etiquette (590, 591). They found both types of mask, and cough etiquette, significantly reduced the dissemination of viable *P. aeruginosa* aerosols compared with uncovered coughing. Comparing the different strategies there was no difference in the effectiveness of the two mask types but both were more effective than cough etiquette. Little *P. aeruginosa* dissemination was observed with talking, either with or without a surgical facemask. Stockwell *et al*. assessed whether duration of mask-wearing had an impact on efficacy (592, 593). They found facemasks worn for 10, 20 and 40 minutes and an N95 mask worn for 20 minutes were similarly significantly effective in reducing dispersal of airborne *P. aeruginosa* compared to uncovered coughing.

4.3.2.5.4 Hand hygiene

One study examined the effect of hand hygiene on environmental *P. aeruginosa* dissemination (520). Zuckerman *et al*. sampled the hands of 100 patients prior to hand hygiene with alcohol gel at the beginning of a clinic visit and again, at the end of the appointment. They found that hand hygiene at the start of a visit did not prevent *P. aeruginosa* contamination at the end; whilst some participants hands were decontaminated, others had evidence of new contamination. The authors suggest that single point hand hygiene is insufficient to prevent

future contamination and therefore it should be performed regularly throughout a clinic visit.

4.4 Discussion

We found use of CFTR modulators, vaccination against *P. aeruginosa*, cohort segregation (alone) and cohort segregation (in combination with other IP&C measures) reduced the rate of *P. aeruginosa* acquisition. We found no effect for azithromycin, BAL-directed therapy, NICU admission or adherence to the 2013 IP&C guidelines (174). The effect of anti-pseudomonal antibiotic prophylaxis, anti-staphylococcal antibiotic prophylaxis, palivizumab, newborn screening and breastfeeding is unclear.

All these findings were graded as low or very low, mostly due to study design since the majority were observational studies. Where RCTs were conducted these were too few to conclusively address the effect of each intervention to a high level of certainty.

Considering time to *P. aeruginosa* acquisition CFTR modulators were found to have a beneficial effect, delaying infection, while anti-staphylococcal antibiotic prophylaxis may have a harmful impact, though this outcome was not considered in the RCT. The effect of newborn screening and cohort segregation was equivocal, and no effect was found for anti-pseudomonal antibiotic prophylaxis, BAL-

directed therapy, palivizumab, breastfeeding, NICU admission and cohort segregation in combination with additional IP&C measures.

Environmental dissemination of *P. aeruginosa* was reduced by UV disinfection of clinic rooms and use of facemasks. Hand hygiene had no impact when performed only at the beginning and the end of a clinic visit and standard cleaning was, in practical terms, probably equivalent to Actichlor solution.

The quality of the evidence for these observations was, although not formally assessed, likely even lower than for the primary outcome.

4.4.1 Therapeutics

The only therapeutic measure which we have found to have an impact on *P. aeruginosa* acquisition was CFTR modulators.

Since CFTR modulators correct the epithelial defect within the lungs, bringing the pulmonary environment closer to that of the non-CF population, it is reasonable that they would reduce the acquisition of *P. aeruginosa* in those not already colonised. CFTR is important in the innate immune response to *P. aeruginosa* (see section 4.1.3.3), therefore once functionality is restored clearance of the organism, as occurs in the non-CF population, is more likely to be achieved. Kawala *et al*. found that the use of Ivacaftor significantly decreased the odds of a *P. aeruginosa* positive sputum culture compared to the same patients prior to treatment. However, they did not examine the effect on

individual acquisition, nor correct for any change in the frequency of sampling by CFTR modulation (595).

The effect of anti-staphylococcal antibiotic prophylaxis on the risk of *P. aeruginosa* infection is unclear. A Cochrane Systematic review could not definitively answer whether such treatment increases the risk (596). It found a trend towards lower *P. aeruginosa* isolation initially but higher rates of infection after four to six years of prophylaxis. The Cochrane review included data from the RCT reported here, and also from two other studies which were excluded from this analysis. One reported the proportion of *P. aeruginosa* positive cultures, rather than individual acquisition (597) and the other did not report *P. aeruginosa* acquisition in the published article (598). The Cochrane authors used individual patient data (IPD), provided by the study authors, which was not part of the protocol for the current review. This question is currently the subject of a registry randomised controlled trial, CF START, which is due to report in 2027 (599).

It is reasonable to hypothesise that anti-pseudomonal prophylaxis would reduce the acquisition of *P. aeruginosa* but the only RCT to look at this found no effect (552). Although the study recruited to target it was powered on the assumption of a 15% per year *P. aeruginosa* acquisition in the control group. In practice over five years only 32% of control participants acquired *P. aeruginosa* therefore it is possible this study was too small to detect any difference. The authors also

speculated as to whether intermittent prophylaxis was sufficient, however the treatment burden of continuous treatment would be high, particularly since concerns about increased treatment burden was a major reason for fear of *P. aeruginosa* infection (220). The cohort study identified found prophylaxis was successful in preventing *P. aeruginosa* infection, but only when this was continuous. A number of participants discontinued treatment due to side effects or treatment burden. These patients were initially treated on the basis of potential *P. aeruginosa* exposures; participants discontinued prophylaxis if no ongoing risk situations were identified but despite this acquisition occurred. Lack of knowledge about risk situations means prophylaxis could not be appropriately targeted. Another observational study reported a lower-than-expected prevalence of *P. aeruginosa* infection in a paediatric population which the authors suggested was due to high usage of prophylactic inhaled antibiotics (600).

We found no clear effect of palivizumab on *P. aeruginosa* acquisition. Three studies found no effect of palivizumab on *P. aeruginosa* acquisition, but it is difficult to explain the increase in infection rates and decrease in time to *P. aeruginosa* in treated patients identified by Groves *et al*. (558). Prophylaxis against RSV infection is not currently recommended in CF (601).

The lack of effect of BAL-directed therapy on *P. aeruginosa* acquisition is to be expected as bronchoscopies, in and of themselves, should not

impact *P. aeruginosa* acquisition unless there is contamination of the bronchoscope (602, 603) or poor IP&C measures in the department. This study was designed to assess whether BAL-directed therapy had an impact on chronic *P. aeruginosa* infection (556).

Azithromycin treatment is recommended in patients without *P. aeruginosa* infection where necessary to reduce pulmonary exacerbations (604). It's mechanism of action is unknown but is suggested to be due to an anti-inflammatory effect. Although it had no significant impact on *P. aeruginosa* acquisition in the current study the duration of follow-up was short, and the study was not powered to answer this question (83).

4.4.2 Vaccination

Vaccination had a beneficial impact on *P. aeruginosa* acquisition in three of the four studies we identified however, despite this, anti-*P. aeruginosa* vaccination is not currently recommended in CF care (174). A Cochrane Systematic review of anti-pseudomonal vaccination in CF concluded that it could not be recommended as there was no impact of vaccination on chronic *P. aeruginosa* infection (605).

4.4.3 Newborn care

No elements of newborn care were conclusively associated with *P. aeruginosa* acquisition. The lack of association of NICU admission with *P. aeruginosa* infection in CF is reassuring given NICU admission has

been associated with *P. aeruginosa* acquisition in the non-CF population (606, 607), though these have been associated with specific outbreaks. Another study of neonates with meconium ileus (MI) found a high rate of *P. aeruginosa* acquisition (72% in the first year) which the authors attributed to a combination of predisposition in MI infants and hospital exposure (608). Larger studies are warranted to fully understand the impact of NICU admission on *P. aeruginosa* acquisition.

We found some evidence of benefit of breastfeeding in reducing *P. aeruginosa* acquisition but in the studies identified numbers were too small to allow any meaningful comparison beyond any breastfeeding with no breastfeeding. Choice of feeding strategy is related to the health status of the child, including the presence of MI and pancreatic status, as well as individual preference (609, 610), though exclusive breastfeeding is the recommended feeding strategy (611). Studies with larger participant numbers are needed to understand the impact of breastfeeding on *P. aeruginosa* acquisition while controlling for potential confounders.

We found no conclusive evidence of association between NBS and *P. aeruginosa* acquisition, with differing results between studies. Initial concern over the implementation of NBS was due to identification of earlier CF diagnosis as a risk factor for *P. aeruginosa* acquisition (612). This concern seemed to be confirmed by the findings from the Wisconsin Newborn Screening Project, but the study was confounded

by increased exposure in the screened group in one clinic (499). Other studies have found no effect of NBS on prevalence of *P. aeruginosa* in childhood (613) or of early diagnosis compared to late diagnosis on acquisition (614).

4.4.4 Environmental strategies (healthcare settings)

We found strategies involving cohort segregation had a beneficial effect on *P. aeruginosa* acquisition, while facemasks and UV cleaning had a beneficial effect on environmental dissemination.

A number of other studies have shown benefits of cohort segregation but were excluded as they did not fit our inclusion criteria (see Appendix B: Chapter 4 excluded studies). Work in Denmark in the 1980s reported reduced incidence of chronic *P. aeruginosa* infection after the introduction of cohort segregation (615-617). Prevalence of LES infection fell in the years following introduction of cohort segregation in the Liverpool CF Centre. Seven patients with novel *P. aeruginosa* infection were all with unique strains; the two cases of LES superinfection were in patients with social contacts with known LES cases (508). Prevalence of multi-resistant *P. aeruginosa* was reduced in centres practicing cohort segregation compared to those with mixed clinics in Italy (618). In Australia prevalence of the epidemic strain reduced, while prevalence of non-epidemic *P. aeruginosa* remained stable, compared to pre-segregation (619).

Disinfection with UV light has a beneficial effect over standard cleaning protocols, particularly for areas less commonly cleaned, leaving negligible traces of any organisms. Furukawa *et al*. recovered bacteria, albeit no CF pathogens, from door frames, door handles and door release buttons in an environmental survey of structural elements at hand and head height in a CF Centre in Belfast. Whilst reassuring that there was no evidence of environmental *P. aeruginosa* contamination at these sites (and indeed in wet areas of the ward), none-the-less current cleaning strategies were insufficient to remove all organisms (620).

We found facemasks to be beneficial in reducing environmental contamination in experimental settings, but little impact was found in a clinical environment. In the clinical study fewer participants were found to contaminate the air with viable *P. aeruginosa* prior to donning a facemask, compared to the experimental scenarios. This may be due to an unselected patient population or the smaller air volumes found in the cough rig compared to a clinic room. There is limited research on the benefit of facemasks to prevent dissemination of airborne pathogens (much is based on the protection of well subjects), however an *in vitro* study comparing facemask use on a “source” and a “receiver” manikin, found use of a mask by the source was more effective at preventing contamination of the air supply in the receiver manikin, than mask use on the receiver (621). Concerns about the risk of airborne pathogen dissemination prompted a recommendation for

mask wearing by people with CF entering a healthcare setting in the 2013 IP&C guidelines (174).

The impact of disinfection solution was equivocal in the included studies, in one due to the very low prevalence of *P. aeruginosa* in the test subjects (586) and in the other due to highly variable sampling at the various time-points rendering comparison difficult (519). Surface *P. aeruginosa* contamination has been found in CF clinical environments (159, 180, 518) and it can survive over three hours on laminate surfaces, over five hours on glass and over six hours on stainless steel (622). Chlorine-containing solution is effective at eliminating *P. aeruginosa* contamination, though *P. aeruginosa* isolates from CF patients are less susceptible than non-CF isolates, requiring least 1000ppm residual free chlorine to ensure complete killing (623).

We found no effect of adherence to the 2013 IP&C guidelines on *P. aeruginosa* acquisition, however there were flaws with both identified studies. The first (577) did not examine the rates of novel infection with transmissible and unique strains, the latter of which would not be expected to change in consequence of following IP&C guidelines. The authors also noted that longer follow-up may be needed to see a difference in a medium-sized CF clinic. The other study, whilst much larger, did not specify which, if any, of the recommended measures had been introduced in each included Centre, treating publication of the guideline as the intervention (575).

The only study of hand hygiene eligible for inclusion found no effect on *P. aeruginosa* contamination of hands, however sampling was done prior to hand hygiene and at the end of clinic, after opportunity for recontamination. Despite lack of studies directly examining the impact of hand hygiene on *P. aeruginosa* contamination, hand hygiene is likely to reduce hand carriage, as long as the sinks and soaps used are not contaminated (517, 624). Performance of hand hygiene is recommended on arrival at clinic, regularly during the visit, after performing pulmonary function testing, after contact with respiratory secretions and at the end of the clinic visit (174).

4.4.5 Environmental strategies (non-healthcare settings)

No studies meeting the inclusion criteria were found in this category. Unfortunately, this is the question that caused most concern to respondents in the survey reported in Chapter 3. As described in the introduction (see section 4.1.4.1), a number of studies have identified *P. aeruginosa* in the households of CF patients, some of which could be strain-matched to the resident CF inhabitant. Studies to determine the direction of infection would however be virtually impossible due to the massive scale of sampling that would be required for an observational study and the ethical constraints of deliberately exposing patients. A previous review of IP&C measures in CF suggested large scale registry studies may overcome these concerns (546) but it is worth noting that in a large, prospective, observational cohort no

environmental strategy was found to delay initial *P. aeruginosa* infection (461).

4.4.6 Strengths

The strengths of this study are its wide examination of strategies which may protect against *P. aeruginosa* acquisition. This provides evidence for clinicians to discuss with patients and their families about what strategies are known to work, what does not work and what strategies have no evidence to guide them. Promotion of unsubstantiated methods of *P. aeruginosa* avoidance has been shown to increase anxiety in families (188).

In addition, the systematic review protocol was published in advance of the work and wide search criteria were used over four research databases to improve the chance of identifying all published material.

4.4.7 Limitations

The specific inclusion criteria for this study means that some studies examining strategies to prevent *P. aeruginosa* infection were excluded. We did not include studies reporting prevalence of *P. aeruginosa*, in patients or in cultures, those reporting after an intervention with no prior comparison data or those reporting only on chronic *P. aeruginosa* infection. It is possible that, while these studies would not have answered the specific question here, they do provide information about strategies to reduce infection with *P. aeruginosa*.

Definitions of *P. aeruginosa* acquisition and freedom from *P. aeruginosa* prior to intervention differed between studies which may have affected the results. Additionally, in many, particularly early studies, initial and chronic infection were not always clearly defined in the study reports so data may have been inadvertently excluded where this was unclear.

4.4.8 Further work

Although patients and parents of people with CF worry about initial acquisition of *P. aeruginosa* in many cases this can be eradicated. Health status is preserved where *P. aeruginosa* is eradicated (191), therefore pursuing strategies to prevent initial infection may be of less clinical use than prevention of chronic infection. A number of studies excluded from this review, along with a body of work on eradication strategies, consider strategies to prevent chronic infection with *P. aeruginosa* in CF. A review of these strategies to identify those which are effective would be useful. Again, this may provide reassurance for patients and their families.

Many of the studies needed to improve the evidence into methods of preventing *P. aeruginosa* acquisition are unethical or logistically impossible (546). Data is awaited from the CF START trial into the effect of anti-staphylococcal antibiotics but there is little scope for additional RCTs of other therapeutic interventions. As the prevalence of transmissible *P. aeruginosa* infection continues to fall and eradication

strategies improve, prevention of initial, unique, P*. aeruginosa* infection may become less important.

4.5 Conclusion

CFTR modulators, vaccination against *P. aeruginosa* and cohort segregation, with or without other IP&C measures all reduce acquisition of *P. aeruginosa* in people with CF. Anti-staphylococcal antibiotics may increase the risk though the results of a definitive trial are awaited. Evidence for all these measures is low or very low and further studies are limited by ethical constraints. Ultimately initial acquisition of *P. aeruginosa* may be less important than development of chronic infection and an assessment of strategies to prevent this would be useful.

Chapter 5: Treatments for preventing recurrence of infection with *Pseudomonas aeruginosa* in people with cystic fibrosis – A Cochrane Systematic Review.

5.1 Introduction

After the failure of primary strategies to prevent the acquisition of *Pseudomonas aeruginosa* in people with CF further, secondary preventative, therapies may be used to reduce the risk of the patient developing chronic infection.

Once initial *P. aeruginosa* infection has occurred treatment with an eradication regimen has been shown to clear the organism from the person's airways (208) and delay commencement of chronic infection (203). As discussed in chapter 1 (section 1.2.4.1) this delay is important in maintaining the health of a person with CF since chronic *P. aeruginosa* infection is associated with increased morbidity and mortality. Additional treatments, given after successful eradication, may prolong the time to chronic infection with a subsequent improvement in morbidity and mortality in CF.

This Cochrane systematic review was designed to explore evidence for secondary preventative treatments which can prevent recurrence of *P. aeruginosa* infection and hence further delay the development of

chronic infection. The work in this chapter is taken from the published Cochrane Review (211) and conducted between June 2015 and December 2019. Details of the contributions made by each co-author are described in Table 5-1.

The title was chosen by myself and my supervisor (AS) from a list of reviews identified by the editorial group of the Cochrane Cystic Fibrosis group. I was involved in every stage of the production of this review. I drafted the protocol and reviewed every study identified for inclusion, at title and abstract and full text stage, with support from one of the second reviewers. I singlehandedly carried out the individual patient data analysis on the included trial, extracted the data and entered this into RevMan. I undertook initial interpretation of the analysis and drafted the final review, with support from colleagues at this stage. I struggled to keep contact with the initial second author due to geographical and time zone challenges and his commitments at medical school and on the wards. I learned the importance of finding the right co-author and making changes early on, experience which I used to better effect when writing Chapter 4.

Roles and responsibilities	
Task	**Who undertook the task?**
Protocol stage: draft the protocol	Dr Sally Palser Dr Edward Nash Prof. Alan Smyth Arnav Agarwal
Review stage: select which trials to include (2+1 arbiter)	Dr Sally Palser Arnav Agarwal Sherie Smith
Review stage: extract data from trials (2 people)	Dr Sally Palser Sherie Smith
Review stage: enter data into RevMan	Dr Sally Palser
Review stage: carry out the analysis	Dr Sally Palser
Review stage: interpret the analysis	Dr Sally Palser Dr Edward Nash Prof. Alan Smyth Sherie Smith

Review stage: draft the final review	Dr Sally Palser Dr Edward Nash Prof. Alan Smyth Sherie Smith
Update stage: Update the review	Dr Sally Palser Dr Edward Nash

Table 5-1: A summary of the tasks carried out by each co-author of the Cochrane Review, Treatments for preventing recurrence of infection with Pseudomonas aeruginosa in people with cystic fibrosis.

5.2 Background

5.2.1 Description of the condition

A description of CF and *P. aeruginosa* can be found in chapter 1.

Acquisition of *P. aeruginosa* is discussed in chapter 4.

5.2.1.1 Detection of P. aeruginosa

Microbiological samples are commonly collected from people with CF at routine clinic appointments and at the time of a pulmonary exacerbation. The frequency of this sampling depends on national practices. The preferred sampling method is a sputum sample, but in many cases this is not possible. Where the individual does not produce sputum, options include cough swabs, oropharyngeal culture (OPC), induced sputum and bronchoalveolar lavage (BAL) (625).

Non-sputum techniques have a number of disadvantages (as discussed in section 1.2.2.1), however for the purposes of this review, detection of *P. aeruginosa* is defined as the detection of *P. aeruginosa* in any respiratory sample. *P. aeruginosa* serology alone will not be accepted as evidence of new infection. First infection with *P. aeruginosa* is defined as the lifetime first identification of *P. aeruginosa* in any respiratory sample.

5.2.1.2 Eradication of P. aeruginosa

Early antibiotic therapy is effective in reducing the chance of chronic infection. A Cochrane Review showed that a number of antibiotic regimens are more effective than no treatment at eradicating *P. aeruginosa*, with an effect that can be sustained for up to two years (208). The current review follows on from this work, focusing on additional treatments given after successful eradication to prevent or delay recurrent *P. aeruginosa* acquisition in people with CF.

Many studies of *P. aeruginosa* eradication use negative respiratory culture at the end of the active treatment period to define successful eradication. The definition of eradication is crucial to understand whether a subsequent positive *P. aeruginosa* sample is truly a new infection, rather than an incompletely cleared index episode.

5.2.2 Description of the intervention

The effect of antibiotic regimens to eradicate initial *P. aeruginosa* infection may be sustained for up to two years, but the risk of a future episode of infection remains (208). Although eradication regimens may be prolonged (626), individuals who have successfully eradicated *P. aeruginosa* revert to their pre-eradication treatment regimens and no ongoing secondary prevention is attempted.

Host factors, such as sputum rheology and mucus plugging, may be equally important as pathogen attributes in the re-acquisition of *P. aeruginosa.* Interventions which affect these host factors, for example, recombinant dornase alfa (rhDNase) or hypertonic saline may also have an effect on recurrence. Treatment of eligible people with CF with CFTR modulators may have an effect on the acquisition and eradication of *P. aeruginosa* (94, 99, 100). The administration of a further course of oral, inhaled or intravenous antibiotics, after eradication of *P. aeruginosa* is complete, may reduce the risk of recurrent infection. Finally, immunotherapy such as IgY (derived from the eggs of hens immunised against *P. aeruginosa*) may have a role in secondary prevention (627).

5.2.3 How the intervention might work

In people with established *P. aeruginosa* infection, antibiotics are used to reduce inflammation, to maintain lung function and to reduce the

chance of a pulmonary exacerbation (625, 628). These antibiotics are administered orally, via nebuliser or in combination with the addition of regular cycled intravenous antibiotics as the disease course progresses (625).

A retrospective study looked at nebulised gentamicin as primary prevention for the acquisition of *P. aeruginosa*. Children meeting high-risk criteria for the development of infection were treated for a period of three years (301), with treatment continued in the event of another risk-event. A final audit of these children in 1999 showed that all those who continued on inhaled gentamicin remained free of *P. aeruginosa,* while seven out of 16 children who stopped prophylaxis (preventative treatment) developed chronic infection (P = 0.01).

Secondary prevention in the form of a similar prolonged course of treatment could sustain the benefits achieved by eradication, delaying the development of chronic *P. aeruginosa* infection and the subsequent negative consequences.

5.2.4 Why it is important to do this review

Long-term antibiotic therapy may be beneficial in delaying chronic *P. aeruginosa* infection, but it exposes individuals to the risk of adverse events (side effects of treatment, for example, allergy, damage to hearing or kidney function). Furthermore, the burden of treatment is one reason that *P. aeruginosa* is feared by people with CF and their families

(220) (see section 3.3.3.2). The use of prophylactic antibiotics, particularly nebulised, in people with established chronic *P. aeruginosa* can impact on school, work and social life (629). As such, strong evidence as to the efficacy of long-term antibiotic treatment is essential before it can be advocated to people with CF. Economically it is also important to assess the cost-benefit of such potential treatments, particularly as the prognosis of people with CF continues to improve (143, 185).

We have therefore examined current evidence to assess the safety, tolerability and cost-effectiveness of secondary prevention strategies proposed to prevent recurrent *P. aeruginosa* infection in people with CF.

5.3 Objectives

To establish whether secondary prevention strategies, using antibiotics or other therapies, increase the chances of people with CF remaining free from *P. aeruginosa* infection following successful eradication therapy.

5.4 Methods

5.4.1 Criteria for considering studies for this review

5.4.1.1 Types of studies

Randomised controlled trials (RCTs), published or unpublished and in any language were eligible for inclusion. Quasi-RCTs were eligible for inclusion if the review authors were satisfied that the groups were similar at baseline.

5.4.1.2 Types of participants

People with CF diagnosed clinically or by genetic or sweat testing. Each participant must have had an episode of *P. aeruginosa* within the last six months which was successfully treated with an eradication regimen. They must have remained free of infection with *P. aeruginosa* between the end of eradication and start of treatment for ongoing prevention.

5.4.1.3 Types of interventions

In people with CF in whom *P. aeruginosa* was successfully eradicated, we compared a time-limited course of therapy (for antimicrobials this could be oral, inhaled, intravenous or any combination of these) to prevent recurrent infection, with usual care, placebo or another therapeutic strategy. Time-limited therapy included all treatment in which a specific duration was pre-specified. Time-limited therapy included regimens where the treatment was intermittent but continued

at specified intervals for a defined duration. Long-term suppressive therapy, given for an indefinite period, was not considered.

5.4.1.4 Types of outcome measures

5.4.1.4.1 Primary outcomes

1. Time to next isolation of *P. aeruginosa* (identified by any method, e.g., sputum culture (spontaneous or induced), BAL or OP culture and as defined by the trial investigators).

5.4.1.4.2 Secondary outcomes

1. Change in quality of life from baseline (as measured by a validated tool (e.g. the Cystic Fibrosis Questionnaire-Revised (CFQ-R) (630), the Cystic Fibrosis Quality of Life Questionnaire (CF-QoL) (631) or any other validated tool)
2. Change (absolute and relative) from baseline for pulmonary function tests:
    a. forced expiratory volume in one second (FEV_1) measured in both L and %-predicted;
    b. forced vital capacity (FVC) measured in both L and %-predicted.
3. Pulmonary exacerbations:
    a. time to next exacerbation;
    b. frequency of exacerbations;
    c. number of days in hospital.
4. Nutritional parameters - change from baseline:

a. weight (kg) and weight centile or Z score;
b. height (cm) (children) and height centile or Z score;
c. body mass index (BMI) and BMI centile.

5. Time to chronic *P. aeruginosa* infection (as defined by the trial investigators).
6. Adherence to treatment:
    a. self-reported measures (e.g., participant diaries);
    b. secondary count measures (e.g., pill counting, days of intravenous antibiotics);
    c. electronic data (e.g., downloaded nebuliser data).
7. Adverse effects of treatment:
    a. mild (self-limiting, not requiring treatment change, e.g., wheeze with inhaled therapy which settles);
    b. moderate (requires treatment discontinuation, e.g., ototoxicity (damage to the ears causing hearing loss or balance problems));
    c. severe (e.g., hospitalisation or death).
8. Mortality.
9. Isolation of resistant bacteria (with detection method, i.e., conventional culture or molecular techniques, described where possible):
    a. *P. aeruginosa* with a new resistance pattern;
    b. methicillin-resistant *Staphylococcus aureu*s (MRSA);

c. resistant gram-negative organisms (e.g., *Stenotrophomonas maltophilia, Burkholderia cepacia, Achromobacter xylosoxidans*);

d. other novel organisms.

10. Cost effectiveness.

5.4.2 Search methods for identification of studies

We searched for all relevant published and unpublished trials without restrictions on language, year or publication status.

5.4.2.1 Electronic searches

The Cochrane Cystic Fibrosis and Genetic Disorders Group's Information Specialist conducted a search of the Group's Cystic Fibrosis Trials Register for relevant trials using the following terms: (pseudomonas aeruginosa OR mixed infections) AND (eradication OR preventative OR unknown).

The Cystic Fibrosis Trials Register is compiled from electronic searches of the Cochrane Central Register of Controlled Trials (CENTRAL) (updated each new issue of the Cochrane Library), weekly searches of MEDLINE, a search of Embase to 1995 and the prospective hand-searching of two journals - Pediatric Pulmonology and the Journal of Cystic Fibrosis. Unpublished work is identified by searching the abstract books of three major cystic fibrosis conferences: the International Cystic Fibrosis Conference; the European Cystic Fibrosis Conference and the

North American Cystic Fibrosis Conference. For full details of all searching activities for the register, please see the relevant sections of the Cochrane Cystic Fibrosis and Genetic Disorders Group's website (https://cfgd.cochrane.org/welcome).

Date of most recent search: 28 October 2019.

We also searched the following trial registries:

- ISRCTN registry (www.isrctn.com; searched 21 August 2019);
- US National Institutes of Health Ongoing Trials Register Clinicaltrials.gov (www.clinicaltrials.gov; searched 21 August 2019);
- World Health Organization International Clinical Trials Registry Platform (WHO ICTRP) (http://apps.who.int/trialsearch/; searched 21 August 2019).

For details of our search strategies, please see Appendix C: Chapter 5 search strategy.

5.4.2.2 Searching other resources

Two authors (SP and SS) checked the bibliographies of all included studies and any relevant systematic reviews identified for further references to relevant trials. The authors contacted the chief investigator of any included trials for unpublished data.

5.4.3 Data collection and analysis

Where we were unable to use all the analysis methods described below due to insufficient studies we plan to do so in future, should sufficient studies be identified.

5.4.3.1 Selection of studies

One author (SP) screened the titles and abstracts of identified trials for inclusion in this review according to the processes set out in the Cochrane Handbook for Systematic Reviews of Interventions (632); one of two further authors (AA or SS) then also screened these references. We excluded trials which were obviously irrelevant and removed duplicates. No trials identified required translation into English.

Two authors (SP and SS) independently screened the identified trials against the review's eligibility criteria and collated multiple reports of the same trial where necessary. We were not blinded to the trial authors. Disagreement was resolved by discussion and in consultation with a third author (AS) where necessary. We contacted the corresponding trial investigator(s) for further information where necessary to decide whether to include a trial in the review.

5.4.3.2 Data extraction and management

Two authors (SS and SP) independently extracted data using a data collection form which was agreed by all authors. The data collection form included information on the trial authors and eligibility, in addition to the trial methods (type of trial, blinding, setting, duration, number of

centres and dropouts) and outcome data. For each trial we documented the length of time after first isolation of *P. aeruginosa* that a participant could be randomised and also the length of time after successful eradication that a participant could be randomised. We additionally recorded the active intervention (antibiotic type, route of administration, dose, duration) and the control intervention. We considered all antibiotic regimens together; subgroup analysis was planned were sufficient trials of different regimens identified. We collected data on the participant demographics, including information on participants who dropped out.

We collected data from the text, tables and online supplements where appropriate and resolved any disagreements by discussion. Only a subset of participants from the included trial were eligible for inclusion in this review, so we requested individual participant data from the trial authors and one author (SP) analysed these using Microsoft Excel 2016 (436) and Stata version 16 (2019) (633) to determine the baseline characteristics of the subset of participants eligible for inclusion and their outcome data.

We planned to present data at two weeks, one month, three months, six months and one and two years. For the included trial we have reported the time points "up to three months", "up to six months", "up to one year" and "up to two years" and entered the data into the Review Manager software (2014) (634).

5.4.3.3 Assessment of risk of bias in included studies

Two authors (SP and SS) independently assessed the included trial for risks of bias using the Cochrane's Risk of Bias tool described in the Cochrane Handbook for Systematic Reviews of Interventions (635). This tool facilitates the identification of bias in the following domains:

- random sequence generation;
- allocation concealment;
- blinding of participants and personnel;
- blinding of outcome assessment;
- incomplete outcome data;
- selective reporting;
- other bias.

We classified each domain as having a high, low or unclear risk of bias. We resolved any disagreement by discussion and sought the opinion of a third author (AS) where necessary.

5.4.3.4 Measures of treatment effect

For dichotomous outcomes, such as mortality, development of resistant bacteria and adverse events, we sought data on the number of participants with each outcome and by allocated treatment group. We planned to conduct an intention-to-treat analysis. Data were available to conduct an intention-to-treat analysis for number of participants with a pulmonary exacerbation, severe adverse events and development of

resistant organisms. Adherence was calculated from returned medication so an available-case analysis was carried out for this outcome. We calculated the risk of the outcome in the treatment group compared to the control group (pooled risk ratio (RR) and its 95% confidence interval (CI)).

For continuous data, such as change in Quality-of-Life scores, pulmonary function tests and nutritional parameters, we calculated the pooled mean difference (MD) and its 95% CI between the intervention and control groups. If in the future different trials use different scales, we planned to calculate the pooled standardised mean difference (SMD). We planned to report skewed data narratively.

We analysed common count data (such as number of days in hospital) as continuous data, as described in the Cochrane Handbook of Systematic Reviews of Interventions (636).

For time-to-event data (such as time to next isolation of *P. aeruginosa*) we calculated the pooled hazard ratios (HRs) and their 95% CIs using the Cox Proportional Hazards model in Stata version 16 (2019) (633). We then created forest plots using the generic inverse variance method in RevMan (634).

5.4.3.5 Unit of analysis issues

We have not included any cross-over trials as this is an inappropriate design for the review question. Firstly, it is unlikely that all participants

would have fulfilled the primary outcome (time until a new growth of *P. aeruginosa*) at the point of cross-over; and secondly, once the outcome was reached cross-over would be meaningless. Cluster-randomised trials were also inappropriate as there may be geographical differences between the *P. aeruginosa* strains which could affect eradication and re-acquisition rates. We planned to analyse factorial trials, where there was no suggestion of an interaction between the two interventions, separately (637). Had we included a trial which compared multiple treatment arms of interest we would have presented these in separate comparisons. We planned to directly compare treatments of differing durations as each represents a separate treatment regimen.

5.4.3.6 Dealing with missing data

We contacted the trial authors for missing data and received individual patient data for all the trial participants who had consented to data sharing. We extracted data for the relevant subset of participants for analysis. We collected data on the number of participants with each outcome according to the allocated group, allowing an intention-to-treat analysis. This was also possible for "time to next pulmonary exacerbation," "adverse effects," and "isolation of resistant pathogens". For each time point we presented an available-case analysis, including all participants for whom data were available at that time point.

Where necessary, we planned to use the methods described in Chapter 7 of the Cochrane Handbook for Systematic Reviews of Interventions to

impute these data (632). Had we needed to impute significant amounts of data, we would have undertaken a sensitivity analysis to compare the effects of the imputed data against the available case data.

5.4.3.7 Assessment of heterogeneity

Had there been sufficient trials to undertake a meta-analysis, we planned to test for heterogeneity using the I^2 statistic, and interpret this according to the Cochrane Handbook for Systematic Reviews of Interventions (636). We note that the usefulness of I^2 depends on the magnitude and direction of the intervention effects and the strength of the evidence of heterogeneity. We would have considered an I^2 value of 0% to 30% to represent little or no heterogeneity, 30% to 50% to represent moderate heterogeneity, 50% to 75% to represent substantial heterogeneity and above 75% to represent considerable heterogeneity.

5.4.3.8 Assessment of reporting biases

We used a number of strategies to minimise reporting bias. We assessed publication bias by a comprehensive search of grey literature and clinical trials databases, as well as by discussion with researchers in the field in an attempt to identify unpublished data; this would also have helped reduce location and citation bias. We are, however, aware of the potential bias inherent in the inclusion of unpublished data. The eligibility of inclusion of trials published in any language reduced the risk of language and location bias. We carefully screened trials at

inclusion to look for evidence of duplicate publication, including author names, sites, interventions and participant characteristics.

We assessed outcome reporting bias by comparing outcomes specified in the 'Methods' section to those reported in the results of the full trial paper. We further investigated this bias by comparing the outcomes reported in the full paper to those stated in the published protocol (638). We compared the stated outcomes to those published on www.clinicaltrials.gov. Had any further concerns remained we planned to contact the trial authors to request the original trial protocol.

Had we identified a sufficient number of trials (i.e. at least 10), we planned to construct funnel plots comparing trial effect to trial size, to inspect these for evidence of asymmetry and, where appropriate, test for asymmetry as discussed in the Cochrane Handbook for Systematic Reviews of Interventions (639).

5.4.3.9 Data synthesis

If we had been able to combine sufficient trials, we planned to assess the extracted data using a fixed-effect meta-analysis. In the case of substantial heterogeneity (I^2 greater than 50%), we would have conducted a random-effects meta-analysis. We present the data from the single included trial using a fixed effect analysis.

5.4.3.10 Subgroup analysis and investigation of heterogeneity

We planned to undertake the following subgroup analyses as appropriate:

- comparison of the effect of route of antibiotic administration (oral versus inhaled versus intravenous);
- comparison between participants who underwent eradication of a first episode of *P. aeruginosa* infection versus those with previous *P. aeruginosa* infection;
- comparison of differing methods of *P. aeruginosa* detection;
- comparison of differing definitions of recurrent *P. aeruginosa* infection;
- comparison of differing definitions of chronic *P. aeruginosa* infection.

5.4.3.11 Sensitivity analysis

A sensitivity analysis was carried out to ascertain whether our decision to define eradication in the included study as *P. aeruginosa* culture negativity at visit 2 had any effect on the primary outcome.

If appropriate we would have undertaken other sensitivity analyses to ascertain whether the results of the review are robust. We would have excluded trials assessed as having a high risk of bias (more than 50% of domains with a high risk) and would have repeated the analysis to see if this had any effect on the results. Little difference in the results of this sensitivity analysis would have given us greater confidence in the

results. Furthermore, in situations where we made arbitrary decisions, such as the time points for analysis, or if we had imputed significant amounts of data, we would have carried out a sensitivity analysis to assess the impact of these decisions. Once again similar results would have strengthened the conclusions of this review, while conversely a marked difference would mean the review results would need to be interpreted more cautiously.

5.4.3.12 Summary of findings table

We constructed a summary of findings table for the single comparison in the review, cycled therapy versus culture-based therapy (see Table 5-2). We considered the following outcomes:

1. time to next isolation of *P. aeruginosa*;
2. Quality of Life;
3. FEV_1 (change from baseline);
4. frequency of pulmonary exacerbations;
5. time to chronic *P. aeruginosa* infection;
6. Adverse events; and
7. emergence of novel bacteria.

We used the GRADE approach, described in Chapter 12 of the Cochrane Handbook of Systematic Review for Interventions (640) to classify the body of evidence for each outcome as high, moderate, low or very low. The quality of the evidence was downgraded across five domains: risk of bias, indirectness, inconsistency, imprecision and

publication bias. Where there was serious risk of bias, we downgraded by one level and where it was very serious we downgraded it by two levels. Where we judged the evidence not to be high quality, we described the rationale for this judgement in footnotes to the table.

5.5 Results

5.5.1 Description of studies

5.5.1.1 Results of the search

The results of the searches are presented in a PRISMA diagram (Figure 5-1). We identified 371 unique references to 155 trials from electronic searches and one further trial was identified following discussion with the lead author of another study. No further trials were identified from searches of other trial databases or the reference lists of other trials.

Following review of title and abstract, we excluded 124 trials, with full texts obtained for the remaining 31 trials. One trial was included for analysis (204) and one study is ongoing (641). The study identified through discussion with the lead author is listed as 'Awaiting classification' since only a subset of participants for this trial will be eligible for inclusion; individual patient data have been requested, but are not available at this time (418). The remaining 28 studies were excluded with reasons.

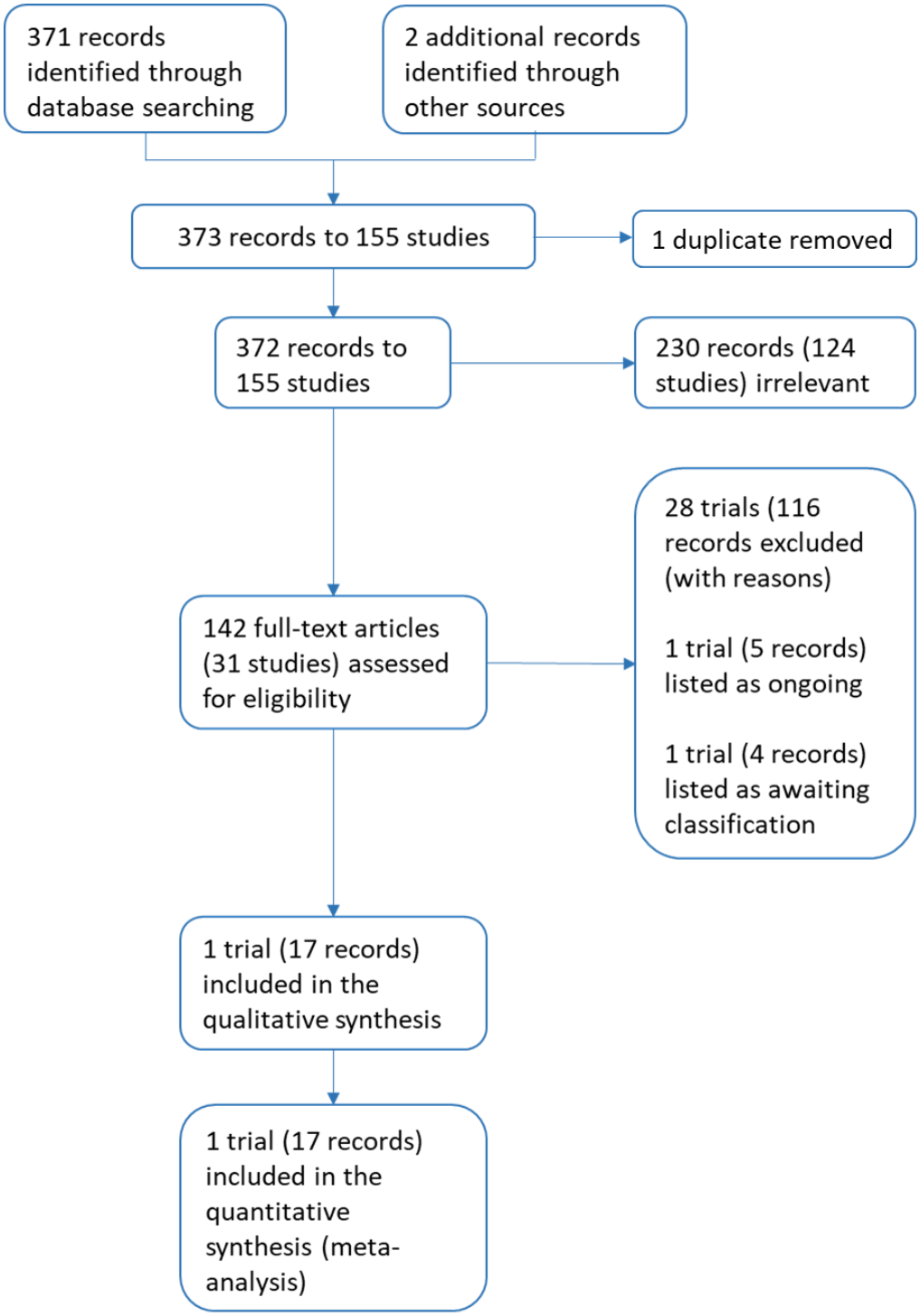


Figure 5-1: PRISMA diagram to show the identification of studies relevant to the Cochrane Systematic Review, Treatments for preventing recurrence of infection with Pseudomonas aeruginosa in people with cystic fibrosis.

5.5.2 Summary of findings

Cycled antibiotic therapy compared with culture-based antibiotic therapy for preventing recurrence of infection with *P. aeruginosa* in cystic fibrosis						
Patient or population: children (aged 1 to 12 years) with CF and a newly isolated *P. aeruginosa* infection **Settings:** outpatients **Intervention:** cycled administration of antibiotics **Comparison:** culture-based administration of antibiotics						
Outcomes	**Illustrative comparative risks* (95% CI)**		**Relative effect (95% CI)**	**No of participants (studies)**	**Quality of the evidence (GRADE)**	**Comments**
	Assumed risk	**Corresponding risk**				
	Culture-based therapy	**Cycled therapy**				

Time to next isolation of *P. aeruginosa* Follow-up: up to 583 days	The participants in the culture-based therapy group were twice as likely to have experienced a recurrence by their final study visit as those in the cycled group.		HR **2.04** (1.28 to 3.26)	253 (1)	⨁⨁⨁⊖ **moderate**[1]	HR was calculated from individual patient data.
Quality of life	This outcome was not measured.					
FEV_1: change from baseline (L) Follow-up: median 494 days	The change in FEV_1 in the culture-based group from baseline was +0.26 L.	The change in FEV_1 in the cycled group was 0L higher (0.09 L lower to 0.09 L higher).	NA	131 (1)	⨁⊖⊖⊖ **very low**[1,2,3]	The difference between the two groups was not statistically significant P = 0.97. FEV_1 was also measured in percent predicted which showed

						a non-significant difference between the groups at up to two years. MD 0.7% (-4.33% to 5.73%) P = 0.79.
Pulmonary exacerbations: frequency Follow-up: up to 583 days	The mean frequency of pulmonary exacerbation in the culture-based group was 1.1.	The mean frequency of pulmonary exacerbation in the cycled group was 0.18 pulmonary exacerbations per person lower (0.51 lower to 0.14 higher).	NA	253 (1)	⨁⨁⨁⊖ **moderate**[1]	The difference between the groups was not statistically significant, P = 0.27.
Time to chronic *P. aeruginosa*	This outcome was not measured.					

Adverse events: severe adverse events (total) Follow-up: 18 months	**231 per 1000**	**150 per 1000** (90 to 256)	**RR 0.65** (0.39 to 1.11)	253 (1)	⨁⨁⨁⊖ **moderate[1]**	There was no significant difference between groups for the total number of participants experiencing a serious adverse event P = 0.11.
Emergence of novel bacteria: isolation of novel gram-negative organisms Follow-up: median 494 days	**276 per 1000**	**276 per 1000** (185 to 414)	**RR 1.0** (0.67 to 1.5)	253 (1)	⨁⨁⨁⊖ **moderate[1]**	No significant difference in the acquisition of novel organisms between treatment groups. P = 0.98

*The basis for the **assumed risk** (e.g., the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CF: cystic fibrosis; **CI:** confidence interval; **HR**: hazard ratio; **RR:** risk ratio.

GRADE Working Group grades of evidence

High quality: Further research is very unlikely to change our confidence in the estimate of effect.

Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.

Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.

Very low quality: We are very uncertain about the estimate.

Footnotes

[1.] Downgraded once due to indirectness. The trial only includes children between the ages of one and 12 years and was not designed to answer the specific question posed in this review.

[2.] Downgraded once due to risk of bias from incomplete outcome data. Although this was taken from individual patient data, only 131 of the 253 participants included in our subset, were included in this analysis.

[3.] Downgraded once from small participant numbers which do not meet the optimum information size.

Table 5-2: Summary of findings table for the main comparison: cycled versus culture-based antibiotic therapy for preventing recurrence of infection with Pseudomonas aeruginosa. This table was prepared by Sherie Smith.

5.5.2.1 Included studies

One trial has been identified for inclusion in this review (204).

5.5.2.1.1 Trial design

The study is a factorial design primarily looking at strategies to eradicate *P. aeruginosa* (204). It was randomised 1:1:1:1 to each of four treatment regimens. Culture-based therapy versus cycled therapy was open label, but the addition of ciprofloxacin or placebo to the tobramycin solution (TIS) regimen was blinded; outcomes assessors were blinded to all treatment allocation. This was a multicentre trial conducted at 55 CF centres in the USA. The planned duration of follow-up was 18 months, which was achieved.

5.5.2.1.2 Participants

Investigators randomised 306 participants (204); 304 were included in the trial intention-to-treat analysis as two participants were subsequently found to have failed screening. The gender split was approximately equal; 154 participants were female (150 male) and eligible participants were aged 1 to 12 years (204). Participants had to have had either a lifetime first isolation of *P. aeruginosa* or a new isolation, defined as at least a two-year absence of *P. aeruginosa* having had at least one respiratory culture examined per year. Participants could have had their *P. aeruginosa* positive sample up to six months prior to enrolment and have had up to one course of anti-pseudomonal antibiotic therapy in that time (204).

The trial did not require specific evidence of eradication prior to commencement of the treatment regimen. For the purposes of this review only participants with documented eradication were eligible; participants with a positive *P. aeruginosa* sample at visit 2 (approximately three weeks after commencement of the initial course of therapy) were therefore excluded.

There were no significant differences between the groups in any of the baseline parameters for our included subset of 253 participants (124 males and 129 females). In the cycled-therapy group 59 out of 119 (49.6%) were female and in the culture-based therapy group 70 out of 134 (52.2%) were female. The mean age in the cycled group was 6.0 years and in the culture-based group was 5.6 years. Baseline weight was 21.5 kg in the cycled group and 20.3 kg in the culture-based group; baseline height was 111.0 cm in the cycled group versus 107.9 cm in the culture-based group. Baseline FEV_1 was 1.53 L in the cycled group and 1.51 L in the culture-based group. No data on the individual variants were available.

5.5.2.1.3 Interventions

All participants underwent an initial course of inhaled TIS 300 mg twice daily for 28 days. This could be extended for a further 28 days if samples taken at the week 3 visit remained positive for *P. aeruginosa*.

The trial compared two separate interventions in a factorial design. The first comparison was cycled therapy, in which participants received TIS 300 mg twice daily every three months regardless of culture results,

compared to culture-based therapy, in which participants received TIS 300 mg twice daily only in the three-month periods in which their respiratory samples were positive for *P. aeruginosa*. The second comparison was between the addition of oral ciprofloxacin 15 to 20 mg/kg/dose (up to 750 mg) twice daily for 14 days with every TIS cycle versus matched placebo. Participants were therefore randomised to one of four groups: cycled therapy and ciprofloxacin; or cycled therapy and placebo; or culture-based therapy and ciprofloxacin; or culture-based therapy and placebo (204). For this review only the cycled versus culture-based comparison was eligible.

5.5.2.1.4 Outcomes

The primary outcome was the time to next pulmonary exacerbation, which was defined *a priori* as a pulmonary exacerbation requiring either intravenous antibiotics or hospitalisation; less severe pulmonary exacerbations were a secondary endpoint. This trial also had a primary microbiological endpoint, the proportion of respiratory samples which were positive for *P. aeruginosa* at each three-month time-point after the first treatment cycle (204). Further secondary endpoints were safety (monitored by adverse events and audiology), changes in height, weight and lung function, additional safety measures including musculoskeletal symptoms, haematological, liver and renal profiles and the emergence of resistant *P. aeruginosa* or other new pathogens (204).

5.5.2.2 Excluded studies

We excluded 28 trials from the review. Nine trials assessed participants with chronic *P. aeruginosa* infection (79, 642-649). In two trials participants were *P. aeruginosa* negative at baseline (i.e., did not have eradication) (650, 651). Four studies compared short-term antibiotic strategies for treating acute pulmonary exacerbations (652-655). Six trials studied eradication with no additional treatment once eradication had been achieved (203, 206, 414, 416, 656, 657); and four studies had no eradication step (658-661). One trial compared three weeks versus three months of eradication treatment but did not look for eradication after the first three weeks (662). One trial was cross-over in design comparing TIS and placebo where participants first treated with TIS could go on to open-label treatment, but this was optional (663).

One further trial required very careful consideration (205). In this trial all participants underwent 28 days of eradication for *P. aeruginosa* with TIS 300 mg twice daily. After this they were randomised to either no further therapy or to a further 28 days of TIS. Randomisation was based on the results of *P. aeruginosa* serology taken at baseline. Following discussion with the lead author, it was clear that no respiratory cultures were taken at day 28 to assess eradication, as participants were still on treatment. Therefore, even with individual participant data this trial did not have definite evidence of eradication after the initial treatment to allow inclusion in this review.

5.5.2.3 Ongoing studies

One ongoing trial seems to meet our inclusion criteria. A phase III study examining the ability of IgY to prolong time to chronic *P. aeruginosa* infection following successful eradication was due to be published in 2019 (NCT01455675) (641). This is a double-blind RCT comparing avian-derived IgY antibodies in a 70 mL gargle to a volume-matched placebo in participants with previously eradicated *P. aeruginosa*. Males and females aged five or over, able to gargle and were *P. aeruginosa* negative at enrolment were eligible. A total of 164 participants were recruited from multiple European CF centres. The primary outcome for this trial is time from enrolment to the first *P. aeruginosa* positive sputum, throat cough swab or endolaryngeal suction culture. Secondary outcomes include the change from baseline in FEV_1 and BMI, number of days of illness, number of days taking antibiotics, number of pulmonary exacerbations, the change in *P. aeruginosa* serum precipitins from baseline, safety and emergence of novel pathogens.

As of March 2024 no results for this study have been posted on clinicaltrials.gov (664) and no paper has been published. For the update of this review we will contact the lead author of the study to discuss whether there data from this study could be made available for inclusion.

5.5.2.4 Studies awaiting classification

The OPTIMIZE trial is a multicentre (45 sites in the USA) parallel group RCT examining the effect of azithromycin in preventing pulmonary

exacerbations in participants who have undergone *P. aeruginosa* eradication (418). Participants were randomised in a 1:1 ratio, but stratified by age group. The trial was blinded four ways (participant, care provider, investigator, outcomes assessor). The trial planned for an 18-month follow-up but was terminated early when it reached a pre-specified interim monitoring boundary for efficacy. The median length of follow-up for this trial was therefore 11.8 months. Investigators enrolled 221 participants aged 6 months to 18 years in this trial. Participants had to have either lifetime first isolation of *P. aeruginosa* or a new isolation, defined as at least a two-year absence of *P. aeruginosa* having had at least one respiratory culture examined per year. The positive *P. aeruginosa* sample had to be within 40 days of the baseline visit; TIS therapy could have started up to 14 days prior to this visit. All participants underwent an initial course of inhaled TIS 300 mg twice daily for 28 days which could be extended for a further 28 days if samples taken at the week 3 visit remained positive for *P. aeruginosa*. The active intervention was oral azithromycin suspension at 10 mg/kg up to a maximum dose of 500 mg, three times a week for the duration of the study; participants in the control group received a volume-matched placebo. The primary end-point was time to next pulmonary exacerbation. This was defined *a priori* as a pulmonary exacerbation treated with any mode of antibiotics (oral, inhaled or intravenous). Secondary endpoints included safety (monitored by adverse events and audiology), changes in height, weight and lung function, time to recurrence of *P. aeruginosa* after the first quarter of

treatment, electrocardiogram (ECG) changes, frequency of exacerbations, frequency of *P. aeruginosa* positive cultures, rates of antibiotic usage, rates of hospitalisations and patient/parent reported changes in the Chronic Respiratory Infection Symptom Score (CRISS).

The study found no significant difference in the rate of *P. aeruginosa* isolation at the end of the first quarter between the two groups, and the hazard ratio for time to *P. aeruginosa* recurrence was 1.0 (418). These data however represent all participants, some of whom were still *P. aeruginosa* positive after the initial period of eradication. Inclusion of this study will require analysis of individual patient data, so that only those with confirmed eradication are included. Such data will be requested for the update of this review.

5.5.3 Risk of bias in included studies

We judged the included trial to have an unclear overall risk of bias (204). There is a low risk of bias for all domains except 'other potential sources of bias' which is unclear due to the potential for additional anti-*P. aeruginosa* therapy to be given prior to commencing the study (204). We have summarised the risk of bias in Figure 5-2.

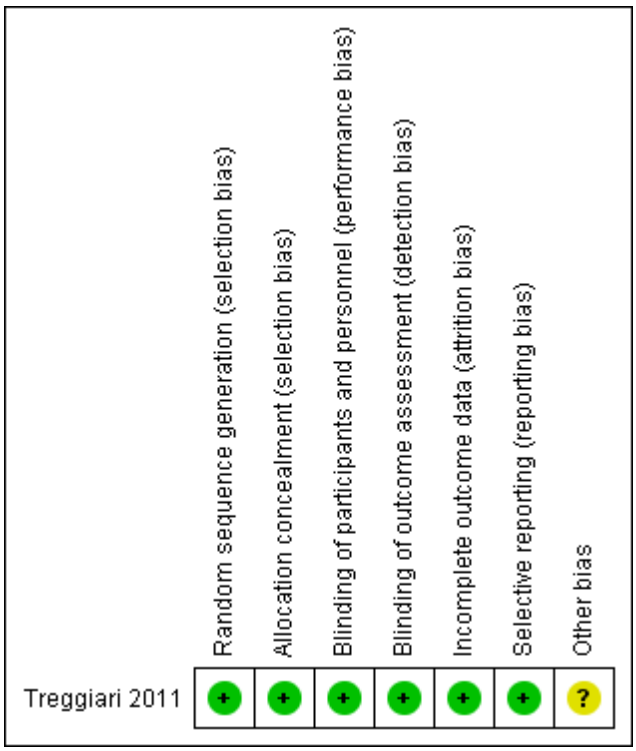


Figure 5-2: Risk of bias summary: review author's judgements about each risk of bias item for each included study for the Cochrane Systematic Review, Treatments for preventing recurrence of infection with Pseudomonas aeruginosa in people with cystic fibrosis.

5.5.3.1 Allocation

5.5.3.1.1 Sequence Generation

The risk of bias from sequence generation was deemed to be low (204). The trial described the processes of randomisation clearly, both in the main paper and in supplementary information. A randomisation code was generated using a computerised random number generator.

5.5.3.1.2 Allocation Concealment

The risk of bias due to allocation concealment was also deemed to be low. Randomisation was performed via an interactive voice response system with email confirmation of treatment allocation (204).

5.5.3.2 Blinding

5.5.3.2.1 Blinding of participants and personnel (performance bias)

The trial blinded all participants and personnel to oral therapy assignment. Active and placebo tablets and suspension were both provided by Bayer Pharmaceuticals Inc. and the placebo suspension was taste-masked. However, in this trial the allocation to cycled therapy or culture-based therapy was open label for participants and care providers (although the core trial investigators were blinded to all treatment allocation for the whole trial). In an attempt to reduce the risk of bias from the lack of blinding the study used an *a priori* definition of a pulmonary exacerbation and all hospitalisation records were reviewed in conjunction with symptom diaries from the participants to ensure that this definition was met. In view of these additional measures the risk of bias for this trial was deemed low (204).

5.5.3.2.2 Blinding of outcome assessors (detection bias)

The risk of detection bias was deemed low as it was explicitly stated that, "The core study investigators were blinded to all treatment allocation for the entire study" (204).

5.5.3.3 Incomplete outcome data

The trial report stated the investigators used an intention-to-treat analysis for their primary outcome measures (204). In reality this was a modified intention-to-treat analysis (all randomised participants who received at least one dose of the trial drug) as two randomised participants failed screening prior to starting any trial medications and were therefore excluded from the analysis; they did not describe how

data were imputed (204). Withdrawals were described; these were small numbers and were balanced across the groups for both numbers and explanation (204). We therefore judged the trial to have a low risk of bias.

5.5.3.4 Selective reporting

We were able to examine the published protocol on the trials registry clinicaltrials.gov (204). In addition, we analysed the published methodology. The trial had a low risk of bias, reporting all the stated outcome measures in the paper, the online supplement and with additional data in the online trial entry (204).

5.5.3.5 Other potential sources of bias

The trial was supported by industry in the provision of medications (204). The paper specifically states that, "The industry sponsors had no role in the analysis, interpretation and writing of the manuscript."

The trial defined new onset *P. aeruginosa* as either a first lifetime *P. aeruginosa* isolation or isolation after two years *P. aeruginosa* -free with at least one culture per year (204). This frequency of respiratory tract sampling is low; USA Infection Control Guidelines recommend sampling be done quarterly (174) and UK guidelines recommend sampling every two months (625). It has been shown that in adults a single sputum culture will identify only 58% of the bacterial diversity identified though more invasive sampling (72); *P. aeruginosa* may therefore have been unknowingly present for longer than the time limits set between positive culture and enrolment, affecting the chance of successful eradication.

In addition, participants were allowed to have had a positive *P. aeruginosa* culture within six months of eradication and to have had up to one course of anti- *P. aeruginosa* therapy prior to enrolment. This additional therapy may have affected the chance of eradication and the subsequent success of the trial regimens (204).

The trial did not specifically answer the question posed in this review (204). We made a practical decision to define eradication as *P. aeruginosa* negative culture at visit 2 and analysed the subset of participants who fulfilled this definition. This may have had an impact on selection bias as not all participants allocated to each group were analysed for our review and the study was not powered for this endpoint.

Overall, we judged the study to have an unclear risk of other potential bias (204).

5.5.4 Effects of interventions

See Summary of findings table, Table 5-2.

5.5.4.1 Cycled versus culture-based therapy

Data are reported below from the eligible subset of the trial population from the only included trial (204). For the whole trial cohort, 304 participants were included in the intention-to-treat population and data for 300 were made available for analysis (consent for data sharing was not provided by the other four).

We defined successful eradication as individuals who were *P. aeruginosa* negative at visit 2 (median 21 days). Whilst trial participants who were *P. aeruginosa* positive at this visit were allowed within the trial design to have a second course of tobramycin (up to 56 days) to maximise the chance of eradication, this would have added bias to our review since not all participants would have had the same eradication regimen. We conducted a sensitivity analysis for the primary outcome to assess the impact of this decision.

At the second study visit 41 participants were *P. aeruginosa* positive and were excluded from the analysis. A further six participants had no result or an inconclusive result at this time point and were also excluded from the analysis. Thus the eligible subset included 253 participants, 119 in the cycled arm and 134 in the culture-based arm. Of these, 64 out of 119 (53.8%) participants in the cycled group were in the ciprofloxacin arm of the ciprofloxacin versus placebo trial and 70 out of 134 (52.2%) participants in the culture-based group were also in the ciprofloxacin arm.

5.5.4.1.1 Primary outcomes

5.5.4.1.1.1 Time to next isolation of P. aeruginosa

Time to next isolation of *P. aeruginosa* was not reported in the published trial report but could be calculated from the individual patient data provided. The median follow-up for the included participants was 494 days and in the cycled group 26 out of 119 participants had recurrent *P. aeruginosa* during follow-up while in the culture-based

group 54 out of 134 participants experienced a recurrence of *P. aeruginosa*. We were unable to measure the median time to recurrence as fewer than 50% of participants in each group had experienced a recurrence at the end of the trial. The time to recurrent *P. aeruginosa* favoured cycled over culture-based therapy. The HR, calculated using the Cox Proportional Hazards Model, for recurrence of *P. aeruginosa* in the culture-based group compared to the cycled group was 2.04 (95% CI 1.28 to 3.26) (Analysis 1.1, see Figure 5-3).

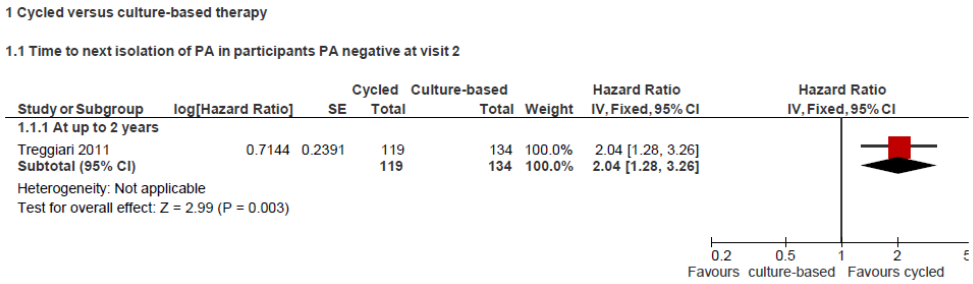


Figure 5-3: Analysis 1.1; Time to next isolation of P. aeruginosa in participants P. aeruginosa negative at visit 2, comparing cycled vs culture-based therapy. Cochrane Systematic Review, Treatments to prevent recurrence of infection with Pseudomonas aeruginosa in people with cystic fibrosis.

The proportional hazards assumption was checked and found to hold (P = 0.68). The GRADE rating for this evidence was moderate; it was downgraded once for indirectness (only examined children aged one to 12 years old) (see Table 5-2). This is in contrast to the main trial report which examined rates of *P. aeruginosa* positivity (rather than time to recurrent *P. aeruginosa*) and found no difference between the cycled and culture-based groups (204).

To assess the effect of the decision to use *P. aeruginosa* negativity at visit 2 to define eradication we conducted a sensitivity analysis using *P. aeruginosa* negativity at visit 3. For this analysis 244 participants were eligible, 123 in the cycled arm and 121 in the culture-based arm. In the cycled group 25 out of 123 (20.3%) participants had recurrent *P. aeruginosa* during follow-up, while in the culture-based arm 44 out of 121 (36.4%) participants experienced a recurrence. Again, the median time to recurrence could not be calculated. The HR, calculated as above using the Cox Proportional Hazards Model, for recurrence of *P. aeruginosa* in the culture-based group compared to the cycled group was 1.98 (95% CI 1.21 to 3.23) (Analysis 1.2, see Figure 5-4). Nineteen of 123 (15.5%) participants in the cycled group had additional TIS eradication therapy compared to 7 out of 121 (5.8%) participants in the culture-based group. The proportional hazards assumption was checked and found to hold (P = 0.96).

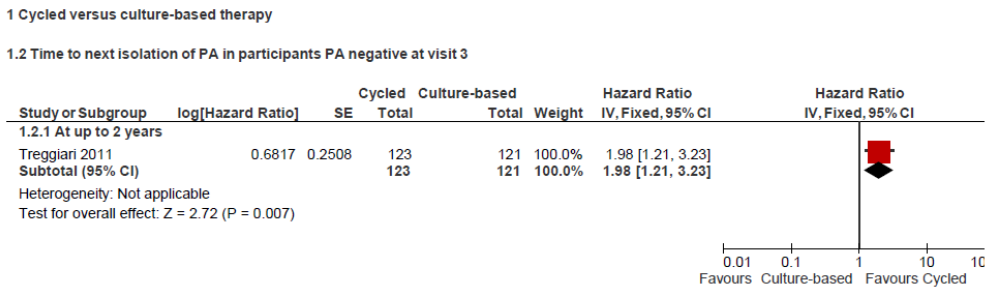


Figure 5-4: Analysis 1.2; Time to next isolation of P. aeruginosa in participants P. aeruginosa negative at visit 3, comparing cycled vs culture-based therapy. Cochrane Systematic Review, Treatments to prevent recurrence of infection with Pseudomonas aeruginosa in people with cystic fibrosis.

5.5.4.1.2 Secondary outcomes

1. Change in Quality of Life from baseline

This was not reported in the included trial (204).

2. Change from baseline for pulmonary function tests

The included trial looked at participants aged between one and 12 years old (204). Pulmonary function testing was only performed in participants aged four years or over who were able to perform spirometry; hence at the end of trial follow-up visit data were only available for 130 participants.

a. FEV_1

The study reported absolute mean (SD) change from baseline of FEV_1 and $ppFEV_1$ at the end of study visit (week 70) (204). Using the individual patient data provided we were able to analyse the mean (SD) absolute and relative change from baseline for FEV_1 and $ppFEV_1$ at "up to three months", "up to six months", "up to one year", and "up to two years". The GRADE of this evidence was very low. It was downgraded three times for indirectness (the results only relate to children), incomplete outcome data (only 131 out of 252 participants) and small sample size (see Table 5-2).

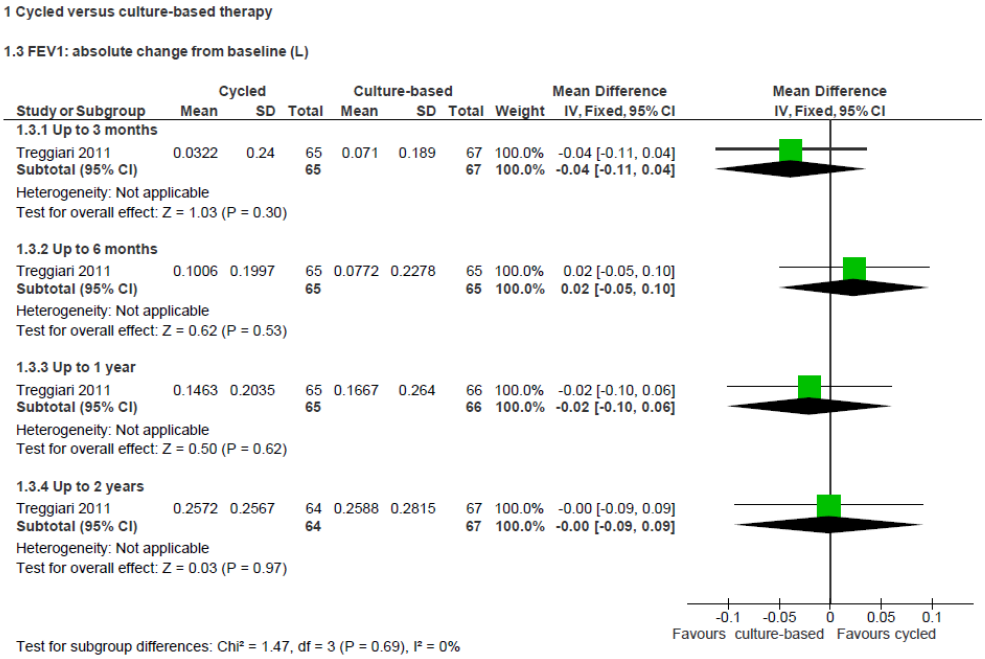


Figure 5-5: Analysis 1.3; FEV_1 absolute change from baseline (L), comparing cycled vs culture-based therapy at up to 3 months, up to 6 months, up to 1 year and up to 2 years. Cochrane Systematic Review, Treatments to prevent recurrence of infection with Pseudomonas aeruginosa in people with cystic fibrosis.

There were no differences identified between the cycled or culture-based therapies for any of the measurements of FEV_1 reported at any time point: absolute change from baseline in L (Analysis 1.3, see Figure 5-5), relative change from baseline in L (Analysis 1.4, see Figure 5-6), absolute change from baseline in % predicted (Analysis 1.5, see Figure 5-7), or relative change from baseline in % predicted (Analysis 1.6, see Figure 5-8) (all very low-quality evidence).

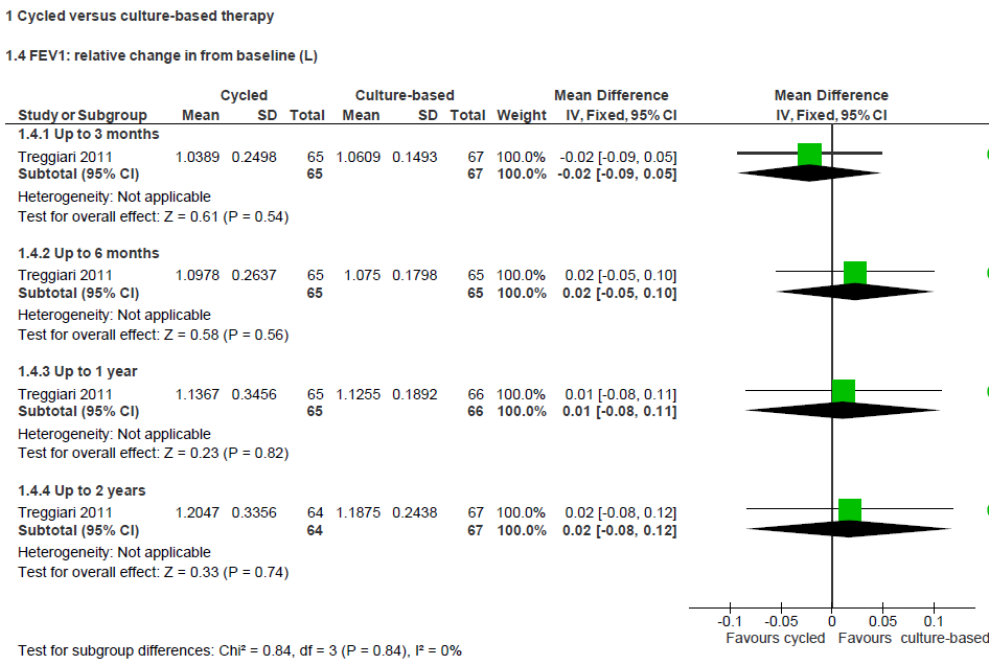


Figure 5-6: Analysis 1.4; FEV_1 relative change from baseline (L), comparing cycled vs culture-based therapy at up to 3 months, up to 6 months, up to 1 year and up to 2 years. Cochrane Systematic Review, Treatments to prevent recurrence of infection with Pseudomonas aeruginosa in people with cystic fibrosis.

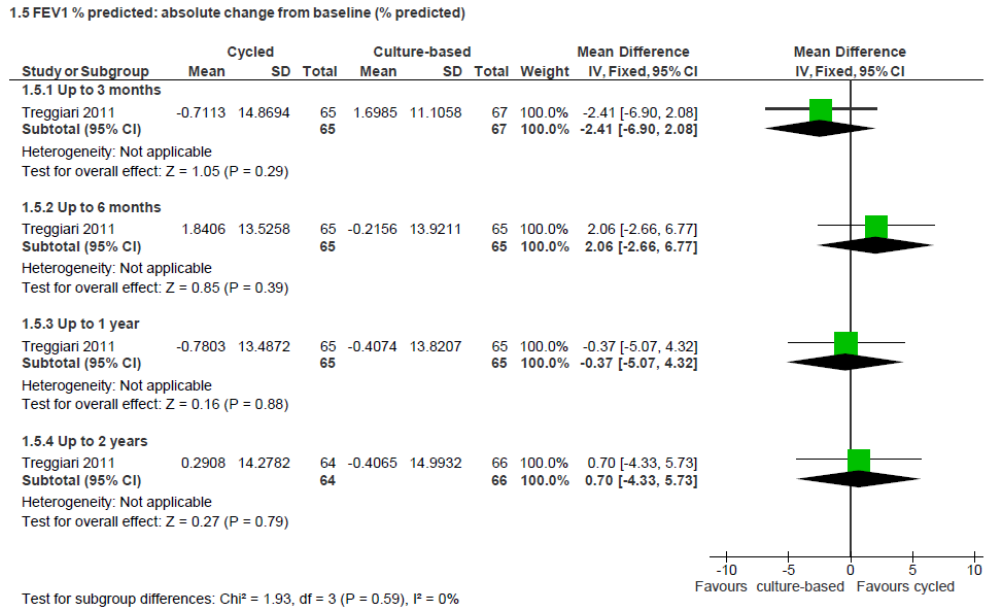


Figure 5-7: Analysis 1.5; $ppFEV_1$ absolute change from baseline, comparing cycled vs culture-based therapy at up to 3 months, up to 6 months, up to 1 year and up to 2 years. Cochrane Systematic Review, Treatments to prevent recurrence of infection with Pseudomonas aeruginosa in people with cystic fibrosis.

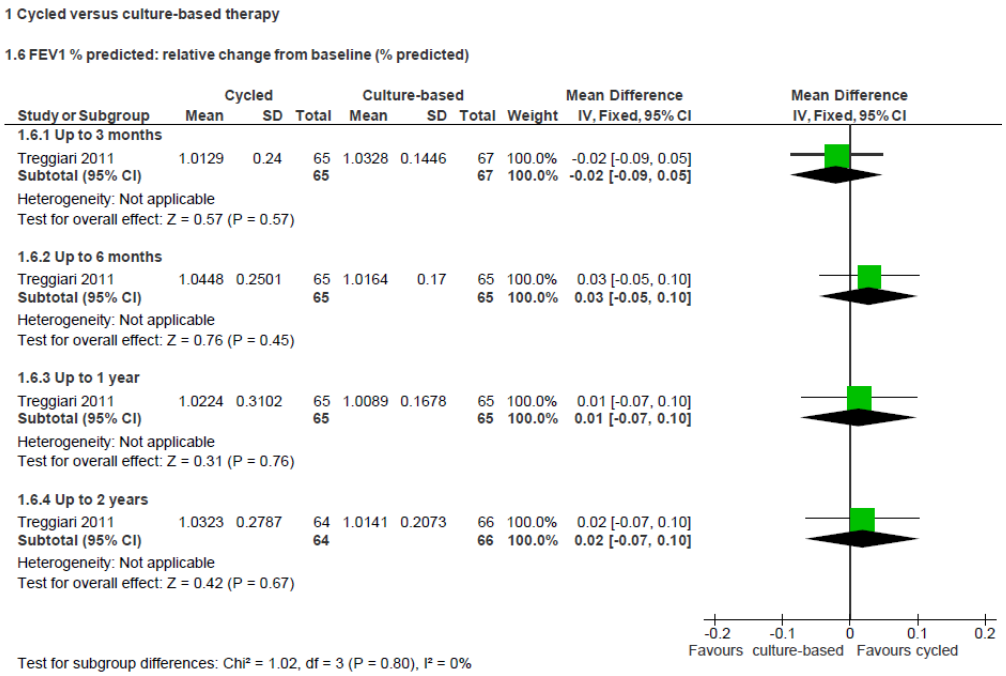


Figure 5-8: Analysis 1.6; ppFEV$_1$ relative change from baseline, comparing cycled vs culture-based therapy at up to 3 months, up to 6 months, up to 1 year and up to 2 years. Cochrane Systematic Review, Treatments to prevent recurrence of infection with Pseudomonas aeruginosa in people with cystic fibrosis.

b. FVC

The published paper does not report changes in FVC, in either litres or % predicted (204). Using the individual patient data provided we were able to analyse the mean (SD) absolute and relative change from baseline for FVC (L) at "up to three months", "up to six months", "up to one year", and "up to two years".

There was no difference in absolute change of FVC (L) from baseline (Analysis 1.7, see Figure 5-9) or relative change of FVC from baseline between the cycled and culture-based groups at any of the time points analysed (Analysis 1.8, see Figure 5-10).

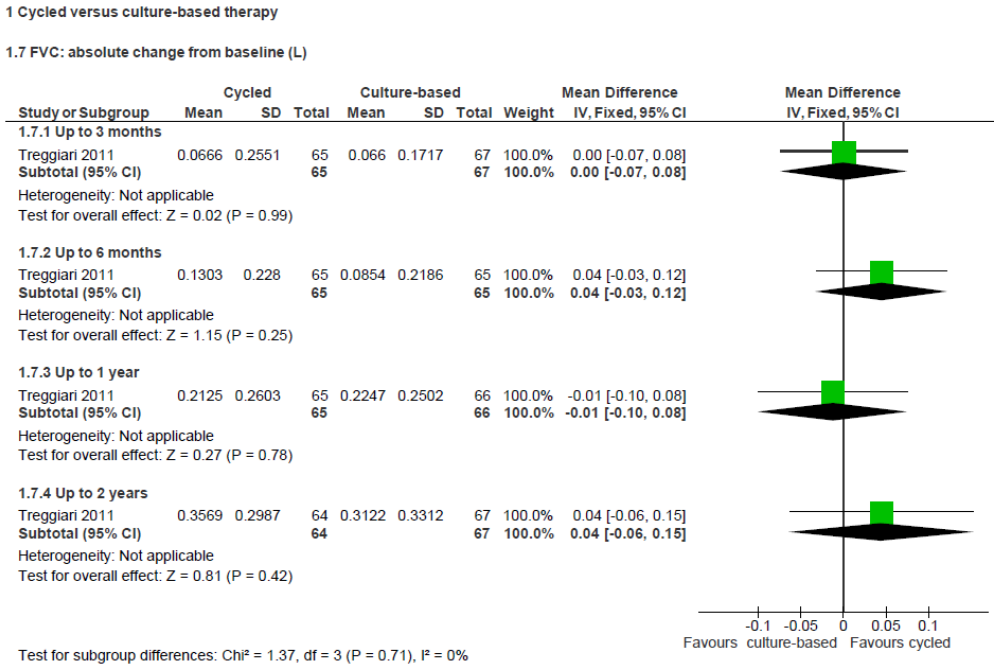


Figure 5-9: Analysis 1.7; FVC absolute change from baseline (L), comparing cycled vs culture-based therapy at up to 3 months, up to 6 months, up to 1 year and up to 2 years. Cochrane Systematic Review, Treatments to prevent recurrence of infection with Pseudomonas aeruginosa in people with cystic fibrosis.

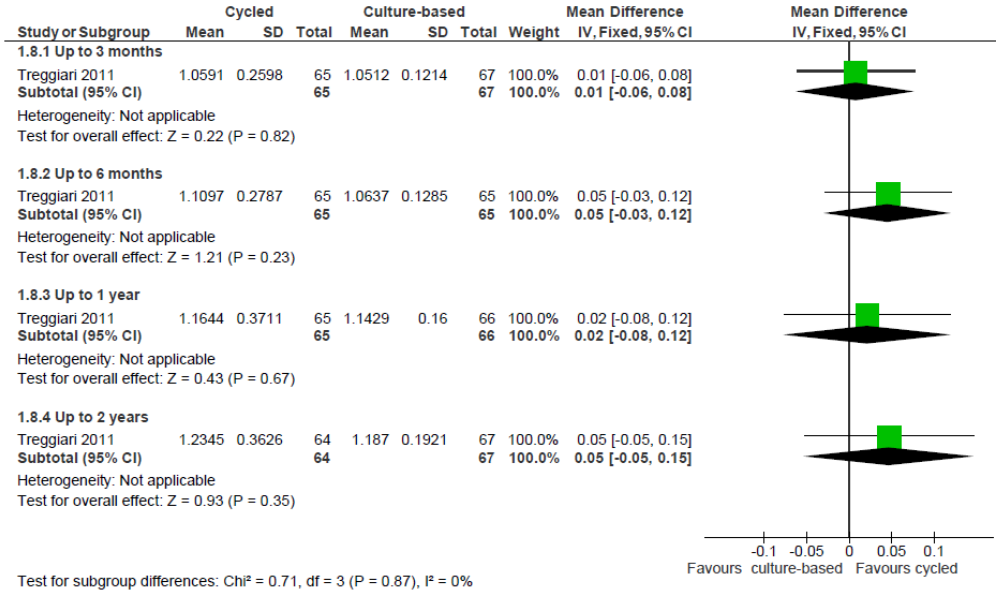


Figure 5-10: Analysis 1.8; FVC relative change from baseline (L), comparing cycled vs culture-based therapy at up to 3 months, up to 6 months, up to 1 year and up to 2 years. Cochrane Systematic Review, Treatments to prevent recurrence of infection with Pseudomonas aeruginosa in people with cystic fibrosis.

3. Pulmonary exacerbations

a. Time to next exacerbation

The primary outcome of the included trial was the time to next pulmonary exacerbation (in days) meeting their *a priori* definition and requiring either intravenous antibiotics or hospitalisation; the investigators reported no difference between cycled therapy and culture-based for the entire cohort, HR 0.95 (95% CI 0.54 to 1.66) (204).

We used the trial's secondary outcome, pulmonary exacerbation (using the study definition) requiring any antibiotic therapy (intravenous, inhaled or oral) for our definition of a pulmonary exacerbation (204). For our subset of participants data were available for 253 participants, 119 in the cycled group and 134 in the culture-based group. Four participants experienced a pulmonary exacerbation prior to commencement of trial medication and were excluded from analysis. Data were therefore assessed for 249 participants. We found no difference in the time to next pulmonary exacerbation (in days) between participants in the cycled arm and those in the culture-based arm (Analysis 1.9, see Figure 5-11). The median time to next pulmonary exacerbation was 518 days in the cycled group and 495 days in the culture-based group. The interquartile range was not calculable for either group as 75% of participants had not undergone a pulmonary exacerbation by the end of the study.

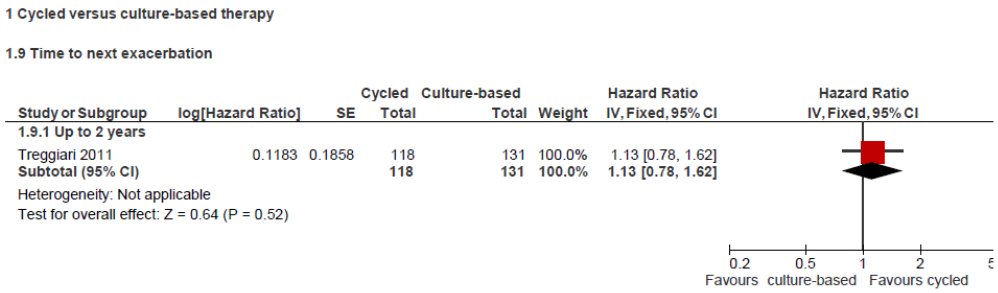


Figure 5-11: Analysis 1.9; Time to next pulmonary exacerbation (defined as a pulmonary exacerbation requiring any antibiotic therapy), comparing cycled vs culture-based therapy. Cochrane Systematic Review, Treatments to prevent recurrence of infection with Pseudomonas aeruginosa in people with cystic fibrosis.

b. *Frequency of exacerbations*

The trial reported the number of participants who experienced a pulmonary exacerbation and for our participant subset data were available for 253 participants (204). There was no difference in the number of participants experiencing a pulmonary exacerbation between groups (Analysis 1.10, see Figure 5-12). The GRADE rating of this evidence was moderate. It was downgraded once for indirectness as the results only relate to children (see Table 5-2).

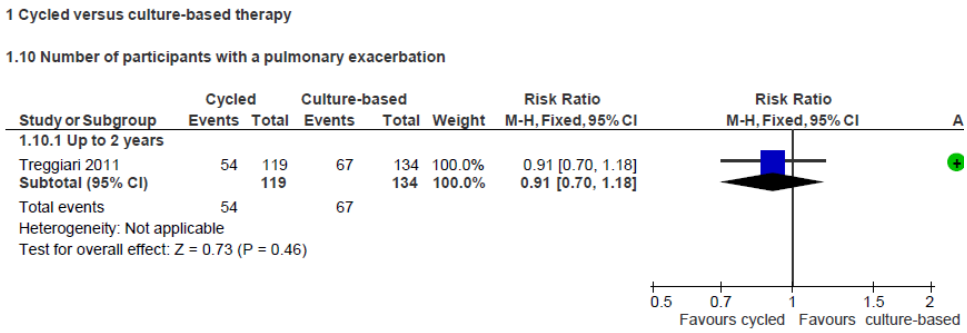


Figure 5-12: Analysis 1.10; Number of participants with a pulmonary exacerbation (defined as a pulmonary exacerbation requiring any antibiotic therapy), comparing cycled vs culture-based therapy. Cochrane Systematic Review, Treatments to prevent recurrence of infection with Pseudomonas aeruginosa in people with cystic fibrosis.

There was no difference in the frequency of exacerbations per participant between the groups (Analysis 1.11, see Figure 5-13).

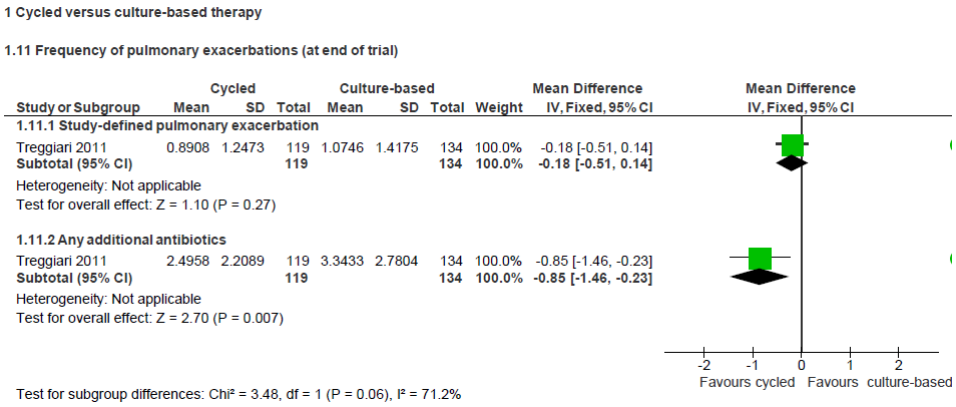


Figure 5-13: Analysis 1.11; Frequency of pulmonary exacerbations (defined as a pulmonary exacerbation requiring any antibiotic therapy) at end of trial, comparing cycled vs culture-based therapy. Cochrane Systematic Review, Treatments to prevent recurrence of infection with Pseudomonas aeruginosa in people with cystic fibrosis.

The mean (SD) frequency of additional courses of antibiotics was 2.50 (2.21) in the cycled group and 3.34 (2.78) in the culture-based group; analysis of these data clearly favoured the cycled group, MD -0.85 (95% CI -1.46 to -0.23) (Analysis 1.11, see Figure 5-13).

c. Number of days in hospital

The trial reported number of hospitalisations, but not the number of days in hospital (204).

4. Nutritional parameters

a. Weight (kg) and weight centile or Z score

The study reports the absolute mean (SD) change in weight (kg) from baseline at the end of study visit (week 70) (204) but does not report changes in weight percentile. Using the individual patient data provided we were able to analyse the mean (SD) absolute change from baseline

for weight and weight percentile at "up to three months", "up to six months", "up to one year", and "up to two years".

There was no difference at any of the time points analysed between the cycled and culture-based groups in either absolute change in weight (kg) or change in weight percentile (Analysis 1.12, see Figure 5-14; Analysis 1.13, see Figure 5-15) .

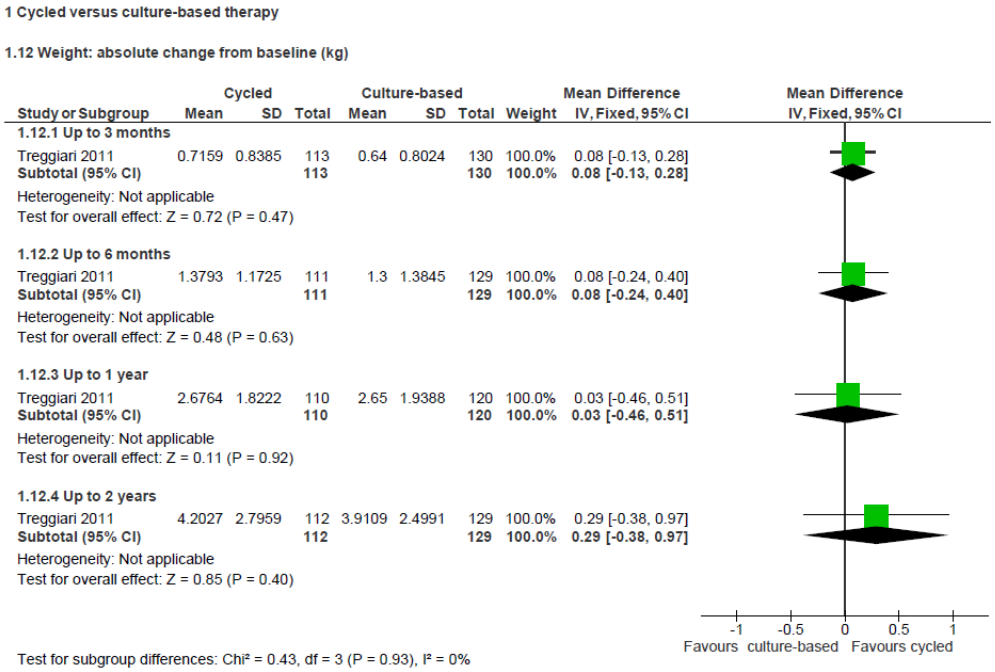


Figure 5-14: Analysis 1.12; Weight – absolute change from baseline (kg), comparing cycled vs culture-based therapy at up to 3 months, up to 6 months, up to 1 year and up to 2 years. Cochrane Systematic Review, Treatments to prevent recurrence of infection with Pseudomonas aeruginosa in people with cystic fibrosis.

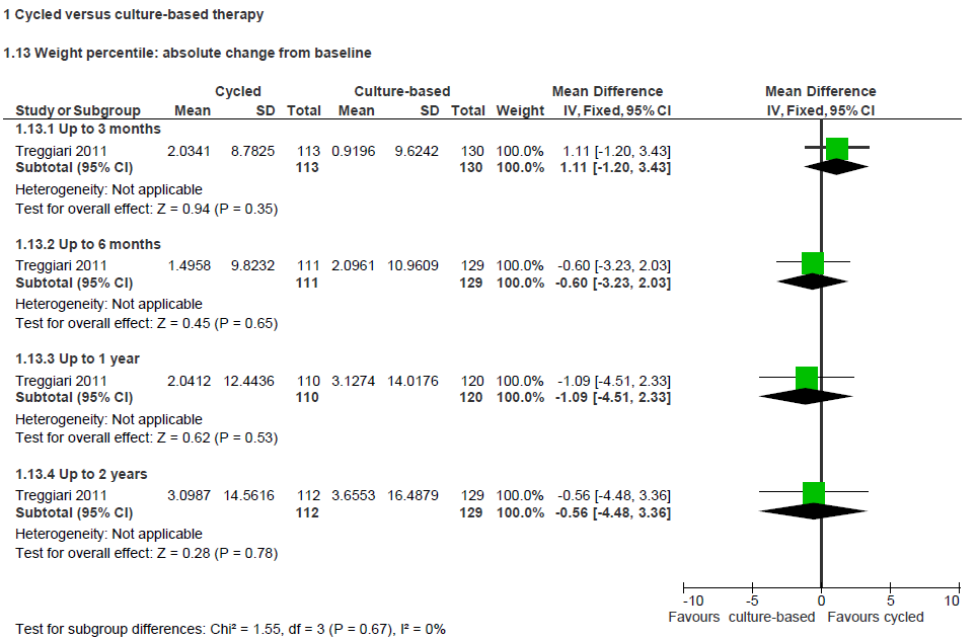


Figure 5-15: Analysis 1.13; Weight percentile – absolute change from baseline (%), comparing cycled vs culture-based therapy at up to 3 months, up to 6 months, up to 1 year and up to 2 years. Cochrane Systematic Review, Treatments to prevent recurrence of infection with Pseudomonas aeruginosa in people with cystic fibrosis.

b. Height (cm) (children) and height centile or Z score

The trial reports the absolute mean (SD) change in height (cm) from baseline at the end of study visit (week 70) (204) but does not report changes in height percentile. Using the individual patient data provided we were able to analyse the mean (SD) absolute change from baseline for height and change in height percentile from baseline at "up to three months", "up to six months", "up to one year", and "up to two years".

There was no difference in absolute change in height (cm) or change in height percentile between the cycled and culture-based groups at any of the time points analysed (Analysis 1.14, see Figure 5-16; Analysis 1.15, see Figure 5-17).

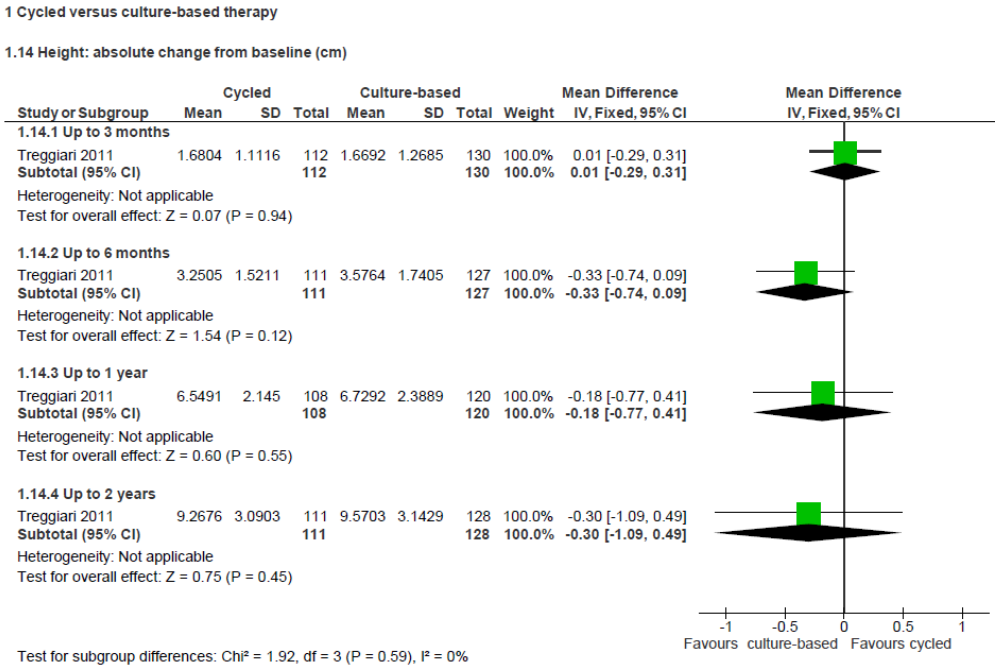


Figure 5-16: Analysis 1.14; Height – absolute change from baseline (cm), comparing cycled vs culture-based therapy at up to 3 months, up to 6 months, up to 1 year and up to 2 years. Cochrane Systematic Review, Treatments to prevent recurrence of infection with Pseudomonas aeruginosa in people with cystic fibrosis.

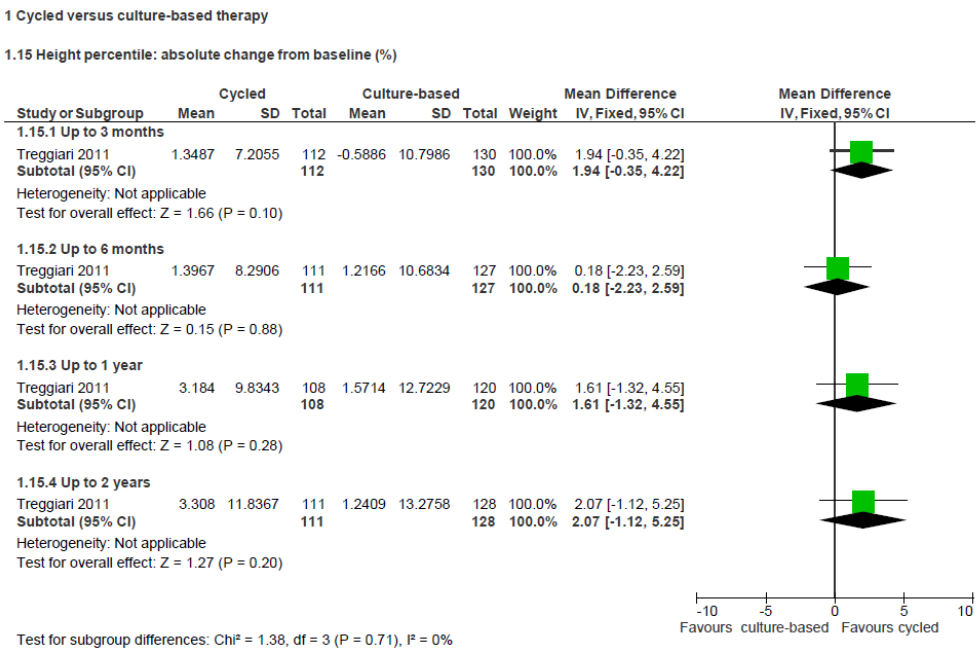


Figure 5-17: Analysis 1.15; Height percentile – absolute change from baseline (%), comparing cycled vs culture-based therapy at up to 3 months, up to 6 months, up to 1 year and up to 2 years. Cochrane Systematic Review, Treatments to prevent recurrence of infection with Pseudomonas aeruginosa in people with cystic fibrosis.

c. BMI and BMI centile

This outcome was not reported in the included trial (204).

5. Time to chronic P. aeruginosa infection

This outcome was not reported in the included trial (204).

6. Adherence to treatment

a. Self-reported measures

This outcome was not reported in the included trial and data were not available to calculate the results (204).

b. Secondary count measures

Adherence was measured by counting returned medication (204). In the published paper there is a comment that, "compliance with study mediations [was] 90% or greater across treatment groups," but no data are presented. Using the individual patient data provided we were able to analyse the number of participants returning any study medication and the number of participants returning tobramycin at "up to three months", "up to six months", "up to one year", and "up to two years".

There was no difference between the cycled and culture-based groups in the percentage of participants returning any trial medication at any of the time-points analysed (Analysis 1.16, see Figure 5-18) or in the proportion of participants returning tobramycin at any of the time-points analysed (Analysis 1.17, see Figure 5-19).

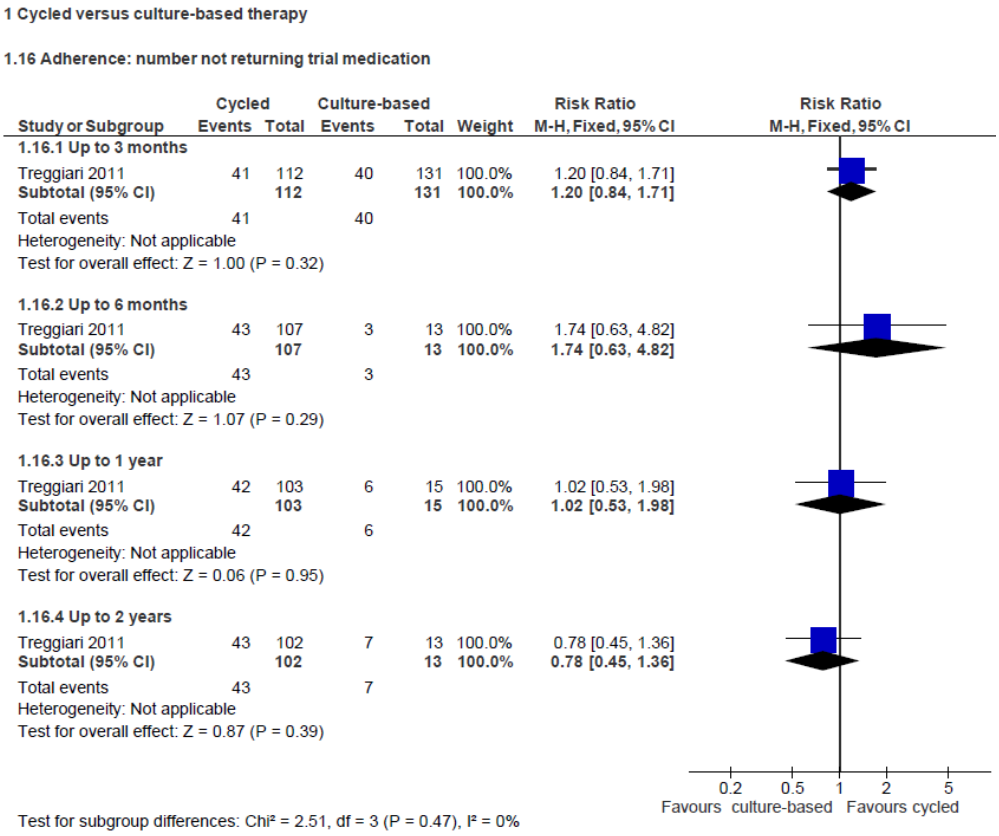


Figure 5-18: Analysis 1.16; Adherence – number of participants not returning any trial medication, comparing cycled vs culture-based therapy at up to 3 months, up to 6 months, up to 1 year and up to 2 years. Cochrane Systematic Review, Treatments to prevent recurrence of infection with Pseudomonas aeruginosa in people with cystic fibrosis.

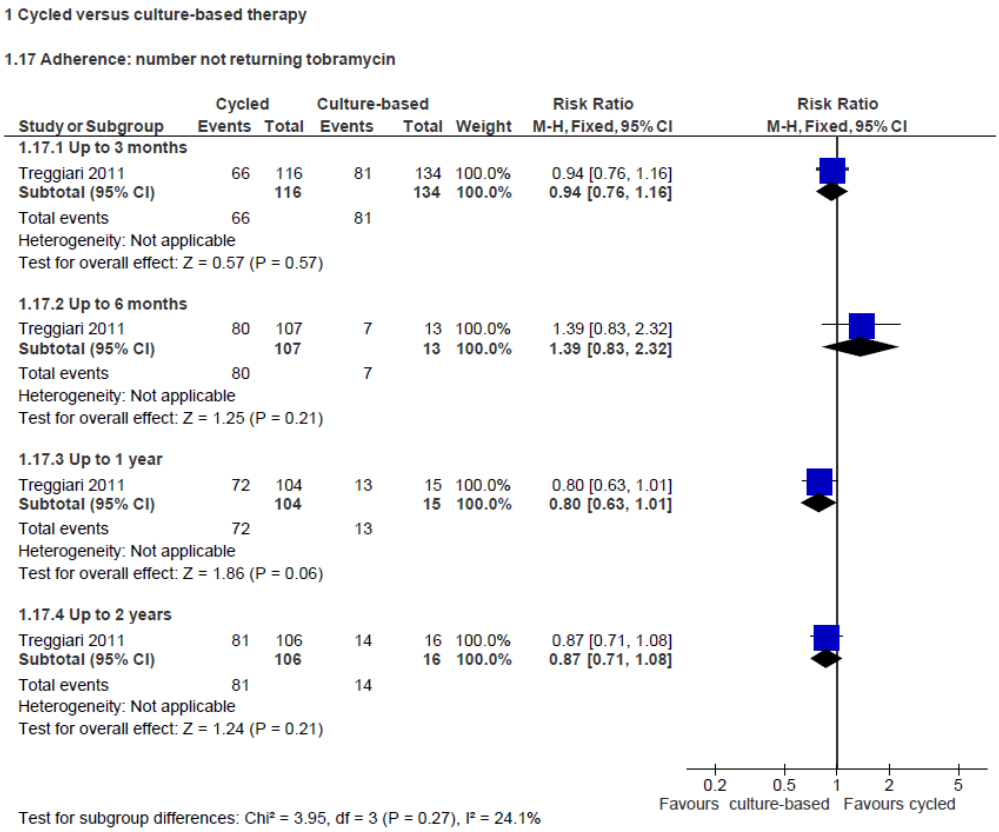


Figure 5-19: Analysis 1.17; Adherence – number of participants not returning any tobramycin, comparing cycled vs culture-based therapy at up to 3 months, up to 6 months, up to 1 year and up to 2 years. Cochrane Systematic Review, Treatments to prevent recurrence of infection with Pseudomonas aeruginosa in people with cystic fibrosis.

c. Electronic data

These data were not reported in the included trial (204).

7. Adverse effects of treatment

The published trial report only reported rates of serious adverse events; they reported no difference in the rate of these between intervention groups (204).

a. Mild

Mild adverse events were not reported in the included trial (204).

b. Moderate

Moderate adverse events were not reported in the included trial (204).

c. Severe

A total of 49 out of 253 (19.4%) participants in our subset experienced a severe adverse event during the course of the trial; this was not significantly different between the cycled and culture-based groups (Analysis 1.18, see Figure 5-20). There was no significant difference in the rate of severe adverse events in any of the categories studied (respiratory, infection, gastro-intestinal, metabolism, musculoskeletal, nervous system, skin or other) (Analysis 1.18, see Figure 5-20). The GRADE rating for this evidence was moderate; it was downgraded once for indirectness since the participants were all children (see Table 5-2).

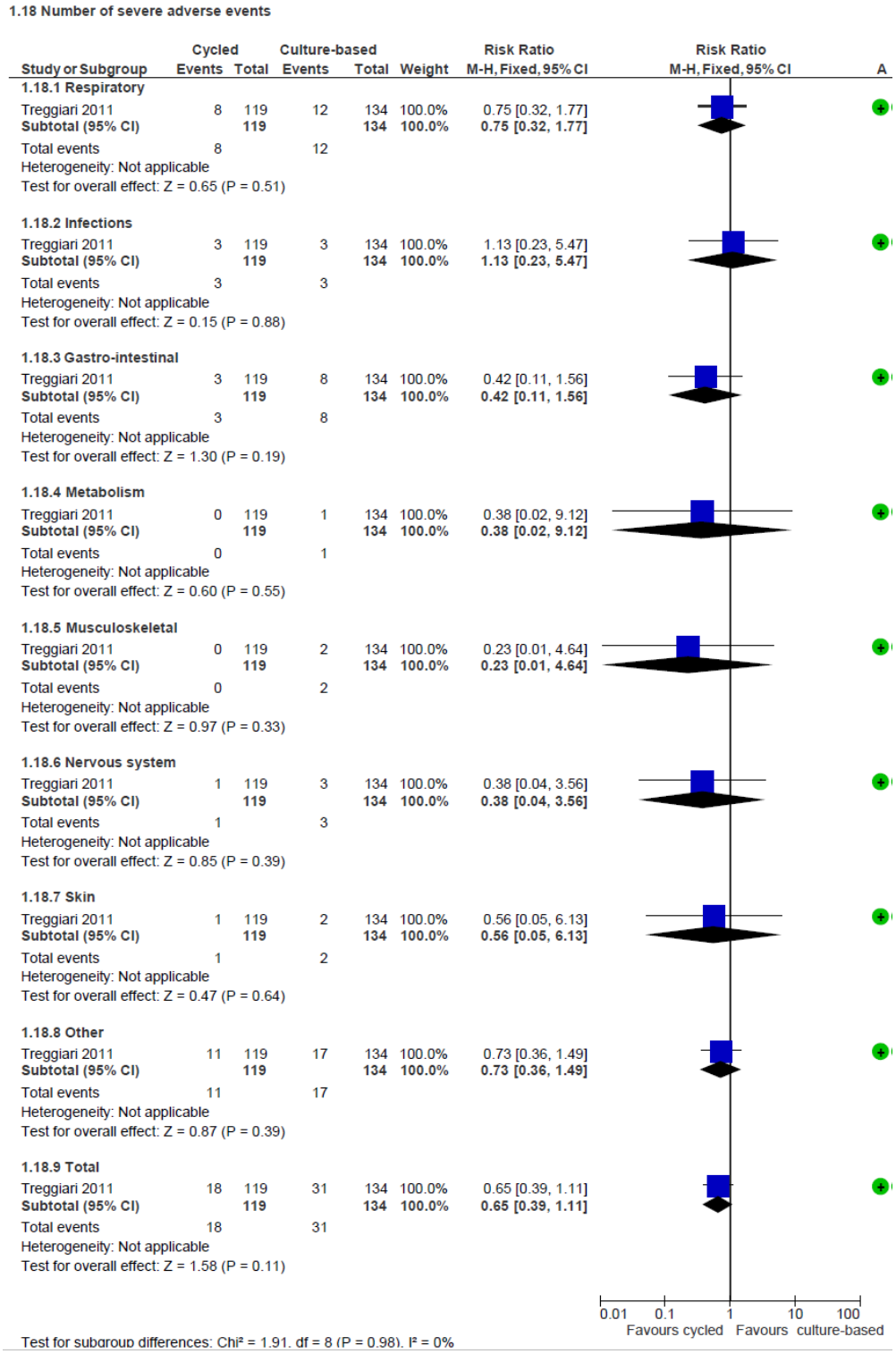


Figure 5-20: Analysis 1.18; Number of severe adverse events by body system and in total, comparing cycled vs culture-based. Cochrane Systematic Review, Treatments to prevent recurrence of infection with Pseudomonas aeruginosa in people with cystic fibrosis. (See overleaf).

8. *Mortality*

This outcome was not reported in the included trial (204).

9. *Isolation of resistant bacteria*

a. *P. aeruginosa with a new resistance pattern or novel mucoid P. aeruginosa*

The trial reported on the emergence of *P. aeruginosa* newly resistant to ciprofloxacin or tobramycin and novel identification of mucoid *P. aeruginosa* (204). The included subset of participants were sampled at six time points after the visit 2 sample, which defined inclusion in our dataset. The 119 participants in the cycled arm provided a median (range) of six (zero to six) samples; in the culture-based arm 134 participants provided a median (range) of six (zero to six) samples.

There was no significant difference in the rate of detection of *P. aeruginosa* newly resistant to either ciprofloxacin or tobramycin between the cycled and culture-based groups (Analysis 1.19, see Figure 5-21).

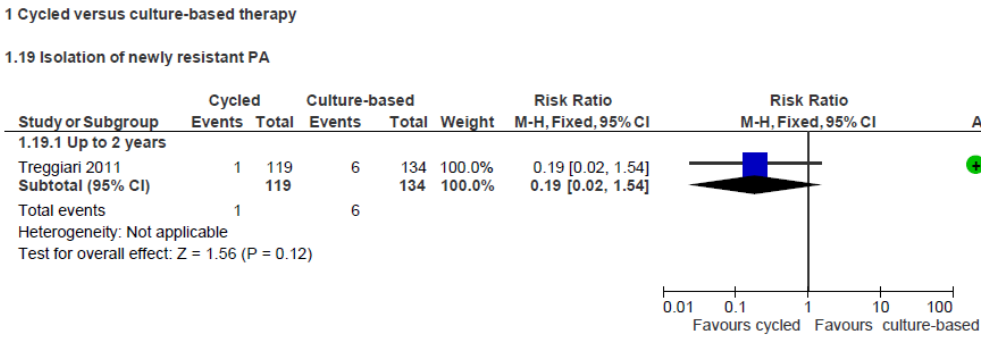


Figure 5-21: Analysis 1.19; Isolation of newly resistant P. aeruginosa, comparing cycled vs culture-based. Cochrane Systematic Review, Treatments to prevent recurrence of infection with Pseudomonas aeruginosa in people with cystic fibrosis.

Isolation of novel mucoid *P. aeruginosa* was not significantly different between the groups (Analysis 1.20, see Figure 5-22).

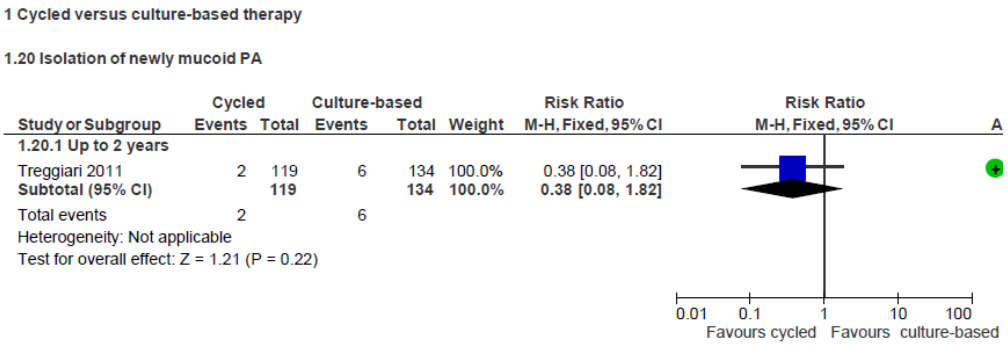


Figure 5-22: Analysis 1.20; Isolation of newly mucoid P. aeruginosa, comparing cycled vs culture-based. Cochrane Systematic Review, Treatments to prevent recurrence of infection with Pseudomonas aeruginosa in people with cystic fibrosis.

b. MRSA

The included trial reported the novel identification of *Staphylococcus aureus*, but this was not further specified as methicillin-sensitive *S. aureus* (MSSA) versus methicillin-resistant *S. aureus* (MRSA) (204).

c. Resistant gram-negative organisms

The trial reported on the emergence of novel *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans* and *Burkholderia cepacia* complex (204). The included subset of participants were sampled at six time points after the visit two sample which defined inclusion in the trial. In the cycled arm, 119 participants provided a median (range) of six (zero to six) samples and in the culture-based arm 134 participants provided a median (range) of six (zero to six) samples. There was no significant difference between groups (Analysis 1.21, see Figure 5-23). The GRADE rating for this evidence was moderate. It was downgraded once for indirectness (see Table 5-2).

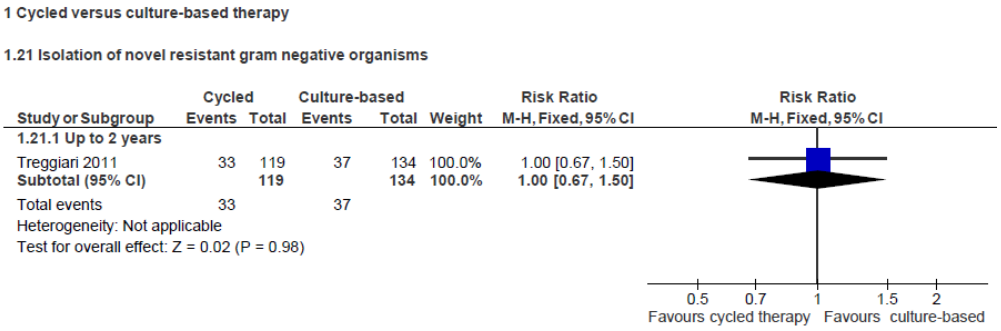


Figure 5-23: Analysis 1.21; Isolation of novel resistant gram-negative organisms, comparing cycled vs culture-based. Cochrane Systematic Review, Treatments to prevent recurrence of infection with Pseudomonas aeruginosa in people with cystic fibrosis.

d. Other novel organisms

The trial did not report on the emergence of any other novel pathogens (204).

10. Cost effectiveness

This outcome was not reported in the included trial (204).

5.6 Discussion

Only one trial which fulfilled our inclusion criteria was identified and only a subset of the 306 participants enrolled in this trial were eligible (204). Individual patient data was obtained for 300 participants, of whom 253 were eligible to be included in our review.

5.6.1 Summary of main results

Additional treatment with inhaled tobramycin every three months (cycled therapy) following eradication of *P. aeruginosa* doubled the time to recurrence of *P. aeruginosa* in a subset of participants from the EPIC study compared to culture-based therapy (204) (Analysis 1.1, see Figure 5-3). This was not affected by changing the definition of

eradication (Analysis 1.2, see Figure 5-4). We deemed the quality of the evidence for our primary outcome to be moderate, suggesting there is probably an improvement in time to recurrence after cycled therapy. Development of newly resistant *P. aeruginosa* and isolation of newly resistant organisms were slightly higher in the culture-based group compared to the cycled group, but this result was not statistically significant. There was no evidence of a difference between the treatment groups in terms of pulmonary function, time to next pulmonary exacerbation, severe adverse events, nutritional parameters, adherence, isolation of newly mucoid *P. aeruginosa* or novel resistant gram-negative organisms. The quality of evidence for secondary outcomes was moderate according to GRADE criteria with the exception of pulmonary function which was deemed to be very low meaning that we are uncertain whether there is an effect. The trial did not report on Quality of Life, time to chronic *P. aeruginosa* infection, mortality or cost effectiveness (204).

In the published report of this trial, the authors concluded that there was no difference between cycled and culture-based therapy in the rate of pulmonary exacerbation or *P. aeruginosa* positivity (204). This is in contrast to the findings of our review, where we found that the time to next isolation of *P. aeruginosa* was doubled by the provision of cycled TIS therapy. The difference in these findings could be due to the fact that only a subset of the trial's enrolled participants were eligible for inclusion in our review; although the baseline characteristics of our subset were similar to those of the whole cohort described in the main

trial report, except that the percentage of participants positive for *P. aeruginosa* at trial baseline in the cycled group was only 27.4% in our subset, compared to 38% in the full cohort. Another explanation could be the different question posed by this review compared to the main trial, namely the time to next isolation of *P. aeruginosa* rather than the rate of *P. aeruginosa* positivity in each group (204). An additional consideration is that the results in the main trial report were age-adjusted, which is not the case for the results presented here.

5.6.2 Overall completeness and applicability of evidence

The included trial only included children up to 12 years of age (204). Eradication can be successful in adults with CF (208); the age at onset of chronic *P. aeruginosa* infection is increasing (665) and the percentage of individuals with a *P. aeruginosa* positive culture is decreasing (666). Strategies to prevent the recurrence of *P. aeruginosa* after successful eradication are therefore important for all age groups, but the findings of this review cannot be directly applied to adolescents and adults with CF. The paucity of trials also meant that we were not able to comment on some of our outcomes (changes in Quality of Life, time to chronic *P. aeruginosa* infection, mortality and cost-effectiveness).

The trial allowed inclusion of participants whose *P. aeruginosa*-positive qualifying sample occurred up to six months prior to the baseline visit. Participants could have undergone up to one course of eradication therapy prior to enrolment. This may have had an effect on the efficacy

of the intervention, either biasing it towards participants who had previously undergone eradication or conversely biasing it towards participants with only a short lag between *P. aeruginosa* identification and the commencement of treatment. Results from the EARLY trial suggest that a delay in the commencement of eradication therapy for *P. aeruginosa* negatively affects the chance of success (663).

The included trial followed-up participants for a median of 494 days. Up to this point there was no evidence of difference between the two groups (cycled therapy or culture-based therapy) in terms of clinical parameters (lung function, nutritional parameters, time to next pulmonary exacerbation), thus although *P. aeruginosa* recurrence was delayed in the cycled group, this was not translated into objective clinical benefit. Chronic *P. aeruginosa* infection is associated with increased morbidity and mortality, but it may be that longer follow-up is required to truly appreciate whether cycled therapy has a clinically apparent benefit.

It is also important to note that the included trial does not directly address the question posed in this review (204). Treggiari *et al.* aimed to compare prolonged eradication regimens to reduce the chance of pulmonary exacerbation after the acquisition of *P. aeruginosa*. Evidence of eradication was not a requirement in the trial protocol prior to the commencement of the trial. We therefore had to use a proxy measure of eradication and could only include a subset of participants from the trial, which reduces the applicability of the evidence to this review question.

5.6.3 Quality of the evidence

We have identified only one trial to include in this review and examined data from 253 participants (204). The included trial was adequately powered to identify differences in its primary outcomes, however, by excluding participants who did not fulfil the inclusion criteria for this review, these results may now be underpowered to show a difference. The risk of bias was deemed to be low across all domains with the exception of 'other bias'. Participants could be enrolled up to six months after a *P. aeruginosa* positive culture and could have had up to one course of eradication therapy during that time. This may have had an effect on the efficacy of the intervention, either biasing it towards participants who had previously undergone eradication or conversely biasing it towards participants with only a short lag between *P. aeruginosa* identification and the commencement of treatment. We have therefore stated that there is an unclear risk of other bias.

The nature of the trial as a four-way factorial trial meant that participants in the cycled and culture-based therapy groups did not receive equal treatment. In our population subset 53.8% of participants in the cycled group and 52.2% of participants in the culture-based group received oral ciprofloxacin in addition to their inhaled tobramycin. Breaking down the analysis into four groups would further reduce the numbers available for each comparison.

Since this trial was conducted in young children many were unable to expectorate sputum. Most respiratory sampling in the trial relied on oropharyngeal swab cultures and the reliability of oropharyngeal

cultures to reflect the microbial populations of the lower respiratory tract is limited (667). This may have caused false positive and false negative results in the sampling, resulting in detection bias, though it is likely that this bias would be equally applied to both groups. Ongoing treatment with anti-Pseudomonal antibiotics theoretically may have a suppressive effect on the growth of *P. aeruginosa* such that it could not be detected, particularly in OP cultures. However, the interval of treatment makes this less likely since the sputum density of *P. aeruginosa* increased almost back to pre-treatment levels during off-treatment intervals in a study of intermittent tobramycin administration in participants chronically infected with *P. aeruginosa* (79).

Using GRADE, we found that the overall quality of the evidence varied from very low to moderate across the outcomes presented in the summary of findings table (see Table 5-2). We deemed the quality of the evidence for our primary outcome to be moderate suggesting there is probably an improvement in time to recurrence after cycled therapy though, since the study only included children, we cannot be certain if the results would be reproducible in an adult population. The quality of evidence for secondary outcomes was moderate, with the exception of pulmonary function which was deemed to be very low due to the fact that younger children were unable to perform spirometry and so there was imprecision in the results from low participant numbers and missing data.

5.6.4 Potential biases in the review process

A potential source of bias in the review process is the lack of a formal definition of eradication in CF clinical trials. The included trial did not formally assess the success of initial eradication therapy; however, a sample was obtained three weeks after therapy commenced. We have therefore defined eradication as a negative *P. aeruginosa* sample at this three-week time point since a later cut-off would add another treatment variation for participants who underwent 56 rather than 28 days of TIS. However, we note that since this sample was taken whilst treatment was ongoing, suppression of *P. aeruginosa* (rather than true eradication) may have occurred. Indeed, in discussion with the authors of the ELITE trial (205), which we had anticipated including, it became evident that for this reason they did not send microbiological samples after the first 28 days of treatment.

5.6.5 Agreements and disagreements with other studies or reviews

We are not aware of any other studies or reviews which recommend strategies to prevent recurrence of *P. aeruginosa* following successful eradication.

However, it is of note that a previous Cochrane Review examining antibiotic strategies for eradicating *P. aeruginosa* in people with CF, also found that cycled therapy was superior to culture-based therapy (208). The authors of that review used different primary outcomes to

ours and did not correct for participant age in their analyses, which may account for some of the differences found.

5.7 Authors' Conclusions

5.7.1 Implications for practice

From the limited evidence available cycled therapy appears to delay the recurrence of *P. aeruginosa* after eradication in children. Further study is required to better understand the benefits of cycled therapy in adolescents and adults with cystic fibrosis (CF).

The burden of additional nebulised therapy needs to be weighed against its efficacy. We were unable to examine the effect of cycled therapy compared to culture-based therapy on treatment burden as quality of life was not measured. Adding 28 days of nebulised therapy every three months for 15 months may seem a small addition to a therapeutic regimen, but we should bear in mind that the addition of nebulised therapy is often cited by people with CF as having a large impact on treatment burden (220).

5.7.2 Implications for research

Now that eradication of *P. aeruginosa* is commonplace in the management of CF, further consideration needs to be made in terms of the next steps. We suggest that randomised controlled trials, specifically dedicated to investigating the prevention of *P. aeruginosa* recurrence are needed. These trials must be conducted in participants of all ages with CF. Treatments other than antimicrobial agents, which

reduce the chance of recurrence by improving host defence or exposure to the organism, could also be considered.

Success in treating infection in CF may necessitate the use of surrogate markers, as evidenced by Treggiari *et al.* (204). In this study, the median time to recurrence of *P. aeruginosa* could not be determined in a trial with a planned 15-month follow-up; time to chronic *P. aeruginosa* would require even longer follow-up. Time to next pulmonary exacerbation may be the most pragmatic outcome and would facilitate comparisons with other CF pathogens. However, as discussed above, the use of time to exacerbation as the primary outcome measure meant that the study reported no difference in the two treatment groups, whilst we have shown a hazard ratio of 2.04 in the time to recurrence of *P. aeruginosa* for those undergoing culture-based therapy compared to cycled therapy.

In addition to time to chronic *P. aeruginosa* infection and time to next pulmonary exacerbation, outcomes to be included in future trials of interventions to prevent *P. aeruginosa* recurrence should include lung function, nutritional parameters, cost-effectiveness and quality of life, including treatment burden.

Since delaying the onset of chronic *P. aeruginosa* infection may reduce the longer-term morbidity and mortality of CF, assessing these outcomes would require longer durations of observation than are feasible in a traditional RCT. We therefore suggest consideration of

alternative trial designs such as registry-based trials may be the best way to examine these outcomes.

These trials need to use internationally standardised definitions for:

1. chronic *P. aeruginosa* infection;
2. pulmonary exacerbations;
3. eradication of *P. aeruginosa*.

Development and widespread use of these definitions will improve the comparability of trials, strengthening the conclusions which can be drawn when they are compared and would form part of a core outcome set for CF. The importance of a core outcome set to reduce selective reporting bias and thereby improve the validity of systematic review in CF has previously been highlighted (668). The results of the Core Outcome Set Taskforce for CF (COST-CF) will be an important development in the care of people with CF (669).

5.8 Conclusions

Secondary prevention of recurrent *P. aeruginosa* infection is an important therapeutic strategy in the prevention of chronic *P. aeruginosa* and therefore an important step in reducing the harm caused by *P. aeruginosa* in people with cystic fibrosis.

Currently there is insufficient evidence to suggest the best therapeutic regimens for effective secondary prevention strategies though cycled inhaled tobramycin shows some promise. Further research is needed

to answer this question and the results of the OPTIMIZE trial (418) and the trial of IgY (641) will provide valuable additional evidence.

Chapter 6: An evaluation of the High Frequency Digit Triplet test as a screening tool for early hearing loss for adults and young adults with CF at a time of clinical stability.

This work was funded by an NIHR RfPB grant, PB-PG-0213-30055.

Details of the contributions made by colleagues in the preparation of this chapter are given in Table 6-1.

Study Stage	**Contributors**
Preparing the grant	Prof. Alan Smyth Dr Heather Fortnum Dr Melanie Ferguson Mr Mark Edmondson-Jones Dr Edward Nash Dr Jane Clarke Dr Jane Dewar Dr Robert MacKinnon Mrs Zoe Elliot
Writing the protocol	Dr Sally Palser Prof. Alan Smyth Dr Heather Fortnum Dr Melanie Ferguson Mr Mark Edmondson-Jones Dr Edward Nash

	Dr Jane Clarke Dr Jane Dewar Dr Robert MacKinnon Mrs Zoe Elliot
Identifying potential participants	Dr Sally Palser CF teams at Nottingham University Hospitals NHS Trust, West Midlands Adult CF Centre and Birmingham Childrens' Hospital
Recruitment	Dr Sally Palser Prof Alan Smyth Ms Sana Anwar
Data collection	Dr Sally Palser Ms Sana Anwar
Statistical analysis plan	Mr Mark Edmondson-Jones Dr Polly Scutt

Table 6-1: A summary of the contributions made by colleagues to assist in the production of this work.

I was employed as the research fellow on the NIHR grant which had already been awarded when I started the project. I wrote the first and final drafts of the protocol, amended this as necessary and prepared all the study documents. I prepared the ethics committee submission and all amendments. I researched the most appropriate equipment and decided what to purchase, in conjunction with my supervisor. I

identified potential participants, provided information sheets in advance by post and discussed the study in clinic. I consented most of the patients and conducted all the hearing tests. I collected all the additional data from the patient's records, corresponded with the GP and referred participants for formal hearing testing when necessary. I co-ordinated the study steering group and the patient and public involvement (PPI) group. I designed the Microsoft Access database and did primary input of all the data. I analysed the results in light of the statistical analysis plan and interpreted them in context.

6.1 Introduction

Hearing loss prevalence in the CF population has been variably estimated between 1 and 51% (273). A calculated weighted mean using data from Prayle *et al.* (2010) (273) estimated the prevalence of hearing loss in UK CF patients at 27%. When EHF- hearing is considered these numbers may be higher (see Table 6-2).

Study	Condition	Participants	HL definition	Prevalence of hearing loss
Mulheran 2001 (366)	CF	43 adults 27 children	2 frequencies ≥ 20dBHL or 1 ≥ 25dB HL (0.25-16 kHz)	12/70 (17%)
Harruff 2021 (363)	CF	18 adults	Any frequency > 15 dB HL (0.25-16 kHz)	Standard PTA: 7/18 (39%) EHF-PTA: 13/18 (72%)
Garinis 2021 (670)	CF	31 adults	Any frequency >25 dB HL (0.25-16 kHz)	Standard PTA: 6/31 (19%) EHF-PTA: 18/31 (58%)
Al-Malky 2015 (145)	CF	70 children aged 4-16	≥20 dB HL at ≥1 frequency (0.25-16 kHz)	Standard PTA: 13/70 (18.6%) EHF-PTA: 15/70 (21.4%)
Blankenship 2021 (314)	CF	57 adults and children aged 6-21 years	Any frequency > 15 dB HL (0.25-16 kHz)	Standard PTA: 38% EHF-PTA: 47%
Kreicher 2018 (671)	PCD, situs inversus and	42 patients with HL and one of these	Any frequency > 15 dB HL (0.5-8 kHz)	SNHL: 2/70 ears (2.9 %)

	Kartagener's syndrome	conditions, in the AudGen database		
Alexandru 2022 (672)	PCD/ bronchiectasis	17 adults with PCD and 17 adults with bronchiectasis	Any frequency > 15 dB HL (0.5-4 kHz)	SNHL (PCD): 3/17 (18%) SNHL (bronchiectasis): 2/17 (12%)
Owusu 2022 (673)	Drug-resistant TB	TB patients treated with AGS and healthy controls	Any frequency > 25 dB HL (0.5-4 kHz)	TB patients: 12/60 (20%) Control patients: 0/60 (0%)

Table 6-2: Prevalence of hearing loss in studies of CF, other causes of bronchiectasis and other groups treated with long-term aminoglycoside antibiotics. PTA, pure tone audiogram; EHF-PTA, extended high frequency pure-tone audiogram; dB HL, decibels hearing loss; PCD, primary ciliary dyskinesia; SNHL, sensorineural hearing loss; AGS, aminoglycosides.

Hearing loss is common in the general population. The World Report on Hearing, 2021 (674), estimates that hearing loss affects 1 in 5 of the world's population. Prevalence increases with increasing age (675), affecting 2.0% of 20-year-olds and 12.7% of 60-year-olds, with more than half of those with hearing loss aged over 60 (674). Evidence for ototoxicity in people with non-CF bronchiectasis is lacking; hearing loss is common in those with primary ciliary dyskinesia (PCD), but this is more commonly related to conductive hearing loss (CHL) (671, 672). Aminoglycoside ototoxicity is common in patients treated for tuberculosis (TB), affecting 20-40% (673, 676). In children treated with aminoglycosides for a variety of conditions the prevalence of ototoxicity varies from 0-57%. Comparisons between the rates of hearing loss between CF and other conditions are difficult due to inconsistent definitions (see Table 6-2).

As described in section 2.7.1, people with CF face additional risk to their hearing as a consequence of the ototoxic effects of medications including aminoglycoside antibiotics (356, 363). It is reasonable to suppose that the prevalence of hearing loss in the CF population will vary with age as the cumulative exposure to multiple hearing insults increases.

Currently the gold standard for hearing testing is the Pure Tone Audiogram (PTA). This tests the ability to perceive, and respond to, pure tones in the frequencies 0.25-8 kHz. Testing requires specialist personnel and equipment and is done in a sound-proofed booth. Practically, for people with CF, this means a hearing test requires an

additional clinic appointment. Current CF guidance in the UK advises clinical review of patients every two to three months; for unwell patients this may be more frequent (412). Increases in hospital attendance for intravenous antibiotics have a negative impact on patients' burden of treatment (see section 3.4.1). It is likely therefore that additional clinic appointments for audiometry would be problematic, interfering with school and work attendance.

Additionally, there is increasing evidence that loss in the extended high frequencies (above 8 kHz) may be more important than previously appreciated, in the ability to identify speech sounds within background noise (333, 335, 338). Difficulties with speech-in-noise hearing is a commonly expressed concern in those seeking assistance for hearing loss (333). Since drug-induced ototoxicity (e.g. aminoglycosides) targets the higher frequencies initially (677-679), with subsequent progression to lower frequencies, testing specific to the higher frequencies may be more effective at identifying early hearing loss (145, 405, 680). Identification of early hearing loss may allow modification of treatment protocols to reduce exposure to ototoxic medications with the aim of reducing progression.

Speech-in-noise tests are increasingly used as adjuncts to hearing testing and in the assessment of hearing amplification effectiveness (333). Speech words, which may be digits (406), consonant-vowel-consonant (CVC) words (681) or sentences (682), are presented within a masking noise. The ratio of the loudness of the speech and masking noise, termed the signal-to-noise ratio (SNR) varies adaptively to

identify the level at which 50% of the speech can be correctly identified, termed the speech reception threshold (SRT). These tests have been developed as internet- (407, 683) and telephone-based (406) tests which can be used to screen for hearing loss with subsequent formal testing only for those with an identified loss.

The High-Frequency Digit Triplet (HFDT) test was designed and validated to screen specifically for noise-induced hearing loss (319). The masking noise was developed using a low-pass filter at 1.5 kHz, meaning sounds below this threshold are selectively masked leaving respondents reliant on sounds within the higher, unmasked, frequencies (see section 2.9). This test is computer-based and needs no special equipment or training to administer. It could therefore be performed during a routine CF clinic appointment with onward referral to specialist hearing services only required for those scoring below the cut-off value.

We conducted a study, the 3D-CF study, to assess the validity of the HFDT test as a screening tool for early hearing loss in a CF population. This study was conducted between February 2015 and January 2019.

6.2 Methods

6.2.1 Participants

Participants were recruited from four CF Centres in the UK, two adult and two paediatric. The two adult centres were the West Midlands Adult CF Centre (WMACFC) and the Wolfson CF Centre, Nottingham

(WCFC, part of Nottingham University Hospitals NHS Trust). The two paediatric centres were Birmingham Children's Hospital (BCH) and Nottingham Children's Hospital (NCH, also part of Nottingham University Hospitals NHS Trust).

Potential participants were identified by the clinical team and sent a patient information sheet tailored to their age in the weeks leading up to a routine clinic appointment. Discussion with potential participants by a member of the research team (SP) occurred at clinic; consent and testing were carried out at this appointment, or a future session arranged where this better suited the potential participant. Patients who declined to take part were not contacted further; those who did not wish to participate at the time but were happy to re-consider in future were contacted again prior to future appointments.

Potential participants were eligible if they:

- Were aged 11 years old or over,
- Had a diagnosis of CF confirmed by sweat test or genetic testing and
- Provided written, informed consent (or assent with parental consent).

All participants provided written, informed consent (or assent with parental consent if under 16). The study was approved by Nottingham 2 Research Ethics Committee (14/EM/1199) and by the local research and innovation team at each study site.

6.2.2 Primary and secondary outcomes

The primary outcomes measured were the validation of the HFDT as a screening tool for early hearing loss in people with CF and the prevalence of hearing loss in a CF population.

Secondary outcomes included the prevalence of extended-high frequency (EHF)-hearing loss, the relationship between hearing loss and aminoglycoside use and whether there is a learning effect for the HFDT in this population.

6.2.3 Definitions

Hearing loss in the standard frequencies was defined as two or more thresholds above 20 dB HL, in one or both ears, in the frequencies 2-8 kHz as described by Mulheran *et al*. 2006 (362) when tested using a standard audiogram (i.e., 2, 3, 4, 6 and 8 kHz). This definition was chosen as it focuses on high frequency hearing. A comparison was made to the British Society of Audiology (BSA) standard definition of hearing loss; a mean threshold of >20 dB HL over 0.25, 0.5, 1, 2 and 4 kHz (404).

EHF hearing loss is defined as one or more frequency in the range 9-16 kHz in one or both ears > 20 dB HL as defined by the International Organisation for Standardisation (ISO 389-5: 2006) (684).

Aminoglycoside exposure was grouped into no exposure (no aminoglycoside courses), low exposure (<10 lifetime courses of

aminoglycosides) and high exposure (≥10 lifetime courses of aminoglycosides (364).

Paediatric participants were defined as those still under the care of a paediatric centre (i.e., had not transitioned to adult CF care).

Middle ear disease was defined as a red, bulging tympanic membrane, in which case tympanometry was not attempted, or the presence of a flat tympanogram.

6.2.4 Test procedure

Testing was carried out in quiet clinic rooms (WCFC, WMACFC) or in dedicated research rooms (BCH, NCH, WMACFC). Demographic data and details of baseline health were collected, and a questionnaire assessing for other potential causes of hearing loss completed. Participants were categorised into 0, ≤10 or >10 lifetime courses of aminoglycosides by case note review. A summary diagram of the testing procedure is shown in Figure 6-1.

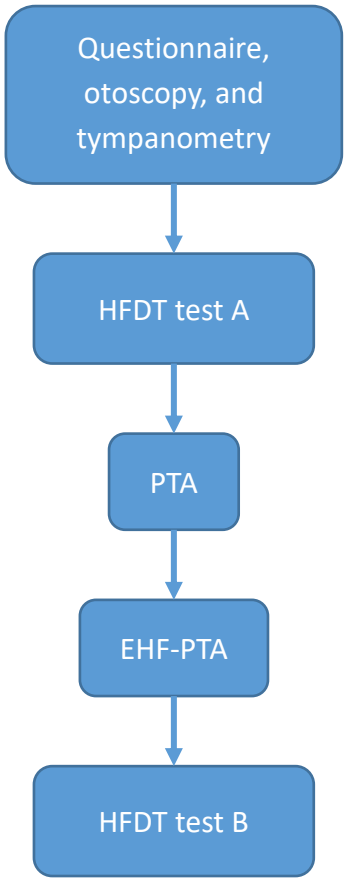


Figure 6-1: A summary of the testing procedure for the 3D-CF study. HFDT test, High Frequency Digit Triplet test; PTA, Pure Tone Audiogram; EHF-PTA, Extended High-Frequency Pure Tone Audiogram.

Each participant underwent an otoscopic ear examination and tympanometry to examine for evidence of middle ear disease. Tympanometry was performed according to the standards set out by the British Society of Audiology (BSA) (685) using a Titan S DPOAE tympanometer with TitanSuite v3.4 software and the OtoAccess v1.5 database (all InterAcoustics, Lanarkshire, UK). Where middle ear disease was identified testing was postponed until after recovery.

The HFDT test was performed twice, before and after the PTA to assess for the presence of a learning effect as described in the initial development of the test (319) (see Vlaming 2014, page 669). The test

was performed using a Lenovo ThinkPad L540 (Lenovo, Basingstoke, UK) with Sennheiser HDA200 circumaural headphones (Sennheiser Electronic Corporation, Connecticut, USA).

Briefly, each subject set their own comfortable volume using a demonstration stimulus presenting at an SNR of -4. A trial test was performed until the participant was confident with the test procedure. Twenty-five digit-triplets were presented at an initial SNR of -14. Responses were entered via the computer keyboard or mouse depending on participant preference. A correct response caused the SNR to decrease by 2 dB (making the test harder) while an incorrect response caused the SNR to increase by 2 dB. Participants were asked to give their best guess if they were unable to identify the digit presented. The final SRT was calculated as the mean of the final 19 SNRs presented plus the SNR which would be presented for a theoretical 26th trial.

PTA was performed according to the standards set out by the BSA (404) using a Callisto AC440 audiometer with TDH39 transducers, CallistoSuite v1.9 software and the OtoAccess v1.5 database (all InterAcoustics, Lanarkshire, UK). The same procedure was also used for EHF- audiometry which in addition used the Callisto High Frequency Module (InterAcoustics, Lanarkshire, UK). All audiometry was carried using the same laptop and headphones as for the HFDT. These headphones have been validated for high frequency audiometry (686).

Participants were positioned so as not to be able to see the laptop screen. They held a response button and were asked to press this whenever they thought they heard a tone in either ear. An initial test was performed at 1kHz at 40 dB(A) to the right ear, increasing in 10 dB steps as required, to ensure the participant understood the test. Participants were instructed to press the response button as soon as they heard the tone and keep it depressed until the tone stopped. Once happy with the understanding the 1 kHz tone was presented in decreasing increments of 10 dB until the participant no longer responded to the stimulus. Tones were then presented in increments of 5 dB until a response occurred. A threshold was accepted when there was a response to two of two, three or four presentations of the ascending tone. Subsequently the frequencies 2, 3, 4, 6, 8, 0.25 and 0.5 kHz were tested in the right ear then the procedure repeated in the left ear. For the right ear only a repeat test at 1 kHz was carried out after 0.5 kHz to check for intra-test variability. EHF-PTA was performed at 9, 10, 11.2, 12.5, 14 and 16 kHz. Where the test had to be curtailed this was limited to 12.5 and 14 kHz as these frequencies have previously been shown to be particularly sensitive in identifying ototoxicity (687).

A tone was deemed perceived when the response button was pressed soon after the beginning of the presentation of the tone and continued until the presentation ended. Care was taken to vary the length of presentation and the time gap between each.

The HFDT was then repeated as described above with the exception of the trial presentation.

Data about prior health conditions, aminoglycoside exposure and allergies were obtained from participant and the medical records. Participants with evidence of hearing impairment were referred for formal audiological testing.

Equipment was calibrated daily with checks of sound output for the audiometer and HFDT test and cavity calibration of the tympanometer. Calibration was carried out yearly by the manufacturer to BS EN 60645-1:2001, 6.1.2 and ISO/FDIS 389-8:2004 and BS EN 60645-1:2001, 7.3.

6.2.5 Statistical analysis

A power calculation was performed for the original grant application by the then study statistician, Mark Edmondson-Jones, as previously described (688) using GPower 3.1.3 software (Informer Technologies, Inc). The study was designed to have 80% power to detect a lower confidence limit of 80% sensitivity. The required sample size according to this calculation was 111 participants.

Data were initially stored in a Microsoft Access database (Microsoft 2016, Seattle, USA). Data were analysed in Microsoft Excel (Microsoft 2016, Seattle, USA) and Stata version 17 (Stata Corp, 2021).

Descriptive statistics are reported as means (standard deviation) for normally distributed data and medians (range) for skewed data. Hearing loss is reported as present or absent according to the above

definitions and the prevalence compared between adult and paediatric populations using a chi-squared test.

Receiver-operating characteristic (ROC) curves were constructed to assess the sensitivity and specificity of the HFDT test at a variety of cut-off SNRs. A paired student's t-test was used to assess for the presence of a learning effect.

The association between courses of aminoglycoside antibiotics and hearing loss was examined using a chi-squared test.

All tests were deemed significant at a value $p<0.05$.

6.3 Results

6.3.1 Demographics

All eligible participants at each centre were offered the opportunity to participate in the study (see Table 6-3).

Site	**Number of eligible patients**	**Number tested when well**
WMACFC	395	32
WCFC	171	43
BCH	47	17
NCH	40	7
Total	653	99

Table 6-3: Numbers of patients aged 11 and over eligible to participate in the 3D-CF study when well or at the start of a course of intravenous antibiotics and the numbers actually tested when well at each site.

Participants	**n = 99***
Age, median years (range)	28.3 (11.1 - 71.6)
Paediatric participant, n (%)	24 (24.2)
Sex, number female (%)	44 (44.4)
$ppFEV_1$, median (range) n = 83	68 (16 – 105)
BMI, median (range) n = 76	22.5 (14.8 – 37.9)
CF complications, n (%)	
Pancreatic insufficiency	89 (89.9)
CF-related diabetes	54 (54.6)
CF-related liver disease	28 (28.3)
Sinus disease	40 (40.4)
Meconium ileus	12 (12.1)
Lung transplant	7 (7.1)
Liver transplant	2 (2.0)
ABPA	16 (16.2)
One or more non-aminoglycoside antibiotic allergy, n (%)	40 (40.4)
Two or more non-aminoglycoside antibiotic allergy, n (%)	20 (20.2)

*Table 6-4: Demographic and baseline health information for the 3D-CF study. * n = 99 unless otherwise stated.*

One hundred and forty-six people consented to do the test when well and unwell. Of these, testing was achieved in 99 people at a period of clinical stability (see Figure 6-2). Twenty-four (24.2%) were paediatric patients and 75 (75.8%) were adults. The median age of the participants was 28 years and three months, with the youngest having just turned 11 and the oldest 71. Forty-four (44.4%) were female. The

severity of lung disease, as measured by the ppFEV$_1$, ranged from none-measurable (105%) to very severe (16%).

Allergy to at least one other non-aminoglycoside antibiotic was reported by 40 (40.4%) of participants while 20 (20.2%) had allergy to two or more non-aminoglycoside antibiotics (see Table 6-4).

Data completeness for each test (with reasons) is shown in Figure 6-2.

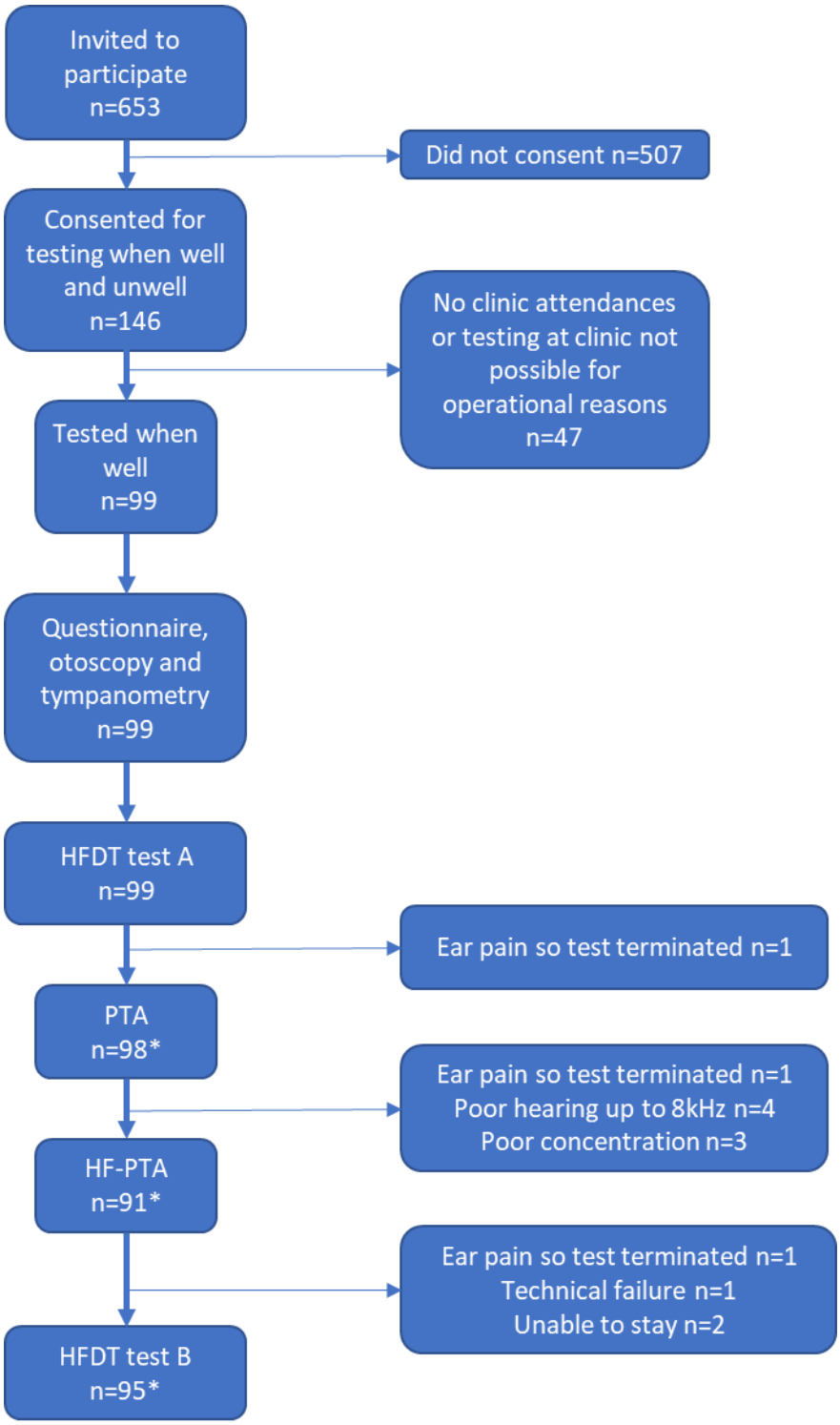


*Figure 6-2: Data completeness by test for the 3D-CF study, with details. HFDT test, High Frequency Digit Triplet test; PTA, Pure Tone Audiogram; HF-PTA, Extended High-Frequency Pure Tone Audiogram. * Attrition numbers are relative to those completing HFDT test A. The number completing each test does not necessarily drop at each stage as the HFDT test was less demanding than the PTA and HF-PTA, so some participants were able to complete HFDT test B but not the audiograms.*

6.3.2 Prior hearing concerns

A total of 43 (43.4%) participants reported hearing concerns (see Table 6-5) and in one further participant problems were evident on review of the case-notes though not mentioned on questioning. The most commonly reported concern was difficulty hearing in conditions of background noise in 12 (12.1%) participants. Five participants reported difficulties hearing speech and six were able to trace their problems back to a course of aminoglycosides. Participant report of hearing loss showed better association with extended high frequency loss, compared to hearing loss in the standard frequencies. Of the 38 participants who both reported hearing loss, and had EHF testing, 30 (79.0%) had EHF hearing loss, while for the standard frequencies only one third of those reporting loss were found to have this on testing.

A history of tinnitus or balance problems was common, occurring in 48 (48.5%) participants; again 11 participants could trace their symptoms to a course of aminoglycosides. This represents 22.9% of participants reporting symptoms of auditory or vestibular impairment, suggestive of damage related to treatment with ototoxic medication. A further three participants who reported tinnitus or balance problems recalled the symptoms as starting after an unknown antibiotic. Of participants reporting tinnitus or balance problems, and who underwent EHF testing, 86.4% were found to have EHF hearing loss.

The most commonly reported risk factor for alternative causes of hearing loss was familial deafness which was reported by 40.4% of participants. Recreational noise exposure was reported by 25 (24.3%)

participants and occupational noise exposure by 15 (15.2%). A recent upper respiratory tract infection had been experienced by 31 (31.3%) participants.

Participant-reported hearing concerns	**n = 99**
Any hearing concern, n (%)	43 (43.43)
Specific concern, n (%) †	
Difficulties with background noise	12 (12.1)
Difficulties hearing speech	5 (5.1)
Needs television loud	4 (4.0)
Hearing has declined over time	4 (4.0)
Problems since aminoglycosides	6 (6.1)
Dislike of loud noises	1 (1.0)
Previously known problems	9 (9.1)
Problems related to noise exposure	2 (2.0)
Mild or intermittent concerns	4 (4.0)
Blocked ears	3 (3.0)
Wax	1 (1.0)
New concern about hearing	1 (1.0)
Concerns expressed by family	4 (4.0)
Tinnitus or balance-problems, n (%)	48 (48.5)
Tinnitus	32 (32.3)
Balance	11 (11.1)
Both	3 (3.0)
Not specified	2 (2.0)
Symptoms relate to aminoglycosides	11 (11.1)
Coryzal illness in last 2 weeks, n (%)	31 (31.3)
Mumps, n (%)	11 (11.1)

Meningitis, n (%)	2 (2.0)
Previous ear surgery, n (%)	3 (3.0)
Deafness in family member, n (%)	40 (40.4)
Chronic middle ear disease, n (%)	4 (4.0)
Prematurity, n (%)	8 (8.1)
Occupational noise exposure, n (%)	15 (15.2)
Recreational noise exposure, n (%)	25 (24.3)

Table 6-5: Self-reported hearing concerns in 3D-CF study participants. †Specific concerns could be reported by more than one participant.

6.3.3 Prevalence of hearing loss

PTA data were available for 98/99 (99.0%) participants; one participant developed ear pain after the first HFDT test, so the procedure was abandoned. Hearing loss was present in 24/98 (24.5%) participants (see Table 6-6). Hearing loss was more common in adult participants with 23/75 (30.7%) affected compared to 1/23 paediatric participants, p=0.01.

Participants	**Hearing loss Mulheran definition n (%)**	**Hearing loss BSA definition n (%)**
All participants, n = 98	24/98 (24.5)	8/98 (8.2)
Paediatric participants, n = 23	1/23 (4.2)	1/23 (4.3)
Adult participants, n = 75	23/75 (30.7), p = 0.01	7/75 (9.3), p = 0.45

Table 6-6: Prevalence of hearing loss in 3D-CF study participants compared to our definition of hearing loss and the BSA standard definition.

The British Society of Audiology definition of hearing loss does not consider the higher frequencies in the standard audiogram. Using this definition only 8/98 participants had hearing loss and the difference between children and adults was not statistically significant, p=0.45 (see Table 6-6).

6.3.4 Prevalence of hearing loss in the extended high frequencies

EHF data was collected for 91/99 (91.9%) participants. Four participants were excluded because their hearing was very poor in the standard frequencies and so EHF testing was not feasible. One participant terminated testing after the first HFDT test. In three participants, all paediatric, testing was not attempted due to concentration difficulties observed during the standard PTA.

Complete data at frequencies 9, 10, 11.2, 12.5, 14 and 16 kHz were available for 81/99 participants. For three participants the highest frequencies were not tested as lower frequencies had been above the limit of the audiometer. Of these, one participant was not tested at 16 kHz bilaterally, one was not tested at 14 and 16 kHz on the left only and one was not tested above 12.5 kHz bilaterally. These 3 participants already met the definition of EHF hearing loss (one or more frequency in the range 9-16 kHz in one or both ears > 20 dB HL). One participant was tested at 9 kHz bilaterally only and met the definition for EHF hearing loss; for this participant the thresholds were above 75 dB HL bilaterally. A further four were tested at 12.5 and 14 kHz only because

of poor concentration. Two participants had a single data point missing; one was unable to complete the test at 16k kHz on the left due to inability to distinguish the tone from their pre-existing tinnitus and the other was missing 12.5 kHz on the left, but the reason was not recorded.

Participants	**EHF hearing loss: ≥1 frequencies HL in one or both ears > 20dB HL n (%)**
Any EHF data, n = 91	70/91 (76.9)
Paediatric participants, n = 21	8/21 (38.1)
Adult participants, n = 70	62/70 (88.6), p < 0.0001
Complete data, n = 81	64/81 (79.0)
Paediatric participants, n = 17	8/17 (47.1)
Adult participants, n = 64	56/64 (87.5), p <0.0001

Table 6-7: Prevalence of EHF hearing loss in 3D-CF study participants.

Of those participants with complete data, 64/81 (79%) demonstrated hearing loss in the extended high frequencies; of those with partial data 70/91 (76.9%) had EHF-hearing loss (see Table 6-7). In both groups adults were significantly more likely to be affected than paediatric participants, p < 0.0001.

6.3.5 Validity of the HFDT test

The first HFDT test and a PTA was completed by 98/99 (99.0%) participants. One participant complained of ear pain following the first HFDT test and did not complete any further observations.

The sensitivity and specificity of the HFDT test for a range of cut-off SRTs is shown in Table 6-8. Using -22.1 dB SNR as the cut-off SRT value gives a sensitivity of over 80% but a specificity of only 45.95%. At a sensitivity above 90%, specificity is reduced to 33.67%. Similarly, to achieve a specificity of over 80% (cut-off SRT of -20 dB SNR) sensitivity is reduced to 62.5%.

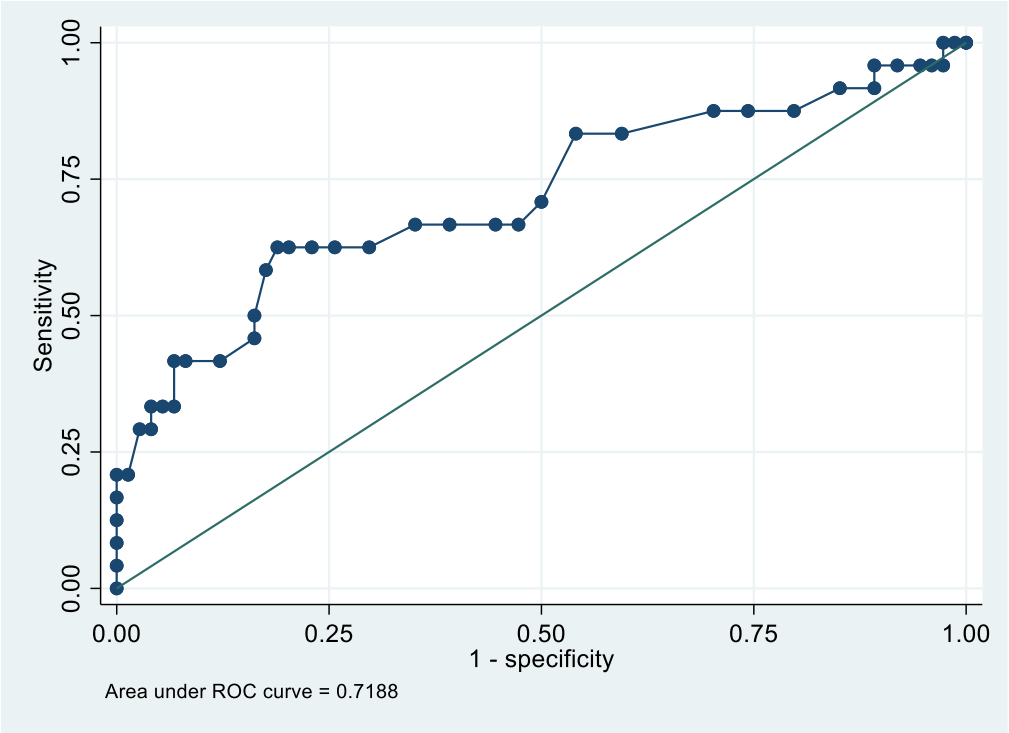


Figure 6-3: ROC curve comparing the HFDT test to hearing loss identified on PTA at 2-8 kHz as previously defined (362).

Detailed report of sensitivity and specificity

Cutpoint	Sensitivity	Specificity	Correctly classified	LR+	LR-
(>= -25.7)	100.00%	0.00%	24.49%	1.0000	
(>= -25.5)	100.00%	1.35%	25.51%	1.0137	0.0000
(>= -25.1)	100.00%	2.70%	26.53%	1.0278	0.0000
(>= -24.6)	95.83%	2.70%	25.51%	0.9850	1.5417
(>= -24.4)	95.83%	4.05%	26.53%	0.9988	1.0278
(>= -24)	95.83%	5.41%	27.55%	1.0131	0.7708
(>= -23.8)	95.83%	8.11%	29.59%	1.0429	0.5139
(>= -23.6)	95.83%	10.81%	31.63%	1.0745	0.3854
(>= -23.4)	91.67%	10.81%	30.61%	1.0278	0.7708
(>= -23.2)	91.67%	14.86%	33.67%	1.0767	0.5606
(>= -22.9)	87.50%	20.27%	36.73%	1.0975	0.6167
(>= -22.7)	87.50%	25.68%	40.82%	1.1773	0.4868
(>= -22.5)	87.50%	29.73%	43.88%	1.2452	0.4205
(>= -22.3)	83.33%	40.54%	51.02%	1.4015	0.4111
(>= -22.1)	83.33%	45.95%	55.10%	1.5417	0.3627
(>= -21.9)	70.83%	50.00%	55.10%	1.4167	0.5833
(>= -21.7)	66.67%	52.70%	56.12%	1.4095	0.6325
(>= -21.5)	66.67%	55.41%	58.16%	1.4949	0.6016
(>= -21.3)	66.67%	60.81%	62.24%	1.7011	0.5481
(>= -21.1)	66.67%	64.86%	65.31%	1.8974	0.5139
(>= -20.8)	62.50%	70.27%	68.37%	2.1023	0.5337
(>= -20.6)	62.50%	74.32%	71.43%	2.4342	0.5045
(>= -20.4)	62.50%	77.03%	73.47%	2.7206	0.4868
(>= -20.2)	62.50%	79.73%	75.51%	3.0833	0.4703
(>= -20)	62.50%	81.08%	76.53%	3.3036	0.4625
(>= -19.8)	58.33%	82.43%	76.53%	3.3205	0.5055
(>= -19.6)	50.00%	83.78%	75.51%	3.0833	0.5968
(>= -19.4)	45.83%	83.78%	74.49%	2.8264	0.6465
(>= -18.7)	41.67%	87.84%	76.53%	3.4259	0.6641
(>= -18.5)	41.67%	91.89%	79.59%	5.1389	0.6348
(>= -17.9)	41.67%	93.24%	80.61%	6.1667	0.6256
(>= -17.3)	33.33%	93.24%	78.57%	4.9333	0.7150
(>= -17.1)	33.33%	94.59%	79.59%	6.1667	0.7048
(>= -16.8)	33.33%	95.95%	80.61%	8.2222	0.6948
(>= -16.6)	29.17%	95.95%	79.59%	7.1944	0.7383
(>= -16)	29.17%	97.30%	80.61%	10.7917	0.7280
(>= -15.8)	20.83%	98.65%	79.59%	15.4167	0.8025
(>= -12)	20.83%	100.00%	80.61%		0.7917
(>= -9.8)	16.67%	100.00%	79.59%		0.8333
(>= -6.6)	12.50%	100.00%	78.57%		0.8750
(>= -6.5)	8.33%	100.00%	77.55%		0.9167
(>= -6.1)	4.17%	100.00%	76.53%		0.9583
(> -6.1)	0.00%	100.00%	75.51%		1.0000

Table 6-8: Detailed report of the sensitivity and specificity of the HFDT test compared to hearing loss identified on PTA at 2-8 kHz as previously defined (362).

6.3.6 Is the HFDT subject to a learning effect?

A repeat HFDT test was available for 95/99 (96.0%) participants. One terminated the test procedure after the first HFDT test due to ear pain, in one there was a technical malfunction and two were unable to stay due to prior commitments.

In 51/95 (53.7%) paired tests the participant improved their score on repeat testing (i.e., had a more negative SNR), 38/95 (40%)

participants' scores decreased while 6/95 (6.3%) remained the same. The mean change in SNR was an improvement of -0.32 dB. This was not statistically significant, p = 0.06.

6.3.7 Does hearing loss associate with aminoglycoside exposure?

Data were available for 93/99 (93.9%) participants in the standard frequencies and 86/99 (86.9%) of participants for EHF audiometry. Complete data on cumulative aminoglycoside exposure was only available for 32/93 (34.4%) participants. Data on previous aminoglycoside courses were frequently missing due to older sets of case notes being unavailable.

There was no significant difference between hearing loss in either the standard or extended high frequencies when compared with aminoglycoside exposure (see Table 6-9). Of those participants with high aminoglycoside exposure, 26/29 (89.7%) participants had EHF hearing loss. However, EHF hearing loss was also present in 6/8 (75.0%) participants with no exposure to aminoglycosides. Two of the participants with no aminoglycoside exposure (25.0%) were found to have hearing loss in the standard frequencies.

Participants	Standard PTA hearing loss: ≥2 frequencies HL in one or both ears > 20dB HL (2-8kHz) n (%)	EHF hearing loss: ≥1 frequencies HL in one or both ears > 20dB HL (9-16kHz) n (%)
No exposure	n = 8: 2/8 (25.0)	n = 8: 6/8 (75.0)
Low exposure (1-9 courses)	n = 51: 12/51 (23.5)	n = 49: 34/49 (69.4)
High exposure (≥10 courses)	n = 34: 9/34 (26.4), p = 0.95	n = 29: 26/29 (89.7), p = 0.12

Table 6-9: Prevalence of hearing loss in the standard and EHF-frequencies by aminoglycoside exposure in 3D-CF study participants.

6.4 Discussion

6.4.1 Prevalence of hearing loss

The prevalence of hearing loss in this CF population was 24.5%; when the extended high frequencies were considered this increased to 76.9%.

Considerable variation in the prevalence of standard and EHF hearing loss has been shown in previous studies. In a study looking at a group comprising 43 adult and 27 paediatric CF patients, 17% were found to have hearing loss in either the standard or extended frequencies (0.25-16 kHz). Only one participant with hearing loss was in the paediatric age group (Mulheran *et al.* 2001) (366). This figure is lower than our results for both standard and EHF hearing loss which may be due in

part to the higher percentage of paediatric participants (38.6%) in the Mulheran study, compared to the present study where only 24.2% were in the paediatric age group.

More recently, studies of adult participants only have found a prevalence of hearing loss closer to our results. Harruff *et al.* (2021), looking at the incidence of ototoxicity in adult CF patients undergoing a single course of tobramycin, found a baseline prevalence of 28% in the standard frequencies and 72% in the extended high frequencies (363). Garinis *et al.* (2021), compared the effect of a single course of tobramycin to non-tobramycin antibiotics in a group of adult CF participants. It is not possible to compare this study directly with our data because the authors used a different definition of hearing loss (a single threshold > 25 dB in either ear). They found that the prevalence of hearing loss (in 0.25-16 kHz range) at baseline was 67% in those scheduled to receive tobramycin and 46% in the non-tobramycin group (670).

In studies of paediatric populations Al-Malky *et al.* (2015) found 13/70 (18.6%) of children had standard frequency hearing loss with a further two having loss above 8 kHz (145). An earlier study by the same group found EHF hearing loss in 8/45 (17.8%) participants but found no loss in the standard frequencies (356). Again, the cause for the difference may be related to differing age-distribution since both these studies recruited participants as young as three years. Blankenship *et al.* recruited participants aged 6-21 years and found hearing losses in 43.0 and 57.0% in the standard and extended high frequencies respectively

(314). The authors noted that the variability in reported prevalence is likely multifactorial, with one factor suggested being differences in definitions of hearing loss.

Hearing loss can be defined as either a fixed value or a change from baseline (see chapter 2, section 2.8.4). Some studies use a fixed value to identify baseline hearing deficits then look for a change from baseline following exposure to an ototoxic agent (363, 670). Other studies have used a single threshold > 20 dB HL but over a wider range of frequencies (0.25-12 kHz) (355, 359).

Our data were insufficiently sensitive to exclude hearing loss due to other causes. In particular, the age of some participants makes it likely that at least some of the observed EHF-loss is attributable to presbycusis. EHF hearing declines with increasing age (689, 690) but there are no accepted age-related normal values to facilitate investigation of the relative effects of ototoxicity and age-related change. It is however important to note that cochlear-damage can be synergistic; people with sensorineural hearing loss (SNHL) of any cause are more vulnerable to an ototoxic insult as they have already sustained damage to their inner and outer hair cells and have less reserve (375, 691). Identification of hearing loss of whatever cause is therefore important to allow modification of antibiotic prescription where needed and reduce the possibility of additional damage.

It is also possible that the prevalence of hearing loss in our population was skewed by selection bias; patients with prior concerns about their

hearing may have been more likely to agree to participate than those who had not noticed any problems. This effect, however, is not unique to the current study.

6.4.2 Validity of the HFDT test

The HFDT test is insufficiently sensitive and specific to be used as a screening tool for early detection of hearing loss in CF. This compares to a reported sensitivity of 87% and specificity of 92% in the original description of the test (319).

There are a number of possible reasons for this. Firstly, the test was initially developed to identify noise-induced hearing loss which characteristically begins with loss at 4 kHz with subsequent spread to higher and lower frequencies. In contrast, ototoxic hearing loss begins in the highest frequencies and progresses apically with continued damage. There are few speech sounds in the frequencies above 4 kHz (see the speech banana diagram, Figure 2-6). English single digits contain these sounds in only five; three (/th/), four (/f/), five (/f/), six (/s/) and seven (/s/). The HFDT test excludes seven, as the only two-syllable digit, and four which could not be validated in the development of the test. This means the HFDT test only includes 3/9 words which contain higher frequency speech sounds; despite selection for higher frequencies through the use of low-pass filtered masking noise there may be insufficient exposure to these higher frequency sounds to be truly discriminatory. However, Motlagh Zadeh *et al.* found better performance on their digits-in-noise test when low-pass filters, even up

to 8 kHz, rather than broad-band filters were applied, suggesting the importance of EHF information in speech perception (338).

Secondly the definition of hearing loss used in the original paper (319) was a mean of the PTA thresholds at 3, 4, 6 and 8 kHz >20 dB. Moderate hearing loss (40-70 dB HL) at a single frequency could therefore be sufficient to fit this definition but would not be recognised as hearing loss in our study.

Thirdly, the study was underpowered with only 99/111 (89.2%) of the required participants recruited. This increases the chance of a type 2 error; the small sample size may mean that the true validity of the HFDT test is underestimated (692).

6.4.3 Is the HFDT subject to a learning effect?

Minimal learning effect was demonstrated in our study with a mean improvement in SNR of only -0.32 dB between the first and second test. This is similar to the learning effect of -0.5 dB seen in the original description of the HFDT test. The learning effect may be smaller for a digits-in-noise test than a words-in-noise test since unless the corpus of word or phrases is large repeat presentations of the same stimulus may be remembered (319).

6.4.4 Prior hearing concerns

Nearly half of participants (43.4%) reported prior concerns with their hearing. Self-reported hearing concerns related better to EHF loss than standard frequency loss, though this may be due to the higher

proportion of participants with EHF loss. This suggests that, where patients report hearing concerns, a standard PTA may be insufficient to provide reassurance, but further studies are needed.

A cross-sectional study of hearing loss in paediatric and young adult CF patients Blankenship *et al.* found self-reported hearing concerns in 32% (314); the older demographic in the current study likely accounts for the increased numbers reporting concerns. The difference between the prevalence of hearing loss in the standard frequencies and the proportion of participants reporting concern with their hearing is likely explained by the higher prevalence of EHF-hearing loss. This has previously been termed hidden hearing loss. Motlagh Zadeh *et al.* found a correlation between self-reported hearing difficulties in noise and hearing loss above 8 kHz (338).

Nearly half of participants reported either tinnitus or balance problems. These are both symptoms which may represent previous ototoxicity. In a study of people with CF exposed to tobramycin 7/19 (36.8%) patients reported dizziness, three of whom were felt to have dizziness related to vestibular damage (360). Our questionnaire was insufficiently sensitive to determine the probable cause for any balance problems which may account for the increased prevalence, though Blankenship *et al.* found similar self-reported levels of balance problems (32%) also using an unvalidated questionnaire (314). Handelsman *et al.* found a much higher prevalence of vestibulotoxicity (79%) in CF patients assessed for vestibular function. Vestibulotoxicity was associated with hearing damage (only measured up to 8 kHz) in 18% of participants in this

study (361). These results underline the importance of directly asking patients about symptoms prior to treatment with aminoglycosides. Vestibulotoxicity in CF patients treated with aminoglycosides remains understudied and further work is needed to understand the true prevalence of this complication.

Over 40% of participants reported allergy to at least one non-aminoglycoside antibiotic and 20% to two or more. Identification of aminoglycoside induced ototoxicity, or indeed pre-existing hearing damage which may predispose to ototoxicity in this group will reduce the antibiotic options available to treat an exacerbation. This is not a reason not to screen for ototoxicity; where alternative antibiotics are limited identification of ototoxicity allows a careful discussion of the risks and benefits of aminoglycoside treatment for the individual.

6.4.5 Is there an association between aminoglycoside courses and hearing loss?

No association between aminoglycoside courses and hearing loss was found in this study. More participants with no history of aminoglycoside exposure had both standard and high-frequency hearing loss than those with a low exposure.

The association between aminoglycoside exposure and hearing loss is still unclear. Martins *et al.* also found no significant association (355). Mulheran *et al.* found a significant difference in the median number of aminoglycoside courses between those with hearing loss (20) and those without (9) (366) however, the relationship between hearing loss

and number of courses was non-linear. Vijayasingam *et al.* pragmatically only collected aminoglycoside exposure data for the preceding five years. They found participants with hearing loss were less likely to have been prescribed aminoglycosides in that time but did find an association between increased total courses of all antibiotics in the preceding 10 years and the chance of hearing loss. They ascribed this to avoidance of aminoglycoside administration to patients with known hearing loss (359). Al-Malky *et al.* found all the children with both standard and EHF-hearing loss had exposure to aminoglycosides; of the 15 children with EHF-hearing loss 11 had “high” exposure (≥10 courses) (145). In another study by the same group all eight children with EHF-hearing loss had received >5 courses of aminoglycosides (356).

The studies with the strongest associations between hearing loss and aminoglycoside exposure are those with a younger participant cohort. Younger patients will have had less cumulative exposure to other ototoxic insults, such as noise, and will not be subject to any age-related reduction in hearing thresholds, both of which may act as confounders. In addition, retrospective collection of aminoglycoside exposure data is dependent on clinical record keeping. In the current study only 32/93 participants had reliable data; in the remainder aminoglycoside exposure is likely to be underestimated. Transfer of participants between CF units, either through transition, shared care or moving centre meant much historical data were not available. Older records were less likely to be complete (for example noting that a

course of antibiotics was given without stating which agents were used). Data reliability improved over time, particularly with the advent of computerised records. Record keeping for younger patients is likely therefore to be more reliable.

The confounding effect of additional hearing insults and difficulties in data collection, particularly for older patients, may explain the lack of association between aminoglycoside exposure and hearing loss in this and other studies.

6.4.6 Strengths of the study

Despite not reaching the planned recruitment target this study examined three tests of hearing in a large number of people with CF, representing just under 1% of the CF population in the UK (10). It was conducted in multiple centres and at a wide spread of ages, making the estimates of prevalence of hearing loss in the standard and extended high frequencies generalisable in the UK adult and adolescent CF population.

The range of ages studied, up to 71 years, gives a better assessment of the hearing needs of older participants with CF than other recent studies (363, 670). The study also attempted to assess for the presence of non-ototoxic risk factors for hearing loss, including noise exposure, family history, prematurity and other infections. All appear more predictive of EHF hearing loss than standard loss, though this may be due to the high prevalence of EHF hearing loss in this cohort.

This shows that simply enquiring about hearing problems is insufficient and testing, including the EHF range, is required.

6.4.7 Study limitations

As discussed in the introduction increasing age is a risk factor for hearing loss. Our patient population had a 60-year age range, with the oldest participant over 70 years old. It is likely that some of the hearing loss we identified is related to age; indeed, in a study of adult patients with CF, frequencies above 12.5 kHz were deliberately not tested due to the likelihood of loss in these frequencies in an adult population (359).

There were insufficient data to be clear about the impact of cumulative aminoglycoside dose on hearing loss. We were also unable to ascertain whether patients with prior aminoglycoside exposure had ever had serum levels above the therapeutic range. Furthermore, we had no data as to the presence or absence of genetic mutations predisposing patients to aminoglycoside ototoxicity, such as the mitichondrial m.1555A>G mutation. More complete data about these issues is required to understand the impact of aminoglycosides in both individuals with CF and the wider CF population.

In addition to the limitations described above, recruiting at multiple sites, whilst an overall advantage to the study, may have affected recruitment. Having one research fellow recruiting participants at four separate sites meant that many patients who expressed an interest in the study were not recruited for logistical reasons. This may have

exacerbated the potential for selection bias since those most keen to participate were more likely to be recruited as they were more likely to make time than those more ambivalent towards the study.

6.4.8 Challenges in conducting the study

In the original grant application for this study recruitment of 117 well participants was planned, in order to have data on the 111 required by the power calculation. It was anticipated that testing these participants would take approximately nine months, with two-three participants tested per clinic.

Unfortunately, there were a number of barriers to this. Very few patients had read the patient information sheet sent out prior to clinic. Since our ethics approval required that the participant had chance to review the information sheet approximately two weeks prior to consent being taken, these patients could not be consented on first review. In patients who agreed to participate there was then a delay of two to three months prior to their next clinic appointment. Some patients did not remember these discussions at the next appointment or had forgotten to allow the extra time. Clinic appointments frequently changed at short notice, or patients did not attend. Additionally, as I was the only research fellow carrying out the testing, I was not always available on the date of the next clinic appointment.

Testing took approximately an hour and, with cleaning of the equipment in accordance with infection control requirements, in most clinics it was not feasible to perform more than one test procedure. Some interested

patients were not able to stay for the additional hour required, particularly if that involved waiting for another test to be finished.

These difficulties meant that recruitment was much slower than anticipated. Recruitment began in February 2015 and continued until June 2016, by which time 60 participants had been tested. Testing recommenced in August 2017 and continued until January 2019.

Participants in this study were primarily identified from the adult CF Centres, partly since they had more clinics per week to attend, and partly because during paediatric clinics I concentrated on recruiting younger children. Although there were more adult patients at the WMACFC I had to balance the additional travelling time required to get there, which impacted on my ability to attend other clinics, and therefore I spent more time recruiting in Nottingham for this part of the study.

The decision to stop recruitment in January 2019 was made by the study steering committee in November 2018. Recruitment after November 2018 was limited to those for whom an appointment had already been arranged. My out-of-program period finished in May 2019 and it was felt that time was needed for data analysis whilst I was still in post.

6.4.8.1 Strategies which could have improved recruitment

With the benefit of hindsight there are a number of changes I would make to the protocol in order to facilitate recruitment and improve the chance of achieving the target sample size.

Firstly, I would aim to provide potential participants with an information sheet and then consent and test them the same day. This study was not invasive and therefore it would be reasonable to reduce the time needed for consideration of participation. This would have reduced the lag time between first contact and testing, speeding up recruitment. Fewer interested patients may have been lost due to logistical constraints in this way. In conjunction with this, secondly, I would not send out patient information leaflets in advance of clinics as this wasted a lot of time and did not achieve many recruits.

Whilst building a relationship with new CF teams I attempted to test across all four sites concurrently. In retrospect this was a mistake as I lost valuable time commuting between sites and often missed some clinics all together. If I were to repeat this study I would concentrate on one city at a time, so that I could attend all the clinics available and ensure the clinical teams would always expect me to attend. This would have the added benefit of embedding me within the clinical teams, potentially improving their promotion of the study. I would exhaust recruitment in the first city prior to starting in the next.

Fourthly, in those patients who expressed an interest but were unable to stay for testing that day, I would contact them prior to their next appointment to ask if they were still willing to participate and if convenient arrange to see them. I would use hospital communications, through the CF specialist nurses, to achieve this as many patients (when I did gain ethical approval to contact interested patients in

advance of an appointment) did not answer an unknown, university, phone number.

Finally, I would be more realistic about how long recruitment would take as it was clear early on that performing three tests in a single clinic was not feasible. This would allow better planning of my time throughout my PhD period.

6.4.9 Further work

The study demonstrated that hearing testing is feasible in a clinic setting so further work to identify valid screening tests is warranted. Vijayasingam *et al.* examined a web self- hearing test and tablet audiometry, comparing them to sound-booth audiometry and found that tablet audiometry at 0.25-12.5 kHz was 93% sensitive and 88% specific at identifying hearing loss in both the out-patient and in-patient setting (359). This type of testing has the advantage of conveying a broader amount of data than the HFDT test, which only provides a single data point, and can be compared with formal audiometry if needed. However simple perception of a pure tone does not provide the cognitive challenge of a speech-in-noise test and therefore does not examine the higher auditory pathways (677).

The high prevalence of hearing loss in people with CF found here and previously supports the need to develop better screening pathways to prevent ongoing hearing damage. It will be interesting to note over the coming years whether the advent of CFTR modulators affects the need

for intravenous antibiotics enough to reduce the prevalence of hearing loss in younger patients.

6.5 Conclusions

This study found a prevalence of hearing loss in CF patients aged 11-71 of 24.5% in the standard frequencies and 76.9% in the extended high frequencies. The HFDT test was insufficiently sensitive to be used as a screening tool for early detection of hearing loss, but we have shown that hearing testing in a clinic setting is feasible. Further work is needed to develop alternative screening tests, such as tablet-based audiometry, to facilitate hearing monitoring and reduce the risk of ototoxicity in this patient group.

Chapter 7: An evaluation of the High Frequency Digit Triplet test in special circumstances

This work was funded by an NIHR RfPB grant, PB-PG-0213-30055.

Details of the contributions made by colleagues in the preparation of this chapter are given in Table 7-1.

Study Stage	**Contributors**
Preparing the grant	Prof. Alan Smyth Dr Heather Fortnum Dr Melanie Ferguson Mr Mark Edmondson-Jones Dr Edward Nash Dr Jane Clarke Dr Jane Dewar Dr Robert MacKinnon Mrs Zoe Elliot
Writing the protocol	Dr Sally Palser Prof. Alan Smyth Dr Heather Fortnum Dr Melanie Ferguson Mr Mark Edmondson-Jones Dr Edward Nash Dr Jane Clarke

	Dr Jane Dewar Dr Robert MacKinnon Mrs Zoe Elliot
Identifying potential participants	Dr Sally Palser CF teams at Nottingham University Hospitals NHS Trust, West Midlands Adult CF Centre and Birmingham Childrens' Hospital
Recruitment of unwell adults and children with CF	Dr Sally Palser Prof Alan Smyth Dr Sana Anwar
Recruitment of healthy control children	Dr Sally Palser Ms Sana Anwar
Data collection in unwell adults and children with CF	Dr Sally Palser (n=118) Ms Sana Anwar (n=7)
Data collection in healthy control children	Ms Sana Anwar (n=84)
Statistical analysis plan	Mr Mark Edmondson-Jones Dr Polly Scutt

Table 7-1: A summary of the contributions made by colleagues to assist in the production of this work.

This project was run concurrently with that described in chapter 6. I completed the same work for this chapter. In addition, I liaised with the CF specialist nurses and medical teams at each site to identify in-

patients who were suitable to approach and liaised with the medical teams where results suggested aminoglycoside antibiotics might be reconsidered. I contacted schools to discuss participation and attended an assembly at one school site to promote the study. I tested all the unwell participants and 33/40 well children. All the school children were tested by Sana Anwar.

7.1 Introduction

As discussed in chapter 6 hearing testing by pure tone audiogram (PTA) is limited by the equipment and specialist personnel required. In some circumstances there are additional barriers, particularly in situations where concentration is reduced. This is the case in children, optimal testing of whom requires experienced personnel (693) and is also likely to be impacted in those unwell at the time of testing (303).

The high frequency digit triplet (HFDT) test is much shorter than a standard audiogram, taking approximately 3-5 minutes to complete (319). In situations where concentration is reduced a shortened test-protocol may improve completion. In addition, speech-in-noise testing better reflects functional hearing (693) so may be a better method of assessing the day-to-day abilities of people with CF to communicate. Speech-in-noise tests are also appealing to children (693).

However, in addition to requiring specialist PTA, primary school aged children may not perform as well at speech-in-noise testing as adults (693) despite some tests being validated in this age-group (694, 695). Speech recognition thresholds (SRTs) improve with increasing age

even where PTA thresholds are normal (694). This is due to later development of higher cortical auditory processing, which is adult-like at age 10-12 years old if speech-shaped masking noise (at frequencies mimicking the distribution of speech sounds) is used for speech-in-noise testing (695) (see section 2.5.1.1). Where more complex forms of masking are used, such as two-person talking, adult-like perception occurs later (696, 697). This may be due a greater distracting effect in children who are less able to ignore competing speech signals (696).

Noise produced intrinsically by an unwell participant, such as coughing or noisy breathing, may also affect the test. People with CF commonly develop periods of additional respiratory symptoms, termed pulmonary exacerbations, in which increased cough is very common (224).

The HFDT test was therefore studied in additional circumstances: unwell adolescents and adults at a time of pulmonary exacerbation and children aged 5-10 years old. The aim of this part of the 3D-CF study was to determine the feasibility and validity of the test in unwell adults and adolescents with CF and the feasibility in primary school aged CF children. Data for this part of the 3D-CF study were collected between March 2015 and January 2019, concomitantly with the data collected for chapter 6.

7.2 Methods

7.2.1 Participants

Participants were recruited from the same sites as described in Chapter 6; the West Midlands Adult CF Centre (WMACFC), the Wolfson CF Centre, Nottingham (WCFC, part of Nottingham University Hospitals NHS Trust), Birmingham Children's Hospital (BCH) and Nottingham Children's Hospital (NCH, also part of Nottingham University Hospitals NHS Trust).

Information sheets were sent out to participants prior to clinic appointments as previously described (see section 6.2.1). Consent was taken at the first instance of the participant agreeing to take part, either in clinic or on the ward, and confirmed at future testing.

Participants with evidence of hearing impairment were referred for formal audiological testing.

All participants provided written, informed consent (or assent with parental consent if under 16). The study was approved by Nottingham 2 Research Ethics Committee (14/EM/1199) and by the local research and innovation team at each study site.

7.2.1.1 Unwell adolescents and adults

Unwell adults and adolescents were tested either at a clinic appointment when a decision to start IV antibiotics was made, or on the wards within the first four days of an admission. In the latter instance the clinical team advised the researcher (SP) when a suitable patient

was admitted. Where the potential participant had previously consented to participate or expressed interest in the study (either at a prior admission or clinic appointment) testing was carried out at the initial visit if consent was confirmed. If no prior discussion had taken place an information sheet was left with the patient and a follow-up visit arranged within the next three days.

Potential participants were eligible if they:

- Were aged 11 years old and over,
- Had a diagnosis of CF confirmed by sweat test or genetic testing,
- Required intravenous antibiotics or other hospital treatment for a pulmonary exacerbation,
- Could be seen prior to starting antibiotics or within four days of starting and
- Provided written, informed consent (or assent with parental consent).

It was initially planned to only recruit participants scoring 4 or more on Fuchs' criteria for a pulmonary exacerbation (67) but initial experience showed that this excluded a large number of patients. The protocol was therefore amended to include any patient whose clinical team felt they required a course of intravenous antibiotics (at hospital or at home) or other in-patient treatment for a pulmonary exacerbation.

7.2.1.2 Children aged 5-10 years

Primary school children with CF, aged 5-10 years, were approached in clinic with an adult with parental responsibility. Consent and testing were performed at this appointment or at a future time if this was preferred.

Patients were eligible to participate if they were:

- Aged 5 to 10 years old,
- Had a diagnosis of CF confirmed by sweat test or genetic testing,
- Were clinically well and
- Provided written, informed parental consent with assent from the child.

Healthy control children were recruited from two local schools. Information was sent to the schools' headteacher and, where they expressed interest, further information was provided, along with age-appropriate information sheets. An assembly was given at each school to inform the children of the project and letters were sent home with each child.

Children whose parents/legal guardian consented, and who returned the consent form to school, were tested in quiet classrooms. Testing continued until sufficient tests were successfully carried out for each age group.

7.2.2 Primary and secondary outcomes

7.2.2.1 Unwell adolescents and adults

The primary outcomes measured were the feasibility and validity of the HFDT test in unwell participants.

Secondary outcomes were the prevalence of hearing loss in this group of participants and whether any changes in hearing could be identified before and after a course of intravenous (IV) antibiotics.

7.2.2.2 Children aged 5-10 years

The primary outcome measure was the lower age limit at which both 80% of children were able to complete the HFDT test and the correlation between the SRT (obtained from the HFDT test) and the PTA was at least half that of adult participants.

The secondary outcome was the prevalence of hearing loss in this age group of children with CF.

7.2.3 Definitions

Hearing loss in the standard frequencies was again defined using criteria set out by Mulheran *et al.* (362); two or more thresholds above 20 dB, in one or both ears, in the frequencies 2-8 kHz. Extended high frequency (EHF) hearing loss was again defined as per the ISO 389-5:2006 (684); one or more frequency in the range 9-16 kHz in one or both ears > 20 dB HL.

Middle ear disease was defined as a red, bulging tympanic membrane, in which case tympanometry was not attempted, or the presence of a flat tympanogram.

Ototoxicity at follow-up testing was defined using the American Speech-Language-Hearing Association (ASHA) guidelines for cochleotoxicity monitoring as an increase in threshold of 20 dB HL at one frequency, 10 dB HL increase at two consecutive frequencies or loss of response at three consecutive frequencies where a response was previously achieved (698).

7.2.4 Test procedure

The test procedure was carried out in the same manner as set out in chapter 6 (section 6.2.4) with the following modifications.

7.2.4.1 Unwell adolescents and adults

Testing was carried out on the wards in participants individual rooms (WCFC, WMACFC, NCH, BCH) or the ward dayroom (BCH). For participants at clinic in whom the need for antibiotic was identified or participants starting home intravenous antibiotics, testing was carried out in quiet, individual clinic rooms.

For logistical reasons the group tested when unwell represents a convenience sample of the whole population who had an exacerbation during the study period.

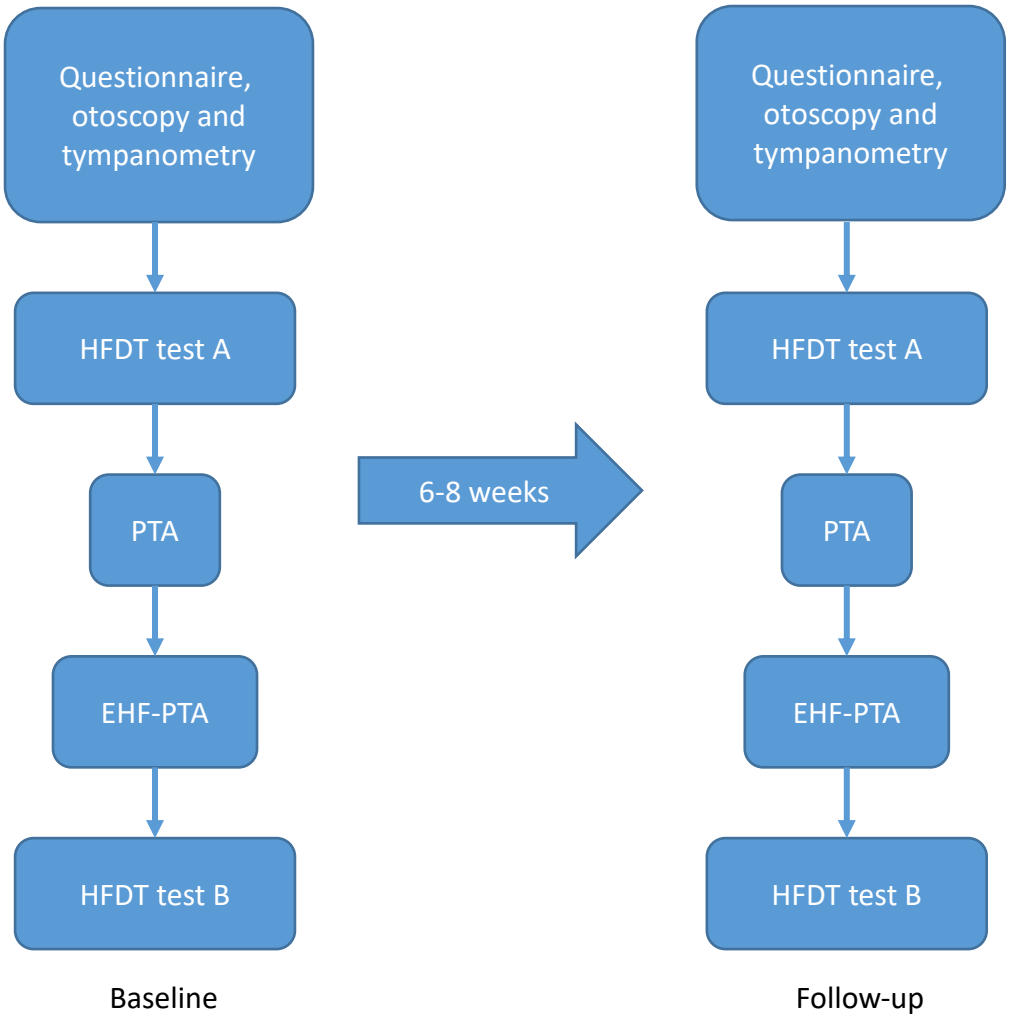


Figure 7-1: Planned test procedure for adults and adolescents at the beginning and end of a course of treatment for a pulmonary exacerbation in the 3D-CF study. HFDT test, High Frequency Digit Triplet test; PTA, Pure Tone Audiogram; EHF-PTA, Extended High-Frequency Pure Tone Audiogram.

The same testing regimen (see Figure 7-1) as for patients at a time of clinical stability was planned, accepting that modifications may be necessary depending on the clinical situation of each individual. A single HFDT test and standard PTA was the minimum required and reasons for reducing the protocol were noted. Data about prior health conditions, aminoglycoside exposure and allergies were obtained from the participant and the medical records.

Formal questioning of participants about the acceptability of the test was added to the protocol near the end of the study. These participants were asked if, in future, were the HFDT test to be adopted, would they be happy to do the test at a clinic appointment and at the beginning of a course of IV antibiotics.

Where possible participants were tested again at follow-up clinic appointment to assess for any changes in hearing acuity following the administration of IV antibiotics. This became very difficult to achieve for a number of reasons:

- Participants who never attend clinic but seek ad-hoc treatment when unwell.
- Participants who are not able to remain out of hospital long enough to come to clinic.
- Conflict in recruiting time between follow-up visits and new participants.

This part of the protocol was subsequently stopped since these difficulties led to low numbers tested.

7.2.4.2 Children aged 5-10 years

Testing of children with CF was carried out in quiet rooms in the clinical research facilities (NCH and BCH). The planned test procedure is shown in Figure 7-2. Data about prior health conditions, aminoglycoside exposure and allergies were obtained from the accompanying adult and the medical records.

Again, there was acknowledgement that protocol modifications may be necessary depending on the individual child's concentration. The BSA guidance for PTA testing states that, in the case of subjects with short attention spans,

> *"...it may be appropriate to test fewer frequencies, as it is better to test fewer frequencies accurately than to attempt a complete test of an uncooperative subject where the accuracy will be in doubt."* (Section 6.8, Variations in method, p.11) (404).

Modifications were made on an individual case basis, prioritising the frequencies needed for the definition of standard hearing loss. Data bilaterally at 1, 2, 4 and 8kHz was deemed a successful PTA. Whilst this does not include all the frequencies required for the Mulheran definition of hearing loss (362), used in the 3D-CF study, an adaptation was necessary for the younger participants. Data bilaterally at 12.5 and 14 kHz was deemed a successful EHF-PTA (687). If a child was found to have flat tympanograms an abbreviated PTA was conducted to look for evidence of conductive hearing loss (CHL) which would require referral. These children were excluded from analysis unless repeat testing could be arranged after resolution.

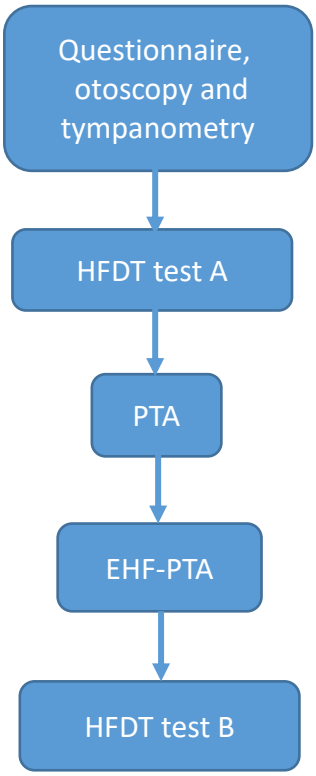


Figure 7-2: Planned test procedure in the 3D-CF study for children aged 5-10 years. HFDT test, High Frequency Digit Triplet test; PTA, Pure Tone Audiogram; EHF-PTA, Extended High-Frequency Pure Tone Audiogram.

Formal questioning about the acceptability of the test was again added to the protocol towards the end of the study. Children tested after this addition, and their accompanying adult, were asked if they felt the HFDT test would be acceptable to do in future, at a clinic appointment or during an admission.

Healthy control children were asked for assent on the day of testing and asked about any changes to the hearing concerns questionnaire returned by their legal guardian. Testing was carried out in quiet classrooms allocated by the school. Again, children with flat tympanograms underwent an abbreviated PTA to look for evidence of CHL. Children with CHL were referred for formal PTA testing and all children with flat tympanograms or CHL were excluded from further

testing. All testing of healthy control children was carried out by Sana Anwar (paediatric research audiologist). PTA in healthy control children did not include 3 and 6 kHz as this was done following standard audiological procedure which, in the standard frequencies, comprises 0.25, 0.5, 1, 2, 4 and 8 kHz (404).

7.2.5 Statistical analysis

A power calculation was performed for the original grant application by the then study statistician, Mark Edmondson-Jones, as previously described (688) using GPower 3.1.3 software (Informer Technologies, Inc). For unwell adults the study was designed to have 80% power to detect a lower confidence limit of 80% sensitivity. The required sample size, according to this calculation, was 133 participants recruited, with complete tests achieved in 111 of these. This assumes that a number of participants would be unable to complete the test protocol.

For children, the test was powered on the feasibility of the HFDT test, rather than for the detection of hearing loss due to small numbers of children predicted to have this condition. To detect a difference in test completion rates ranging from 95% in older children to 80% in younger children the required sample size is 134. It was planned to test 11 children with CF and 12 control children at each age from 5 to 10 years: in total 66 children with CF and 72 control children.

Data were stored in a Microsoft Access database (Microsoft 2016, Seattle, USA). Data were analysed in Microsoft Excel (Microsoft 2016, Seattle, USA) and Stata version 17 (Stata Corp, 2021).

Descriptive statistics are reported as number (%) for frequencies, means (standard deviation) for normally distributed data and medians (range) for skewed data. Hearing loss is reported as present or absent according to the above definitions and the prevalence compared between groups (adult and paediatric populations in the unwell participants, CF and non-CF in the children) using the chi-squared test or Fisher's exact test (where observations were fewer than 5).

Receiver-operating characteristic (ROC) curves were constructed to assess the sensitivity and specificity (with 95% confidence intervals) of the HFDT test at a variety of cut-off SNRs. The negative- and positive-predictive values were calculated (with 95% confidence intervals).

All tests were deemed significant at a value $p<0.05$.

7.3 Results

7.3.1 Participants tested when unwell

7.3.1.1 Demographics and prior hearing concerns

All eligible patients at each centre were offered the opportunity to participate in the study (see Table 7-2).

Site	Number of potentially eligible patients aged 11 and over*	Number tested when unwell
WMACFC	395	21
WCFC	171	46
BCH	47	3
NCH	40	1
Total	653	71

*Table 7-2: Numbers of patients aged 11 and over eligible to participate in the 3D-CF study when unwell or at the start of a course of intravenous antibiotics.*Not all potentially eligible patients would require IVs during the study period.*

One hundred and forty-six participants consented to do the test when well and unwell. Of these testing was achieved in 71 (48.6%) patients at the start of a course of intravenous antibiotics (see Figure 7-3). Follow-up testing at the clinic visit after completion of intravenous antibiotics was performed in 14 participants (19.7%).

The median age of participants tested at baseline (at the start of a course of intravenous antibiotics) was 31 years. The youngest participant was 16 years old, the oldest was 69 years and 5 months and 31 (43.7%) were female. The severity of lung disease, as measured by the ppFEV$_1$, ranged from normal spirometry (100%) to very severe lung disease (16%) (see Table 7-3).

Allergy to at least one non-aminoglycoside antibiotic was reported by 45 (63.4%) participants while 22 (31.0%) had allergy to two or more non-aminoglycoside antibiotics. Data about aminoglycoside exposure

were available for 70/71 (98.6%) of participants. All participants with available data had been exposed to intravenous aminoglycoside antibiotics. Forty-five (63.4%) participants had received ten or more courses of intravenous aminoglycosides and 25 (35.2%) had received between one and nine courses. Twenty-one participants (29.6%) were treated with an aminoglycoside for the index course (see Table 7-3).

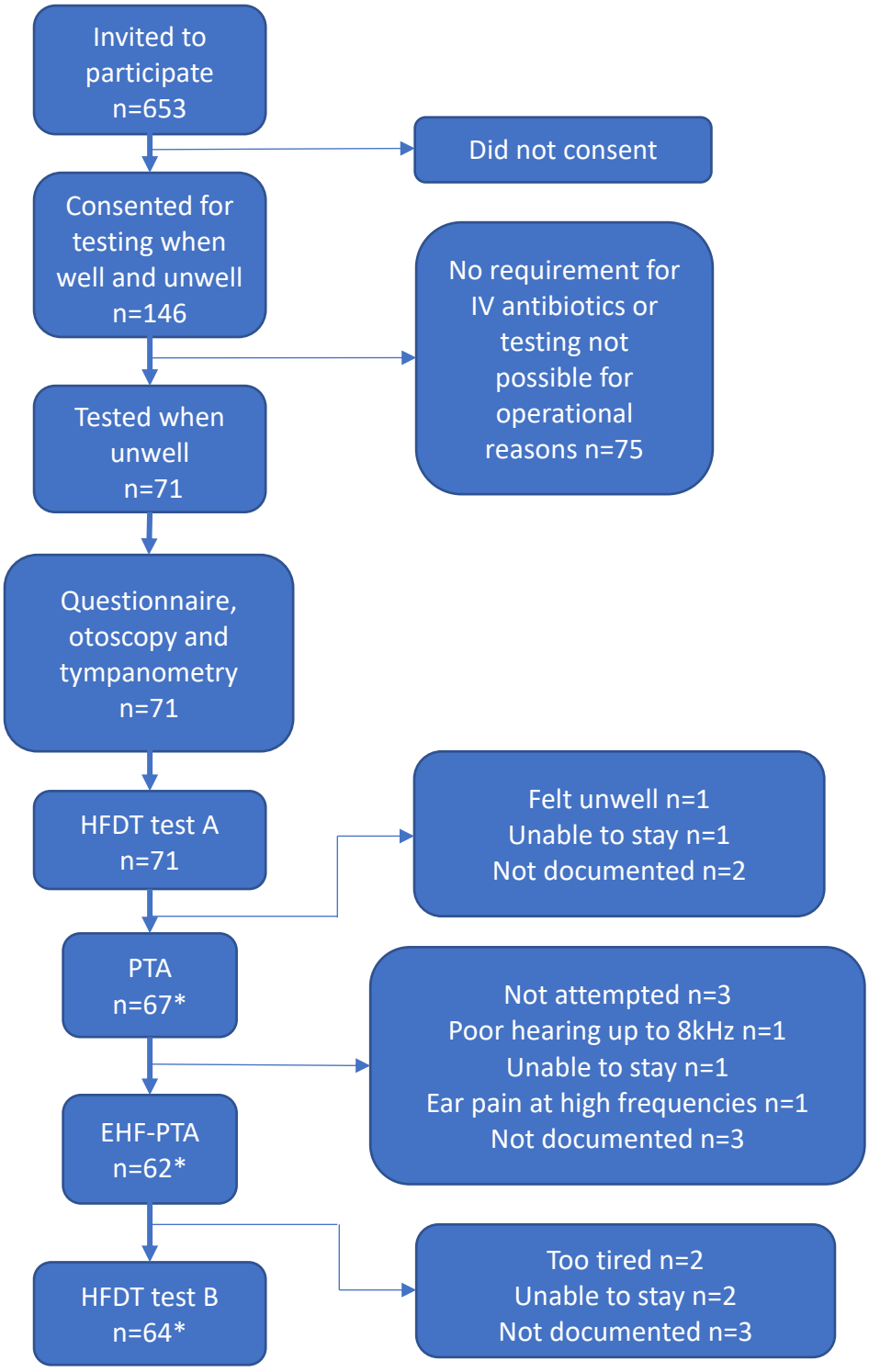


*Figure 7-3: Data completeness by test with reasons in unwell adults and adolescents in the 3D-CF study. HFDT test, High Frequency Digit Triplet test; PTA, Pure Tone Audiogram; EHF-PTA, Extended High-Frequency Pure Tone Audiogram. * Attrition numbers are relative to those completing HFDT test A. The number completing each test does not necessarily drop at each stage as the HFDT test was less demanding than the PTA and EHF-PTA, so some participants were able to complete HFDT test B but not the audiograms.*

Participants	**Baseline** **n = 71***	**Follow-up group** **n = 14***
Age, median years (range)	31.0 (16.0 – 69.4)	30.5 (18.2 - 69.4)
Paediatric participant, number (%)	4 (5.6)	0 (0.0)
Sex, number female (%)	31 (43.7)	6 (42.9)
$ppFEV_1$, median (range)†	49 (16 – 100) n = 46	50 (40 – 67) n = 9
BMI, median (range)†	22.1 (17.6 – 32.2) n = 41	23.0 (18.2 - 30.8) n = 5
CF complications, n (%)		
Pancreatic insufficiency	64 (88.9)	11 (78.6)
CF-related diabetes	38 (52.8)	9 (64.3)
CF-related liver disease	23 (31.9)	7 (50.0)
Sinus disease	25 (34.7)	5 (35.7)
Meconium ileus	12 (16.7)	4 (28.6)
Lung transplant	3 (4.2)	1 (7.1)
Liver transplant	1 (1.4)	0 (0.0)
ABPA	11 (15.3)	3 (21.4)
One or more non-aminoglycoside antibiotic allergy, n (%)	45 (62.5)	11 (78.6)
Two or more non-aminoglycoside antibiotic allergy, n (%)	22 (30.6)	6 (42.9)
Aminoglycoside courses		
0	0 (0.0)	0 (0.0)
1-9	25 (35.2)	5 (35.7)
≥ 10	45 (63.4)	9 (64.3)
Unknown	1 (1.4)	0 (0.0)

Current treatment with aminoglycosides	21 (29.6)	3 (4.2)
Tobramycin	20 (28.2)	3 (4.2)
Amikacin	1 (1.4)	0 (0.0)

*Table 7-3: Demographic and baseline health information for participants with CF tested before and after a course of treatment for a pulmonary exacerbation in the 3D-CF study. * n = 71 for baseline testing and n = 14 for follow-up testing unless otherwise stated. † Not all adult participants had this measured at every encounter. Height especially was often not recorded.*

Thirty-one (43.7%) participants reported concerns about their hearing (see Table 7-4). The commonest were difficulties hearing speech (9.9%) and non-specific concerns (9.9%). Of the 31 participants reporting hearing concerns only 10 (32.3%) had standard frequency hearing loss, while 23/28 (82.1%) of those reporting hearing concerns had loss in the extended high frequencies. Tinnitus or balance problems were reported by 40/71 patients (54.8%); this was most commonly tinnitus in 21/71 (28.8%). Twelve (16.4%) participants related their symptoms of hearing loss and tinnitus or balance problems to aminoglycoside use. Similar to self-reported hearing concerns, 12/39 (30.8%) of patients reporting tinnitus had standard frequency hearing loss, while 30/36 (83.3%) had EHF loss.

Unsurprisingly in participants with an exacerbation of their CF lung disease recent coryzal illness was the most commonly reported alternative risk factor for hearing loss, present in 31/71 (43.6%). Once again noise exposure was common, with 24/71 (33.8%) participants having recreational exposure and 16/71 (22.5%) having occupational exposure. A quarter of participants reported a hearing loss in a family member (see Table 7-4).

Participant-reported hearing concerns	**n = 71**
Any hearing concern, n (%)	31 (43.7)
Specific concern, n (%) *	
Difficulties with background noise	3 (4.2)
Difficulties hearing speech	7 (9.9)
Needs television loud	2 (2.8)
Hearing has declined over time	1 (1.4)
Problems since aminoglycosides	3 (4.2)
Previously known problems	2 (2.8)
Mild or intermittent concerns	2 (2.8)
Blocked ears	1 (1.4)
Concerns expressed by family	5 (7.0)
Non-specific concerns	7 (9.9)
Tinnitus or balance-problems, n (%)	40 (56.3)
Tinnitus	21 (29.5)
Balance	10 (14.1)
Both	7 (9.9)
Not specified	3 (4.2)
Symptoms relate to aminoglycosides	12 (16.9)
Coryzal illness in last 2 weeks, n (%)	31 (43.6)
Mumps, n (%)	6 (8.6)
Meningitis, n (%)	0 (0.0)
Previous ear surgery, n (%)	1 (1.4)
Deafness in a family member, n (%)	18 (25.3)
Chronic middle ear disease, n (%)	3 (4.2)
Prematurity, n (%)	4 (5.6)
Occupational noise exposure, n (%)	16 (22.5)
Recreational noise exposure, n (%)	24 (33.8)

*Table 7-4: Self-reported hearing concerns in 3D-CF study participants tested at the start of a pulmonary exacerbation. * More than one specific concern could be reported by a participant.*

7.3.1.2 Prevalence of hearing loss

Hearing loss in the standard frequencies (up to 8 kHz) was present at baseline in 18 (25.4%) participants. No paediatric participants had standard frequency hearing loss, but the difference in prevalence between adult and paediatric participants was not statistically significant (p=0.566) (see Table 7-5).

Participants	**Hearing loss in the standard frequencies, Mulheran definition (362) n (%)**
All participants, n = 71	18/71 (25.4)
Paediatric participants, n = 4	0/4 (0.0)
Adult participants, n = 67	18/67 (26.9) p = 0.566
Complete data, n = 67	17/67 (25.4)
Paediatric participants, n = 4	0/4 (0.0)
Adult participants, n = 63	17/63 (27.0)

Table 7-5: Prevalence of hearing loss in 3D-CF study participants tested when unwell according to our definition of hearing loss in the standard frequencies; ≥2 frequencies above 20 dB HL in the frequencies 2-8 kHz (362).

PTA data were incomplete in four participants (see Figure 7-3). One participant, who felt, “really rough,” was missing data at 6kHz bilaterally. Two participants were missing data at 3 kHz bilaterally though the reason for this was not documented. In these cases, there was no effect on the categorisation of hearing loss since each participant either already met the definition without these data or did not have >20 dB HL at any other frequency. In one participant 3 kHz was not tested on the

left as the participant had to leave and the test was truncated. This participant had a hearing threshold of 25 dB HL on the left at 8 kHz and so the result at 3 kHz could have changed the categorisation if it had shown a loss of > 20 dB HL. Since this participant had thresholds of 5 dB HL at 2 kHz and 15 dB HL at 4 kHz it is unlikely that the threshold would be high enough to change the categorisation.

Hearing loss in the extended high frequencies was more common. For participants with complete EHF data 50/62 (80.6%) had loss at one or more threshold over 8 kHz, this included two paediatric participants (see Table 7-6).

Participants	**EHF hearing loss (684): ≥ 1 frequencies HL in one or both ears > 20dB HL, n (%)**
Any EHF data, n = 68	56/68 (82.4)
Paediatric participants, n = 4	2/4 (50.0)
Adult participants, n = 64	54/64 (84.4) p = 0.141
Complete data, n = 62	50/62 (80.6)
Paediatric participants, n = 4	2/4 (50.0)
Adult participants, n = 58	48/58 (82.5)

Table 7-6: Prevalence of hearing in the extended high frequencies (EHF) in 3D-CF participants tested when unwell according to the definition of EHF hearing loss in ISO 389-5: 2006 (684).

Complete EHF-PTA was available for 62 participants (87.3%), while EHF-PTA was not attempted in 3 participants (see Figure 7-3); one due to ill health, one due to poor concentration and one due to significant

loss in the standard frequencies. In six participants high frequency testing was abbreviated. One had poor hearing to 8 kHz and all tested frequencies above 8 kHz showed hearing loss above the measurement limit of the audiometer. One had a further appointment so asked to stop, one had ear discomfort during testing at 14 kHz on the left so no further testing at 14 and 16 kHz was attempted. In three participants no reasons for an abbreviated protocol were documented. All participants in whom EHF-PTA testing was incomplete fulfilled the criteria for EHF-hearing loss on the basis of the frequencies tested so no change in classification was caused by the incomplete data.

7.3.1.3 Feasibility of the HFDT test in unwell participants

All eligible participants at each of the four sites were invited to participate (see Table 7-2). One hundred and forty-six participants consented to participate, of these 71 (48.6%) were tested at the start of a course of IV antibiotics. Reasons for failure to test participants who had previously given consent included the participant not having IV antibiotics during the study period, inability of the researcher to attend within the specified time period, participant unavailability (for example, presence of other healthcare professionals or participant had left the ward) and inability of the participant to return for testing (for example, if antibiotics were given at home and started when the researcher was unavailable).

All tested participants were able to complete one HFDT test (see Figure 7-3). Fifty-five participants (76.4%) were able to complete the entire

test protocol. In four participants (5.6%) tiredness or ill-health required a shortened test protocol (see Figure 7-3).

A second HFDT test was obtained in 64/71 participants (90.1%). This test was optional in the protocol and could be omitted if the participant was tiring. Two participants were noted to be tired, so testing was not attempted, two had other commitments so had to leave and for three no reason was given, though it is likely that this decision was made on clinical grounds.

More participants were able to complete the HFDT test than the PTA. More participants were able to complete a second HFDT test than were able to complete the EHF-PTA.

Coughing, a predicted concern, did not adversely affect test completion as there was scope to pause all tests until a bout of coughing had subsided.

7.3.1.4 Changes from baseline in patients with repeat tests

Fourteen (19.7%) participants were followed up at repeat clinic appointment. The median time to follow-up test was 55 days. In view of the small numbers tested at follow-up the results have been reported narratively and no statistical testing has been carried out.

Two participants (2/14; 14.3%) reported new ear concerns at follow-up. One described as “vibrating in both ears”. This participant had not been treated with aminoglycosides. The other had been treated with tobramycin and reported intermittent earache, but felt their hearing was unchanged.

	Baseline **n = 14**	**Follow-up** **n = 14**
Time to follow-up, median days (range)	n/a	55 (33 – 121)
Hearing loss, n (%) Standard frequencies Extended high frequencies	 2/14 (14.3) 9/13* (69.2)	 3/14 (21.4) 10/14 (71.4),
N (%) with ototoxic increase in hearing thresholds (defined as per ASHA guidelines) Standard frequencies Extended high frequencies	 n/a n/a	 3/14 (21.4) 2/14 (14.3)
HFDT SRT, median (range)	-22.1 (-6.0 to -24.8)	-22.9 (-6.2 to -25.1)
Treated with aminoglycosides	3 (21.4)	n/a

*Table 7-7: Changes in hearing at baseline and follow-up in 14 participants tested before and after a course of aminoglycoside antibiotics as part of the 3D-CF study. *One participant who felt unwell and had poor hearing up to 8 kHz did not undergo EHF-PTA at baseline. HFDT, high frequency digit triplet test. SRT, speech reception threshold.*

Of the participants with data at both baseline and follow-up 2/14 (14.3%) had hearing loss in the standard frequencies and 9/13 (69.2%) in the EHF at baseline (see Table 7-7). At follow-up 3/14 (21.4%) participants had standard frequency hearing loss; one participant had new hearing loss in the standard frequencies. EHF hearing loss was present in 10/14 (71.4%) at follow-up; the additional participant had not had EHF-PTA testing at baseline.

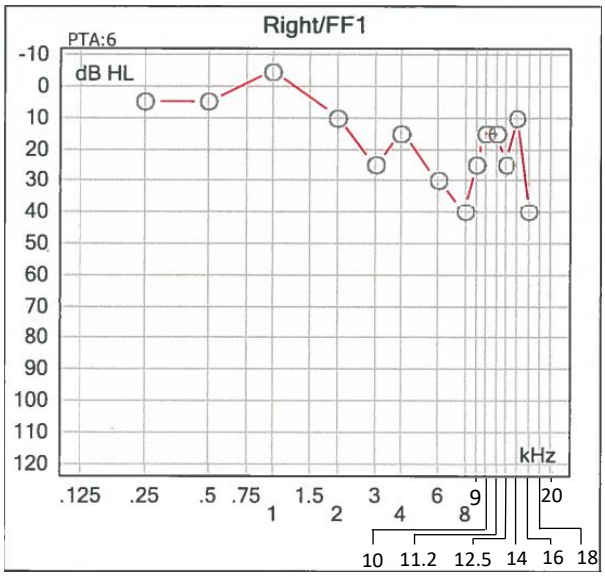
Three participants had a hearing change from baseline at follow-up testing in the standard frequencies (see Table 7-7). One participant had normal hearing at baseline but fulfilled the criteria for both hearing loss in the standard frequencies and ototoxicity in the right ear at follow-

up. There was an increase in threshold of +10 dB HL at 4 and 6 kHz and +25 dB HL at 8 kHz (see Figure 7-4). No clinically significant changes occurred in the left ear. This participant could not be followed up until 121 days after initial testing and again presented unwell, requiring a further course of IV antibiotics. A second participant did not fulfil the criteria for hearing loss at either baseline or follow-up but had a threshold increase of +10dB HL at 4, 6 and 8 kHz on the right at repeat testing and so fulfilled the ASHA criteria for ototoxicity.

A third participant fulfilled the criteria for hearing loss in the standard frequencies at both baseline and follow-up testing. This participant had a 10dB HL threshold shift at 0.25 kHz and 0.5 kHz on the right but no changes to meet ototoxicity criteria at any other frequencies. This participant met the ASHA criteria for ototoxicity though it is unusual for ototoxicity to present with changes solely in the lowest frequencies.

No participant, who had previously not met the criteria for EHF hearing loss, newly met these criteria following antibiotic treatment. Two participants had increased thresholds meeting the definition of ototoxicity in the extended high frequencies (see Table 7-7). One had increases of at least +10 dB HL at 9 – 12.5 kHz on the right and 9 – 10 kHz on the left. This participant had not been treated with tobramycin but was unwell, requiring a further course of IV antibiotics, at follow-up testing (see Figure 7-4). A second participant had a threshold increase of +20 dB HL 11.2 kHz on the left only. The adjacent frequencies were stable. This participant had been treated with an aminoglycoside.

Three participants were treated with an aminoglycoside (all tobramycin). Of the three participants treated with an aminoglycoside only one had a change in hearing thresholds consistent with ototoxicity. The two other participants did not meet the criteria for either ototoxicity or hearing loss at standard frequencies or EHF hearing loss at baseline or follow-up.



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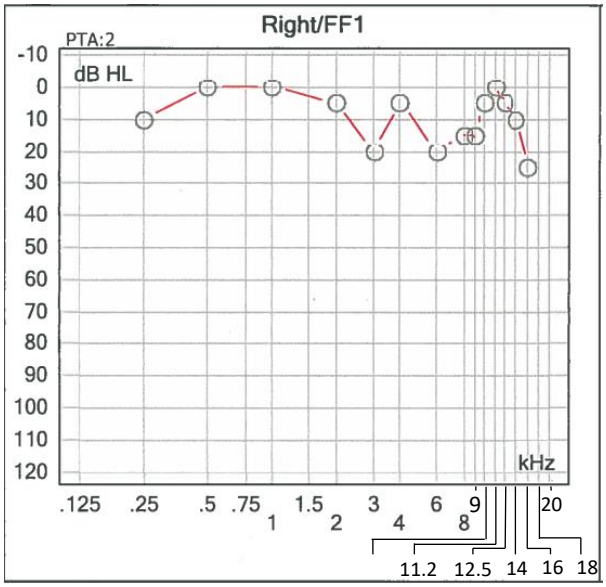

B

Figure 7-4: Deterioration in hearing between initial test and follow-up in the right ear of a participant in the 3D-CF study initially tested when unwell. A – initial test. B – follow-up test.

7.3.1.5 Reliability of the HFDT test in unwell participants

An initial HFDT test was completed by all participants. Whilst only 67 participants had complete PTA data since the categorisation of hearing loss would not have been changed in three and was unlikely to have changed in the last patient, all participants have been included in the analysis.

The sensitivity and specificity of the HFDT test for a range of SRT cut-offs in the setting of unwell participants is shown in Table 7-8 and the corresponding ROC curve is shown in Figure 7-5. Where a cut-off SRT of -21.7 dB SNR is applied, then 88.9% (95% CI 67.2% - 96.9%) of participants with hearing loss are identified, with a specificity of 71.7% (95% CI 58.4% - 82.0%). This would give the test a positive predictive value (PPV) of 51.6% (95% CI 34.8% - 68.2%); a participant with hearing loss identified on the HFDT test has a 51.6% chance of actually having hearing loss. The negative predictive value (NPV) is 95.0% (95% CI 83.5% - 98.6%); a participant without hearing loss on the HFDT test has a 95% chance of having normal hearing. Using the optimum cut-off identified in participants tested when clinically well, -22.1 dB SNR, the sensitivity remains 88.9% (95% CI 39.7% - 65.6%) with a specificity of 52.8% (95% CI 67.2% - 96.9%). At this cut-off SNR the PPV is 39.0% (95% CI 25.6% - 54.3%) and the NPV is 93.3% (95% CI 78.7% - 98.2%).

Detailed report of sensitivity and specificity

Cutpoint	Sensitivity	Specificity	Correctly classified	LR+	LR-
(>= -24.8)	100.00%	0.00%	25.35%	1.0000	
(>= -24.2)	100.00%	1.89%	26.76%	1.0192	0.0000
(>= -23.8)	100.00%	5.66%	29.58%	1.0600	0.0000
(>= -23.6)	100.00%	11.32%	33.80%	1.1277	0.0000
(>= -23.4)	100.00%	22.64%	42.25%	1.2927	0.0000
(>= -23.2)	100.00%	26.42%	45.07%	1.3590	0.0000
(>= -22.9)	100.00%	30.19%	47.89%	1.4324	0.0000
(>= -22.7)	100.00%	33.96%	50.70%	1.5143	0.0000
(>= -22.5)	100.00%	47.17%	60.56%	1.8929	0.0000
(>= -22.3)	94.44%	47.17%	59.15%	1.7877	0.1178
(>= -22.1)	88.89%	52.83%	61.97%	1.8844	0.2103
(>= -21.9)	88.89%	64.15%	70.42%	2.4795	0.1732
(>= -21.7)	88.89%	71.70%	76.06%	3.1407	0.1550
(>= -21.5)	83.33%	77.36%	78.87%	3.6806	0.2154
(>= -21.3)	83.33%	79.25%	80.28%	4.0152	0.2103
(>= -21.1)	77.78%	79.25%	78.87%	3.7475	0.2804
(>= -20.6)	77.78%	81.13%	80.28%	4.1222	0.2739
(>= -20.4)	72.22%	81.13%	78.87%	3.8278	0.3424
(>= -20)	72.22%	86.79%	83.10%	5.4683	0.3200
(>= -19.8)	66.67%	88.68%	83.10%	5.8889	0.3759
(>= -19.6)	66.67%	92.45%	85.92%	8.8333	0.3605
(>= -19.4)	55.56%	92.45%	83.10%	7.3611	0.4807
(>= -19.2)	55.56%	96.23%	85.92%	14.7222	0.4619
(>= -18.9)	55.56%	98.11%	87.32%	29.4445	0.4530
(>= -17.7)	44.44%	98.11%	84.51%	23.5556	0.5662
(>= -15.6)	38.89%	98.11%	83.10%	20.6112	0.6229
(>= -14.3)	33.33%	98.11%	81.69%	17.6667	0.6795
(>= -13.1)	27.78%	100.00%	81.69%		0.7222
(>= -12)	22.22%	100.00%	80.28%		0.7778
(>= -8.4)	16.67%	100.00%	78.87%		0.8333
(>= -6.5)	11.11%	100.00%	77.46%		0.8889
(>= -6)	5.56%	100.00%	76.06%		0.9444
(> -6)	0.00%	100.00%	74.65%		1.0000

Obs	ROC area	Std. err.	Asymptotic normal [95% conf.	interval]
71	0.8868	0.0445	0.79960	0.97398

Table 7-8: Detailed report of the sensitivity and specificity of the HFDT (high frequency digit triplet) test compared to hearing loss identified on pure tone audiogram (PTA) at 2-8 kHz as previously defined (362) in participants with CF tested in the 3D-CF study when unwell. Correctly classified – the percentage of participants correctly classified as having hearing loss or not by the HFDT test using each cut point. LR+ - positive likelihood ratio, LR- - negative likelihood ratio.

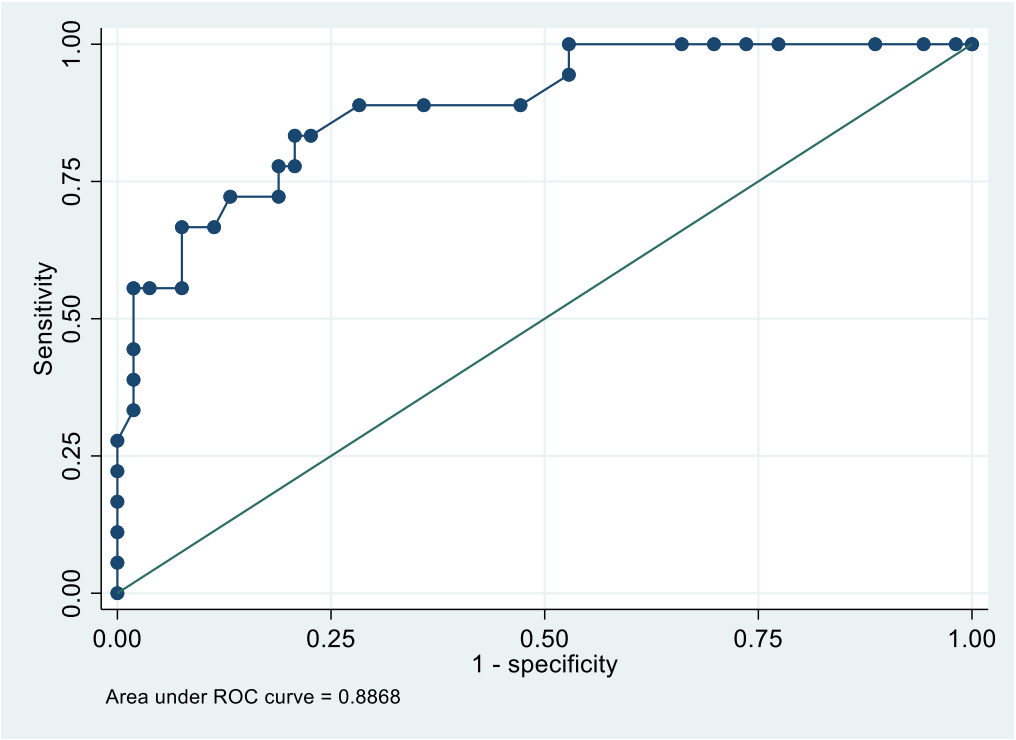

Figure 7-5: ROC curve comparing the HFDT (high frequency digit triplet) test to hearing loss identified on pure tone audiogram (PTA) at 2-8 kHz as previously defined (362) in participants with CF tested in the 3D-CF study when unwell.

7.3.1.6 Acceptability of the HFDT test

Fifteen unwell participants were formally asked to report their feelings about the HFDT test. Two people commented that the test was frustrating, one found it, “a bit hard,” and one struggled with the test due to dyslexia. Despite these comments all fifteen were happy with the test and would be happy to perform it at a clinic appointment or at the start of a course of IV antibiotics. Of note 12/12 (100.0%) well participants reported they would also be happy to perform the HFDT test at the start of a course of IV antibiotics.

7.3.2 Participants aged 5-10 years old

7.3.2.1 Demographics and prior hearing concerns

Site	Number of eligible patients aged 5-10 years	Number tested
BCH	54	21
NCH	25	19

Table 7-9: Number of patients aged 5-10 years eligible to participate in the 3D-CF study at each paediatric site and numbers actually tested.

Forty children aged 5-10 years, with CF were recruited into the study (see Figure 7-6). The median age of these children was 8.0 years and the recruitment by age category is shown in Table 7-10. Fifteen children (37.5%) were female. Disease severity, as measured by the ppFEV$_1$, ranged from normal spirometry (120%) to moderate lung disease (60%). Five children (12.8%) had allergy to one non-aminoglycoside antibiotic, but no children had more than one allergy.

Fifteen children (37.5%) had no exposure to intravenous antibiotics and 25/40 (62.5%) had received between one and nine courses. No child in this cohort had received ten or more courses of intravenous aminoglycosides.

Eighty-four control children were recruited from local schools (see Figure 7-7). The median age of these children was 8 years and two months and 43/84 (51.2%) were female. The recruitment by age is shown in Table 7-10.

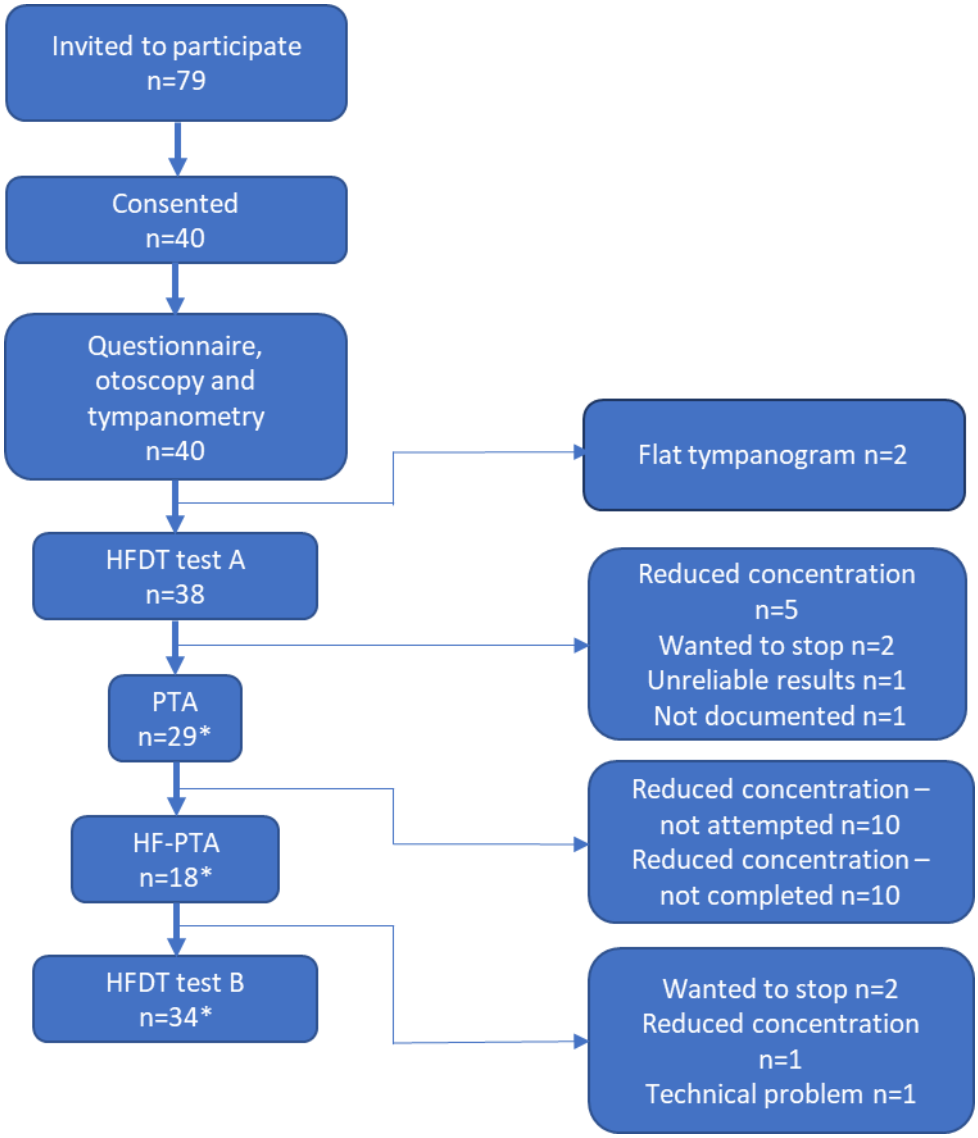

*Figure 7-6: Data completeness by test with reasons for children with CF tested in the 3D-CF study. HFDT test, High Frequency Digit Triplet test; PTA, Pure Tone Audiogram; EHF-PTA, Extended High-Frequency Pure Tone Audiogram. * Attrition numbers are relative to those completing HFDT test A. The number completing each test does not necessarily drop at each stage as the HFDT test was less demanding than the PTA and EHF-PTA, so some participants were able to complete HFDT test B but not the audiograms.*

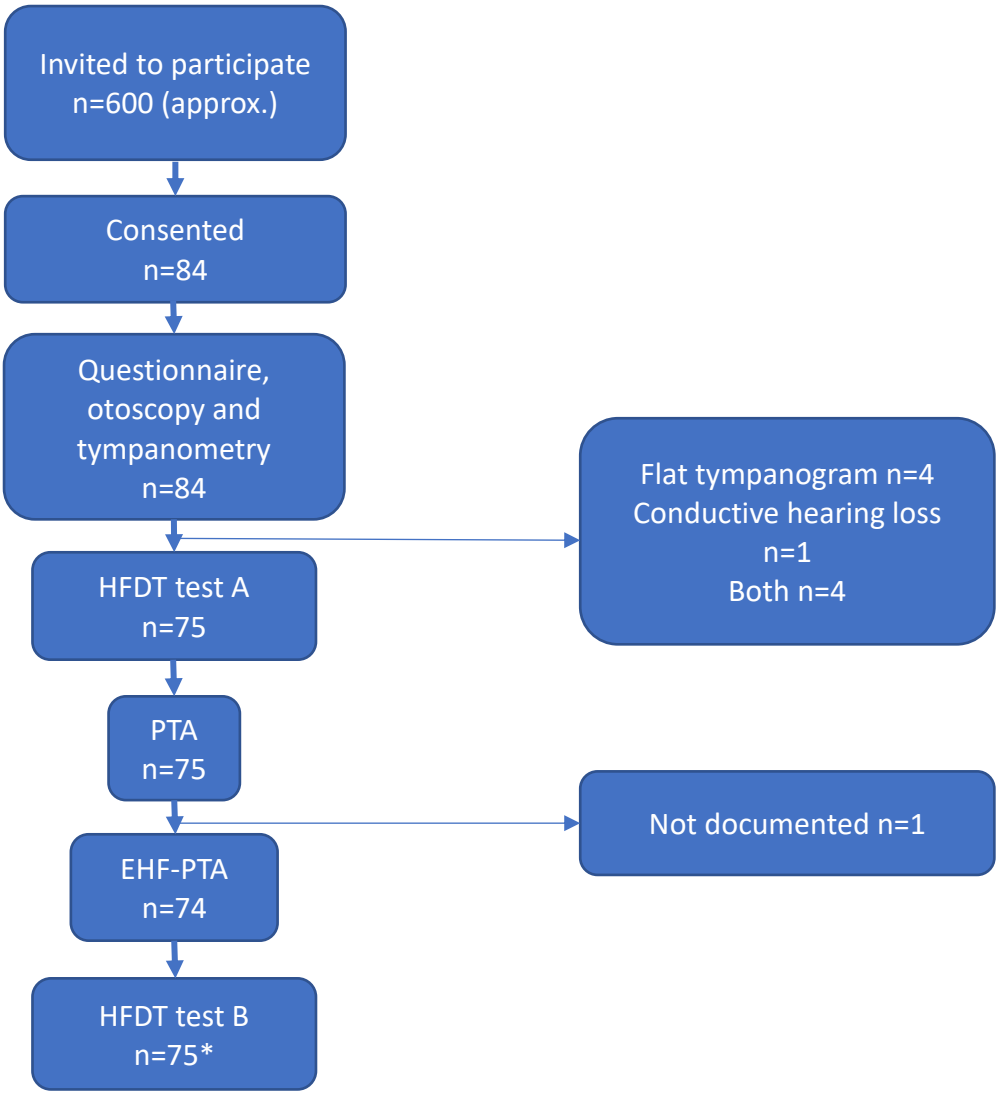

*Figure 7-7: Data completeness by test with reasons for control children tested in the 3D-CF study. HFDT test, High Frequency Digit Triplet test; PTA, Pure Tone Audiogram; EHF-PTA, Extended High-Frequency Pure Tone Audiogram. * Attrition numbers are relative to those completing HFDT test A. The number completing each test does not necessarily drop at each stage as the HFDT test was less demanding than the PTA and EHF-PTA, so some participants were able to complete HFDT test B but not the audiograms.*

5–10-year-old participants	**Children with CF n = 40**	**Non-CF control children n = 84**
Age, median years (range)	8.0 (5.5 – 10.8)	8.2 (5.1 - 10.9)
5-year-olds, n (%)	6 (15.0)	15 (17.9)
6-year-olds, n (%)	11 (27.5)	12 (14.3)
7-year-olds, n (%)	3 (7.5)	12 (14.3)
8-year-olds, n (%)	9 (22.5)	15 (17.9)
9-year-olds, n (%)	4 (10.0)	13 (15.5)
10-year-olds, n (%)	7 (17.5)	17 (20.2)
Sex, number female (%)	15 (37.5)	43 (51.2)
$ppFEV_1$, median (range) n = 39*	94 (60-120)	n/a
BMI, median (range) n = 39*	16.1 (13.4 – 22.6)	n/a
CF complications, n (%)		
Pancreatic insufficiency	38 (95.0)	
CF-related diabetes	0 (0)	
CF-related liver disease	8 (20.0)	
Sinus disease	9 (22.5)	n/a
Meconium ileus	8 (20.0)	
Lung transplant	0 (0)	
Liver transplant	0 (0)	
ABPA	2 (5.0)	
One or more non-aminoglycoside antibiotic allergy, n (%)	5 (12.8)	n/a
Two or more non-aminoglycoside antibiotic allergy, n (%)	0 (0)	n/a
Aminoglycoside courses		n/a
0	15 (37.5)	

1-9	25 (62.5)	
≥ 10	0 (0.0)	

*Table 7-10: Demographic and baseline health information for children with CF and control children in the 3D-CF study. * No data available for one child.*

Participant-reported hearing concerns	**Children with CF n = 40**	**Non-CF control children n = 84**
Any hearing concern, n (%)	7 (17.5)	9 (10.7)
Specific concern, n (%) *		
Parental concerns	2 (5.0)	5 (6.0)
Glue ear	1 (2.5)	1 (1.2)
Hearing vs. Attention	2 (5.0)	2 (2.4)
Loud TV	1 (2.5)	0 (0.0)
Not specified	1 (2.5)	0 (0.0)
School concern	2 (5.0)	0 (0.0)
Unilateral hearing loss	0 (0.0)	4 (4.8)
Muffled hearing	0 (0.0)	2 (2.4)
Blocked ear	0 (0.0)	1 (1.2)
Tinnitus or balance-problems,	4 (10.0)	0 (0.0)
Tinnitus, n (%)	4.0 (10.0)	0 (0.0)
Balance, n (%)	0 (0.0)	0 (0.0)
Coryzal illness in last 2 weeks, n (%)	12 (30.0)	13 (15.5)
Mumps, n (%)	0 (0.0)	0 (0.0)
Meningitis, n (%)	1 (2.5)	0 (0.0)
Previous ear surgery, n (%)	1 (2.5)	0 (0.0)
Deafness in a family member, n (%)	8 (20.0)	6 (7.1)
Chronic middle ear disease, n (%)	2 (5.0)	2 (2.4)
Prematurity, n (%)	3 (7.5)	4 (4.8)

Occupational noise exposure, n (%)	0 (0.0)	0 (0.0)
Recreational noise exposure, n (%)	1 (2.5)	0 (0.0)
Other ear problem, n (%) Recent ear infection Previous investigations Ear pain Wax	0 (0.0) 0 (0.0) 0 (0.0) 0 (0.0) 0 (0.0)	7 (8.3) 1 (1.2) 1 (1.2) 3 (3.6) 2 (2.4)

*Table 7-11: Self-and parent-reported hearing concerns in children with CF and control children in the 3D-CF study. *Specific concerns could be reported by more than one participant.*

Seven (17.5%) children with CF reported concerns with their hearing (see Table 7-11). In two concerns had been raised by the school. Parents had some concerns about the hearing of two children while in another two the parents were unsure if the problem was related to hearing or attention. One of the children reporting hearing concerns had hearing loss in the standard frequencies. This child was found to have flat tympanograms and was, therefore, excluded from the rest of the study. In the 38 children completing high frequency testing, EHF loss was found in 1/5 (20%) of those reporting hearing concerns.

In contrast only 9/84 (10.7%) control children reported any hearing concerns (see Table 7-11). Parental concerns were raised about five of these children, with four (4.8%) reporting unilateral hearing loss. Again, in two children the parents were unsure whether the problem was hearing or attention. Of these nine participants, six were excluded due to flat tympanograms. No hearing loss was found in the remaining three, in either the standard or extended high frequencies.

Four children with CF (10.0%) reported tinnitus or balance problems but no control children reported these symptoms. Of the four children reporting these symptoms, none had standard frequency hearing loss, but 2/2 (100.0%) of those who underwent EHF testing, had loss in these frequencies. Recent coryzal illness was more common in children with CF (30.0%) compared to control children (15.5%).

7.3.2.2 Prevalence of hearing loss

Of the forty children with CF for whom consent was given, 2 (5.0%) were found to have either flat tympanograms or evidence of conductive hearing loss and so were excluded from analysis, which was conducted on data from 38 children (see Table 7-12).

In 29/38 (76.3%) children with CF the criteria for a successful PTA were met (i.e., data at 1, 2, 4 and 8 kHz bilaterally). In those children not meeting this standard, 5 struggled with concentration, 2 were bored and wanted to stop, 1 had very unreliable results as continually wanted to press the button and for 1 the reason was not documented (but was the second child in a pair of siblings to be tested so possibly had reduced concentration by this stage) (see Figure 7-6).

No child (0/38) met the criteria for standard frequency hearing loss, in either those with or without a successful PTA. The child with conductive hearing loss (not analysed for HFDT test) had thresholds over 20 dB HL at 0.5 and 1 kHz on the right and 0.5, 1 and 2 kHz on the left. This was outside the standard frequencies included in the definition of hearing loss (2-8 kHz) (362).

Children with CF, age	PTA success, n (%)	Standard hearing loss, n (%)	EHF-PTA attempted, n (%)	EHF-PTA success, n (%)*	EHF-hearing loss, n (%)*
5 years (n=5)	4/5 (80.0)	0/5 (0.0)	4/5 (80.0)	2/4 (50.0)	0/4 (0.0)
6 years (n=11)	5/6 (45.5)	0/6 (0.0)	6/6 (54.5)	2/6 (33.3)	0/6 (0.0)
7 years (n=3)	3/3 (100.0)	0/3 (0.0)	2/3 (66.7)	1/2 (50.0)	2/2 (100.0)
8 years (n=9)	9/9 (100.0)	0/9 (0.0)	6/9 (66.7)	4/9 (66.7)	1/9 (16.6)
9 years (n=4)	3/4 (75.0)	0/4 (0.0)	4/4 (100.0)	3/4 (75.0)	1/4 (25.0)
10 years (n=6)	5/6 (83.3)	0/6 (0.0)	6/6 (100.0)	6/6 (100.0)	1/6 (16.7)
Total (n = 38)	29/38 (76.3)	0/38 (0.0)	28/38 (73.7)	18/38 (64.3)	5/38 (17.9)

*Table 7-12: Hearing loss in the standard and extended high frequencies by age (years) in children with CF tested in the 3D-CF study. Standard hearing loss was defined according to Mulheran et al. (362) and EHF-hearing loss by ISO 389-5:2006 (684). EHF, extended high frequencies, i.e., above 8 kHz. PTA, pure tone audiogram. *Of those in whom EHF-PTA was attempted.*

EHF-PTA was attempted in 28/38 (73.7%) children with CF and 18/28 (64.3%) met the criteria for a successful test (see Figure 7-6). Extended high-frequency hearing loss was present in 5/28 (17.9%)

children in whom testing was attempted, four of whom were successfully tested, and one who was tested at 12.5 kHz on the right only. Testing success and presence of hearing loss by age is shown in Table 7-12.

Control children, age	**PTA success, n (%)**	**Standard hearing loss, n (%)**	**EHF-PTA success, n (%)**	**EHF-hearing loss, n (%)**
5 years (n=15)	15/15 (100.0)	0/15 (0.0)	14/15 (93.3)	2/15 (13.3)
6 years (n=10)	10/10 (100.0)	0/10 (0.0)	10/10 (100.0)	1/10 (10.0)
7 years (n=11)	11/11 (100.0)	0/11 (0.0)	11/11 (100.0)	1/11 (9.09)
8 years (n=14)	14/14 (100.0)	0/14 (0.0)	14/14 (100.0)	1/14 (7.14)
9 years (n=12)	12/12 (100.0)	0/12 (0.0)	12/12 (100.0)	1/12 (8.3)
10 years (n=13)	13/13 (100.0)	0/13 (0.0)	13/13 (100.0)	1/13 (7.7)
Total (n = 75)	75/75 (100.0)	0/75 (0.0)	74/75 (98.7)	7/75 (9.3)

Table 7-13: Hearing loss in the standard and extended high frequencies by age (years) in control children tested in the 3D-CF study. Standard hearing loss was defined according to Mulheran et al. (362) and EHF-hearing loss by ISO 389-5:2006 (684). EHF, extended high frequencies, i.e., above 8 kHz. PTA, pure tone audiogram.

Nine of the 84 control children consented were excluded due to flat tympanograms (n = 4), conductive hearing loss (n = 1) or both (n=4) (see Figure 7-7). Analysis is therefore presented for 75 children.

All the control children had a successful PTA at 1, 2, 4 and 8 kHz, though none was tested at 3 and 6 kHz (see Table 7-13). No control child, not already excluded, had hearing loss in the standard frequencies. EHF-PTA was successful in all but one five-year-old child (see Table 7-13). EHF hearing loss was found in 7/75 control children (9.3%).

There was no significant difference between the rate of EHF hearing loss in children with CF and non-CF control children (p = 0.23).

7.3.2.3 Feasibility of the HFDT test in children aged 5-10 years

The initial analysis plan was to delineate the age at which 80% of children could complete the HFDT test and the correlation between the SRT (obtained from the HFDT test) and the PTA is at least half that of adult participants. Since the HFDT test in adults proved unreliable, this was not possible. The number of children able to complete the test at each age and the median SRT at each age has therefore been presented. The results for children with CF were underpowered due to difficulties with recruitment so no further statistical analysis has been performed.

All 38 children were able to complete one HFDT test though 5/38 (13.2%) required assistance. All children needing help struggled to recall the numbers for long enough to type them into the test. Two

were able to overcome this problem by calling out the numbers as they heard them prior to typing; three also needed an adult (parent or researcher) to type the numbers recalled by the child into the test (see Figure 7-8).

All seventy-five control children were able to complete the HFDT test successfully and independently (see Figure 7-9).

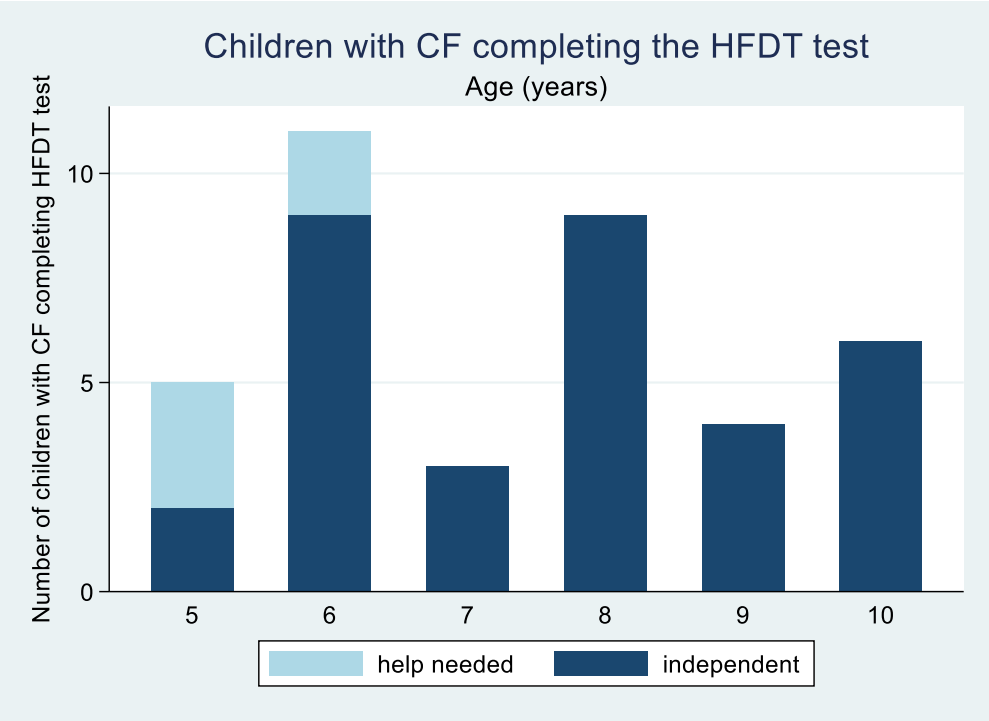

Figure 7-8: Bar chart to show the number of children with CF at each age between 5 and 10 years successfully completing the High Frequency Digit Triplet (HFDT) test independently and with help in the 3D-CF study.

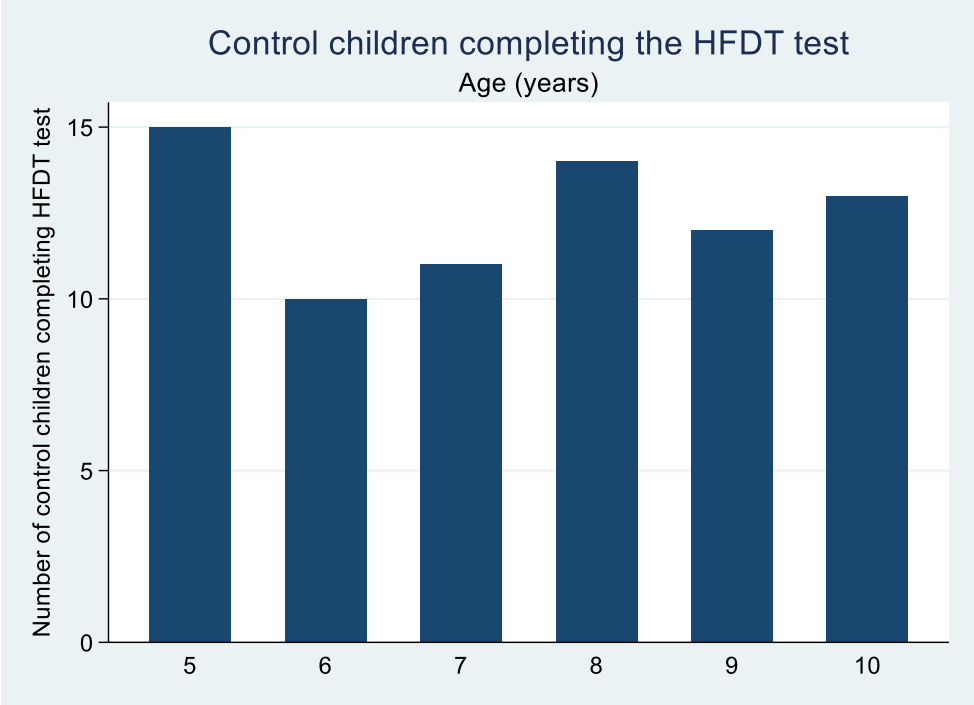

Figure 7-9: Bar chart to show the number of control children at each age between 5 and 10 years successfully completing the High Frequency Digit Triplet (HFDT) test independently and with help in the 3D-CF study.

There was a trend towards lower scores in the five-year-olds in both the children with CF and the control group (see Table 7-14); during the study it was felt that children of this age, whilst able to complete the test struggled more than older children. For example, one five-year-old with CF became bored and frustrated if more than one answer was wrong in a row. This child had an SRT of only -8.7 dB HL and it was felt during testing this child that turning off the “correct” and “incorrect” responses after each triplet might help with concentration in young children.

	Children with CF	Control Children
SRT by age, median (range)	**-20.5 (-8.7 to -26.3)** (n = 38)	**-19.2 (-6.1 to -23.6)** (n = 75)
5 years	**-16.4 (-8.7 to -19.6)** (n = 5)	**-17.5 (-6.1 to -20.4)** (n = 15)
6 years	**-20.2 (-18.5 to -22.5)** (n = 11)	**-19.4 (-9.5 to -20.6)** (n = 10)
7 years	**-23.4 (-20.2 to -24.0)** (n = 3)	**-19.2 (-13.7 to -20.8)** (n = 11)
8 years	**-20.6 (-17.9 to -24.2)** (n = 9)	**-19.6 (-15.8 to -23.4)** (n = 14)
9 years	**-22.2 (-17.9 to -26.3)** (n = 4)	**-21.2 (-14.9 to -23.6)** (n = 12)
10 years	**-21.5 (-16.8 to -24.6)** (n = 5)	**-19.2 (-11.4 to -22.7)** (n = 13)

Table 7-14: Median SRT (in dB SNR) obtained from the HFDT test of children with CF and control children at each age bracket between 5 and 10 years in the 3D-CF study. SRT, speech reception threshold. dB SNR, decibels signal-to-noise-ratio. HFDT, high frequency digit triplet. (SRT is more negative with better hearing.)

7.3.2.4 Acceptability of the HFDT test in children

Ten children with CF, tested after the addition of formal questioning about the acceptability of the HFDT test, were asked if they found the HFDT test acceptable and if they would be happy to perform the test at a clinic visit in future. All felt that they would be happy to perform the test in clinic. Two volunteered that they liked the HFDT better than the PTA, with one saying it was, “fun listening”. One child preferred the “beep-beep test”. One further child reported, before direct questioning was introduced, that, “I like the new test”. This child felt that the PTA took too long while the HFDT test, “used your brain.”

7.4 Discussion

7.4.1 Prevalence of hearing loss

Hearing loss in the standard frequencies was present in a quarter of unwell adults. Extended high frequency hearing loss was more common, occurring in over 80% of adults and 50% of paediatric participants. These figures are similar to those found in well participants (see section 6.4.1).

No children with CF or control children had evidence of sensorineural hearing loss (SNHL) in the standard frequencies. This is similar to some studies looking for evidence of SNHL in children with CF, which have reported prevalences of 0-3.7% (356, 364, 366), though other studies have reported a much higher prevalence. Cheng *et al.,* in a retrospective review of paediatric medical records found SNHL in 14% of children; this was significantly related to the number of courses of IV aminoglycosides received (354). Al-Malky *et al.,* in a prospective study, found hearing loss in 18.6% of children aged 3.1-16.4 years (145). Again, children with a higher exposure to aminoglycosides were more likely to have hearing damage. Kreicher *et al.,* in a retrospective case review of paediatric CF patients who had undergone PTA, found SNHL in 2.3% of ears but any hearing loss in 32% of participants (372). Blankenship *et al.,* found SNHL in 41.2% of ears in children and young adults with CF aged 6-21 years, tested on admission to hospital for treatment with intravenous aminoglycosides (314). Hearing loss in the standard frequencies was present in 38% of ears compared to 17% in

the non-CF control group. The higher prevalence of hearing loss was attributed to the very stringent definition of hearing loss (one or more frequencies >15 dB HL in the standard audiogram, not including 3 and 6 kHz). The same definition of hearing loss was used by Kreicher *et al.,* and the definition used by Al-Malky *et al.,* one or more threshold ≥ 20 dB HL, is functionally identical (145, 372). In contrast the studies with lower prevalences used variable definitions but all considered thresholds of 20 dB HL normal (356, 364, 366).

The definition of hearing loss is key. Solmaz *et al.*, examining the effect of amikacin administration on 32 children with CF and 35 control children, noted that, when they defined SNHL as a mean threshold of >25 dB HL over 0.5, 1, 2 and 4 kHz, then SNHL was present in 4/32 (12.5%) CF participants. This number increased to 30/32 (93.8%) when the mean threshold cut-off was decreased to > 15 dB HL. None of the 35 control children met either definition of SNHL (699). Forman-Franco *et al.* compared the number of children with CF found to have hearing loss in a number of studies using either a cut-off of 15 dB HL or 25 dB HL. In almost all the studies considered, more children with hearing loss were identified using the lower threshold for hearing loss (312). The authors supported the use of 25 dB HL as the cut-off value, in line with the contemporaneous definition used by the American Academy of Ophthalmology and Otolaryngology (700). With current understanding, this cut-off may be too insensitive (314).

The data presented in this thesis show that though there was a trend towards higher rates of hearing loss in the EHF in children with CF, with

EHF hearing loss found in 17.9% of children with CF and 9.3% of healthy control children, this was not statistically significant. This is similar to the EHF rates reported by the group at Great Ormond Street of 14.8% (356) and 21.4% (145) but in contrast to other work. Geyer *et al.* found EHF hearing loss in 30.8% of children with CF but only 2.8 % of control children (p=0.004). However, their population was older (mean age 13.0 years), and a higher proportion (15.6%) had received more than 10 courses of aminoglycosides (364). Blankenship *et al.* also found EHF hearing loss in 47% of participants with CF compared to 30% in the non-CF control group (reported as significant though p-value not given) (314). Again, they examined older children and young adults with greater aminoglycoside exposure and both studies used the lower threshold for hearing loss of any one threshold >15 dB HL (145, 314).

Conductive hearing loss was twice as common in control children compared to children with CF (6.0% versus 2.5%). The reported prevalence of conductive hearing loss in children with CF is variable. Kreicher *et al.*, found that CHL was the commonest specified cause of hearing loss. This pattern was found in 5.3% of ears compared to SNHL which occurred in 2.3% (372). David reported CHL in 4/106 (3.8%) children with CF (701). In contrast, Kulczycki *et al.,* examining 70 children with CF found mild conductive hearing loss in 27%, far higher than their predicted prevalence in school age children of 5-7% (702). Blankenship *et al.* included bone conduction testing in their study protocol and found CHL in 6%, with mixed hearing loss in a

further 10%. A non-CF control group had rates of 1.6% and 5% respectively (314). The authors attributed the higher rate in their study to the use of bone conduction to specifically assess for CHL, rather than relying on tympanometry alone. Other studies have found no difference in rates of CHL in children with CF and non-CF control children (312, 703).

Many more recent studies of hearing loss in CF have focussed on SNHL, excluding participants with evidence of middle ear disease. Al-Malky *et al.* excluded 5/76 (6.6%) participants due to middle ear disease (145); other studies have excluded between 0 and 4% of participants (356, 362, 366) on the basis of abnormal tympanometry, though there are no data on whether these participants had associated hearing loss.

7.4.2 Feasibility of the HFDT test

All tested participants were able to undertake at least one HFDT test.

In unwell adolescent and adult participants all were able to undertake at least one HFDT test, whilst only 76.4% of participants were able to complete the entire test protocol. More were able to complete the HFDT test than the PTA. This was not solely because the HFDT test was performed at the start of the protocol since more participants (90.0%) were able to complete the second HFDT test at the end of the protocol than the EHF-PTA (87.3%).

When asked about the acceptability of the test all participants tested at the time of an exacerbation who were specifically asked, felt that the

HFDT test would be feasible at the start of a course of intravenous antibiotics.

In unwell adolescent and adult participants, the research team was reliant on information from the clinical team about potentially eligible patients. It is therefore unclear as to how many patients were not deemed suitable for approach. Other studies have looked for hearing loss in CF patients at the beginning of a course of IV antibiotics (314, 362, 363, 670, 704-706). These studies, as with the present example, required participation in a research protocol lasting up to an hour. The feasibility of a test taking five minutes versus a test protocol lasting up to an hour is very different, such that even patients not able to undergo a research test protocol may well be able to undergo an HFDT test if this was clinically indicated. Equally for very unwell patients the balance of using an aminoglycoside, regardless of current hearing state, may be outweighed by the potential clinical benefit.

In younger children all were able to attempt at least one HFDT test. In children with CF 3/5 (60.0%) five-year-olds and 2/11 (18.2%) six-year-olds needed some assistance. There was a trend towards improving SRTs with increasing age, and particularly a change between five-year-old and six-year-old children. (SRT is more negative where an individual has better hearing.) This fits with the clinical impression at the time of the study that children aged six and over were able to perform the test to a reasonably reliable standard. All the children who were asked said they would be happy to undertake the HFDT test at a clinic appointment.

In other studies of speech-in-noise testing children have been found to score lower than adults, despite similar or better hearing thresholds (693). Rashid *et al.*, testing ninety-five 5–12-year-old school children using the Dutch online speech-in-noise screening test, found that there was a significant effect of age on SRT, with an improvement of 0.3 dB SNR per year of age (694), though there was no cut-off age at which children were unable to perform the test. Blankenship *et al.* did not find that age was a predictor for SRT but, though they tested CF patients from six-years-old and control children from seven-years-old, it is unclear what proportion of the tested subjects were of primary school age (mean ages 15.0 years for CF patients and 14.6 years for non-CF controls) (314). The differences between SRT scores in children and adults are likely related to central audiological processing which matures after the age of 10-12 years, rather than difficulties in peripheral hearing perception (693) so the different findings are not unexpected.

7.4.3 Validity of the HFDT test in unwell adults and adolescents

The sensitivity and specificity of the HFDT test to identify participants with hearing loss appears better in this sample of unwell participants than in participants tested at a time of clinical stability, at either selected cut-off value. It is unclear why the test should perform better in unwell participants as it was hypothesised that it would be less sensitive and specific in this scenario. However, the study is underpowered,

recruiting only 71/111 required participants, and these results must be interpreted with caution.

The optimum cut-off result for the HFDT in both arms of this study was found to be more negative than that identified by Vlaming *et al.* when describing the development of the test. Using a cut-off SRT value of -17.1 dB SNR they achieved a sensitivity of 87% and a specificity of 98% compared to hearing loss defined as mean threshold >20 dB HL over the range 3 – 8 kHz (319). They validated the HFDT test in a population of 24 people with normal hearing (mean age 29.8) and 50 people with hearing impairment (mean age 63.4). The hearing-impaired population had greater loss in the low-mid-frequencies (0.25 – 4 kHz) than our population. It is likely that the larger number of older participants with hearing loss in the Vlaming study, compared to our population, contributed to the differences in these findings. In addition, the definition of hearing loss used by Vlaming *et al.,* is less sensitive to early change than the definition used here. Many of the participants in the present study would be classified as normal hearing using the Vlaming definition, meaning early loss could be missed. As understanding of the importance of high frequency hearing in speech comprehension, particularly in noise, increases (338) there is need for a screening test that will reliably identify the earliest changes so that interventions can be made before hearing loss in the standard frequencies occurs.

7.4.4 Acceptability of the HFDT test

All participants asked about the HFDT test found it acceptable. All the participants felt that the test could reasonably be performed both during a clinic visit, for example as part of annual assessment, and during a pulmonary exacerbation. Unfortunately, those asked about the acceptability of the test were a minority of participants since formal questioning was only added at the end of the study. It is therefore possible that other participants who did not feel that it would be possible in these circumstances were missed. Blandy and Lutman found that a speech-in-noise test using Bamford-Kowal-Bench (BKB) sentences was appealing to children and therefore could be used as a hearing screening tool in schools (693).

7.4.5 Changes in hearing after a course of intravenous antibiotics

Only fourteen participants were followed up after their course of IV antibiotics. Of these only three were treated with tobramycin. Only one of four participants with a change from baseline which reached the definition of ototoxicity had been treated with an aminoglycoside. The small numbers make it difficult to draw any conclusions.

Similarly, Mulheran *et al.*, reporting on the effect of once vs three-times daily tobramycin administration, found no significant difference in hearing thresholds in either the standard or extended high frequencies, defined as a persistent increase in the PTA threshold above 20 dB HL in two or more frequencies in the range 2-8 kHz, at day 14 or 6-8-weeks

post therapy. They also struggled to achieve follow-up testing, with fewer than half of the participants included in the audiological analysis returning for their testing at 6-8 weeks post treatment (362).

Studies which used the ASHA criteria for monitoring of cochleotoxicity (see section 7.2.3) (698) found higher rates of ototoxicity after a single course of aminoglycosides. Harruff *et al.* tested 18 participants before and after a course of intravenous tobramycin. They found evidence of cochleotoxicity in 33% and 89% of participants in the standard and extended high frequencies respectively at two weeks after treatment cessation; four weeks after treatment cessation this remained 27% and 87% (363).

Garinis *et al.* tested 31 participants, 18 of whom were exposed to IV tobramycin and 13 control participants. They found evidence of cochleotoxicity in the standard frequencies in 11% of participants treated with tobramycin and 31% of control participants at follow-up at least 30 days after cessation of tobramycin. In the extended high frequencies 67% of tobramycin-treated participants had cochleotoxicity at follow-up compared with 46% of those with no tobramycin. This could suggest that any illness can affect hearing thresholds, which would be consistent with the findings of the current study, though it is unclear that Garinis' control participants underwent any alternative IV antibiotic treatment (670).

7.4.6 Prior hearing concerns

A similar proportion of well and unwell participants reported hearing concerns, though fewer unwell participants reported difficulties with speech-in-noise and more reported general problems hearing speech. The proportion of participants with exposure to noise was higher in the unwell cohort for both occupational and recreational noise. The proportions of participants reporting hearing concerns who were subsequently found to have hearing loss was similar to the well participants. It is unclear whether self-report of hearing concerns is actually predictive of EHF loss, since the proportion of participants found to have hearing loss after self-report is very similar to the overall percentages found to have each type of loss. Further work is needed to assess whether self-report of hearing problems predicts EHF loss on testing.

A slightly higher proportion of unwell participants reported tinnitus and balance problems (54.8% versus 48.5%) and the numbers relating these symptoms to aminoglycoside use was also higher (16.4% vs 11.1%). This may be related to the higher use of aminoglycosides in the unwell group with 63.4% of this group receiving ten or more courses of IV antibiotics, compared to 34.4% of the well cohort. As discussed in chapter 6 (see section 6.4.4) vestibulotoxicity is common in patients with CF (314, 361) and more studies are needed to assess the prevalence, monitoring and impact of the condition.

More hearing concerns were reported in children with CF than non-CF control children. Again, the proportion of children found to have hearing

loss after reporting concerns is very similar to the proportion found to loss overall. No control children reported tinnitus or balance symptoms, but these were present in 10% of children with CF. This accords with the findings of Blankenship *et al.* who found significantly more self-reported hearing loss, tinnitus and balance problems in CF participants than the non-CF controls, again though the age cohort was higher than that reported here (314).

7.4.7 Strengths of the study

This study has assessed the hearing of 71 unwell adults and adolescents with CF, a far higher number than many other studies looking at hearing in the context of IV antibiotics in CF (314, 363, 670, 704-706). We were also able to study the hearing of 38 children with CF of primary school age. These results contribute to the understanding of the prevalence of hearing loss in both the standard and extended high frequencies in the CF population.

The study has proved that speech-in-noise testing is feasible in both children and unwell patients. Speech-in-noise testing is an important method of assessing real-life hearing (693) and inclusion of this method of assessment in monitoring CF patients for hearing loss has been recommended (314), though recent guidelines for routine monitoring of cochlear health in people with CF do not include these tests (707). This may be due to the relatively few studies currently available using speech-in-noise testing in CF (314, 363).

In demonstrating high levels of extended high frequency hearing loss in children and adults with CF the study highlighted the importance of monitoring this (680) in addition the standard frequency audiology which is far more widely available in clinical practice. It also emphasised the prevalence of symptoms which may be associated with vestibulotoxicity in the CF population and the need for better assessment and monitoring of this complication (361).

7.4.8 Limitations of the study

Audiological testing in this study was predominantly performed by a non-audiologist. In well adults this was feasible, but the limitations became more obvious in participants with reduced concentration. Compared to the non-CF control children, in whom PTA data completeness was 100% for the standard frequencies and 98.7% for the EHF frequencies, completeness was much lower for the children with CF (76.3% and 47.4% respectively). Some of this variation is explained by children being tired and bored at the end of a clinic visit compared to those having time out of a school lesson. In children who were obviously struggling with concentration paradoxically the data were often more complete as the test protocol was abbreviated. However, in those who managed well initially more frequencies were attempted, sometimes precipitating early abandonment without the basic frequencies being achieved in the second ear due to the frequency test order. In the CF children tested by the research paediatric audiologist (n = 7) all required data were collected. PTA

testing of children requires experienced personnel to maintain concentration in order to obtain optimal results (693).

The definition of hearing loss used may have been less sensitive for identifying early hearing loss (314). However, using a single threshold (for EHF hearing loss) or two thresholds (for standard frequency hearing loss) above 20 dB HL does not discriminate between a participant with thresholds of 25 dB HL at 6 and 8 kHz, or 16kHz and a participant with thresholds of over 90 dB HL (consistent with profound hearing loss) or unobtainable thresholds at multiple frequencies. Correlating the results of the HFDT test with the mean of a set number of thresholds may have given us a better understanding of the hearing levels that the test was able to discriminate.

Once again, having a single researcher to recruit and test at four separate sites inevitably meant that some participants were missed, including those who had consented to participate when unwell. This affected recruitment and subsequently the study was underpowered. Recruitment was further affected by the initial requirement to have a Fuchs' score of 4 or more to be eligible to participate when unwell. Many patients are admitted for semi-elective or planned IV antibiotics when they are clinically deteriorating but not especially unwell. The eligibility criteria were therefore relaxed to allow participation of any eligible patient whom the clinical team felt needed treatment for a pulmonary exacerbation. This could include steroid treatment for allergic bronchopulmonary aspergillosis (ABPA) if in-patient treatment was required. This decision was made in January 2016 in discussion

with my supervisor (AS) and was validated by the study steering committee. However, it is worth noting that the inclusion of participants who were less acutely unwell may have falsely improved the feasibility of the test in unwell patients.

Repeat testing of participants in the aftermath of a course of IV antibiotics was formally abandoned in January 2018, in conjunction with my supervisor (AS) and the study steering committee, after repeat testing was found not to be possible in multiple participants. Some only had contact with the CF Centre for admissions, some did not attend clinic appointments, and some did not have clinic appointments within the specified time period. Furthermore, this is another example where the study was limited by logistical constraints and the decision was made to prioritise data acquisition in well participants and those at the start of IVs at the expense of repeat testing. This was a shame. Such testing would have given additional data on the feasibility of the HFDT test, and its accuracy in unwell patients. We would also have added to the literature on the effect of a course of aminoglycosides on hearing acuity.

Formal questioning about the acceptability of the HFDT test was introduced in September 2017, in accordance with a decision made by myself, AS and the study steering committee. Whilst any comments made about the HFDT test prior to this point were noted there was no objective questioning. This information would have been useful to support the acceptability of the test, particularly in unwell participants. It is possible that the test became more acceptable over time as I became

more adept at the testing protocol and the time taken reduced. Therefore, although the participants questioned reported satisfaction with the test, this is not a representative sample of the population and must be interpreted accordingly.

7.4.9 Challenges in the conduct of this study

As described in 6.4.8 there were a number of challenges in recruiting to this study, which was run concurrently with the work described in chapter 6.

For the unwell participants the required sample size was 111 participants, therefore recruitment of 133 participants was planned to allow for attrition, for example due to ill health. As these patients were due to be tested twice, this required 266 hearing tests. It was estimated that this testing would take 18 months, overlapping with the well participants and the children.

Again, there were a number of challenges to recruitment. Initially the ethics agreement required patients to have been sent an information sheet prior to clinic and be consented in clinic. An ethics amendment was submitted in January 2016 after it became clear that many patients on the ward were being missed as they had not previously been seen in clinic.

Not all the eligible patients at each clinic required IV antibiotics during the study period. Many of the admitted patients were of a small group who had recurrent admissions, other had more sporadic admissions and if missed might not be available again. By definition, these

admissions were often ad-hoc and, due to my split site commitments, I was not always available when patients were admitted. I relied on the CF specialist nurses to inform me of admissions and let me know who was suitable to approach. Understandably they were very busy and so I was not always aware of patients being admitted in time. It quickly became apparent that it was necessary to run this part of the study concurrently with the other workstreams; if no clinic patients were available I would go to the ward to see if there were any suitable admissions.

We initially planned to test participants in the first day of admission. It became clear that this was not feasible due to the many healthcare professionals who needed to review a patient on admission. In January 2016 our ethical approval was amended to allow testing within the first four days of admission. This may have reduced the acuity of illness and falsely improved the ability of participants to complete the test when admitted, but this concern was balanced by the practical need for recruitment. The 24-hour requirement also meant patients admitted over a weekend were not eligible. Even within the first four days it could be difficult to see potential participants. Many, if well enough, left the ward for extended periods or slept late in the mornings. My visits had to fit around the essential clinical reviews which often took up much of the afternoon. In 2015 and 2016 I would often test unwell participants out of hours to avoid clashing with clinicians. After testing recommenced in August 2017, due to breaks in my working week, patients admitted Thursday evening or Friday were out of their four

days before I returned to work the next week. For participants under the age of 16 I required parental consent, and it was difficult to time my visits with those of the parents, especially when I was less able to visit out of hours.

Recruitment for this part of the study took place between March 2015 and November 2018, when the study steering Committee decided to stop recruitment.

The sample size for the 5-10 year old children was 66, with 11 participants at each age between 5 and 10 years old. In order to achieve these numbers this required testing 24/25 eligible patients at NCH and 42/54 patients at BCH. The study initially aimed to find the youngest age at which children could perform the study, defined as the age at which 80% of children could complete the test and the correlation between the SRT and the PTA was at least half of that of adult participants. Unfortunately, as an acceptable cut-off SRT for adults could not be identified, the second of these aims was not possible.

Consenting and testing such a high percentage of each clinic was challenging since some of the patients were shared care between the Centre and a local hospital. These patients typically attended the Centre between once and twice a year; if they were missed at an appointment, it could be a year before they could be seen again and if at that time they had not read the information leaflet, then further delays were unavoidable.

Many parents expressed concerns about prolonging clinic appointments due to the impact on their children's schooling. Some preferred to arrange an alternative time for testing but for many, especially those who lived far away, this was not possible.

Again, the problem of one research fellow covering multiple sites, meant that potential participants were missed.

Recruitment of the children took place between April 2015 and January 2019. Some of these participants were tested by Sana Anwar, the paediatric audiologist who tested the school children. She was able to concentrate on the BCH site, but we were limited by only having one set of equipment.

7.4.9.1 Changes to improve recruitment

As with the well adult participants, changing the ethical requirements to allow recruitment, consent and testing of potential participants on the same day would have reduced the numbers of patients missed because I was unable to be present at their subsequent clinic appointments. Contacting the interested patients using hospital communication channels prior to the next appointment would have given parents a chance to decide if testing was feasible in advance.

Focus on one city at a time would have assisted recruitment for these parts of the study, as well as the well adult participants. Again, I would have become more embedded with the clinical team so that staff would think to contact me about potential participants, I could have gone to the wards each day to enquire, rather than on an ad-hoc basis and less

time would have been spent commuting, improving the time available for recruiting.

Finally had I been able to carry out all the planned tests in well adults and young people, unwell adults and young people and children, this would have equated to 449 testing sessions. Having two research fellows, one in each city, and recruiting simultaneously, would have improved the chances of reaching our target sample size.

7.4.10 Further work

This study has highlighted the impact of the definition of hearing loss noted elsewhere (312, 314). Further work to determine an accepted definition of hearing loss in both the standard and extended high frequencies for use in future research is needed. In addition, extended high-frequency thresholds are known to decrease with increasing age (708, 709). Vijayasingam *et al.*, examining the utility of web- and tablet-based audiometry for ototoxicity monitoring in adults with CF, excluded frequencies above 12.5 kHz due to the effect of age (359). It is likely that some of the extended high frequency hearing loss reported here and elsewhere is related to age rather than aminoglycoside ototoxicity. A better understanding of the interaction of age and ototoxicity on extended high frequency thresholds may prevent some older CF patients being labelled with ototoxicity, though clinically the synergistic effect of cochlear insults means age should still be taken into consideration before prescribing aminoglycosides to patients with any hearing loss.

Strategies to facilitate ototoxicity monitoring in CF, are important to reduce the prevalence of both cochleo- and vestibulotoxicity. A recent study showed that the implementation of an algorithm to assess the risk of aminoglycoside ototoxicity in CF significantly improved the rates of audiogram provision in patients treated with intravenous and inhaled aminoglycosides (710). However, the authors noted that reliance on audiology services was a barrier to this provision. Vijayasingam *et al.* demonstrated the validity of tablet-based audiometry, delivered by non-audiologists in assessing for the presence of hearing loss in adult CF patients and McKinzie *et al.* have successfully piloted the provision of tablet-based audiometry by pharmacists at the beginning of a course of intravenous antibiotics (359, 704). Extension of this work to determine the optimum monitoring regimen, including the role of speech-in-noise testing, is needed, balancing the recommendation for ototoxicity monitoring set out by the American Academy of Audiology (which comprises baseline audiogram, then weekly or biweekly testing during treatment and follow-up PTA a few weeks after discontinuation of the aminoglycoside) (303) and the practical logistics in patients treated with recurrent or long-term aminoglycoside therapy.

7.5 Conclusions

The prevalence of hearing loss in unwell adolescents and adults with CF, as well as primary school aged children is high, particularly in the extended high frequencies. Speech-in-noise testing is functionally important and feasible in both these groups. Development of

internationally accepted definitions for hearing loss in this population will allow comparison of the prevalence of hearing loss between centres and countries. Development of a screening and monitoring regimen that balances the monitoring required to pick up early change with the practical implications of additional appointments will be important to reduce the incidence of aminoglycoside ototoxicity in CF patients in future.

Chapter 8: Conclusions

Harm caused by *P. aeruginosa* is more insidious than simply lung damage, with effects on mental health, family life and social functioning. In addition, indirect physical harms, such as side effects of anti-pseudomonal treatment can lead to increased morbidity and therapeutic burden. The aim of this thesis was to examine these additional, more nuanced, sources of harm, and strategies which may mitigate this.

8.1 Summary of findings

In this thesis I have identified a number of harmful psychological and physical consequences of *P. aeruginosa* infection, alongside strategies which may mitigate some facets of this harm. In chapter 3, I showed that patients and their families are afraid of *P. aeruginosa* infection, even prior to it occurring, fearing negative health consequences and increased treatment burden. These fears lead, in some cases, to the adoption of impractical avoidance strategies that can have a negative impact on mental health. A better understanding of evidence-based approaches to avoiding *P. aeruginosa* infection amongst patients and families was suggested as a way to mitigate these fears.

In Chapter 4 I examined the evidence for strategies to prevent initial acquisition of *P. aeruginosa*. The quality of the evidence was low or very low for all strategies, but there was some evidence to suggest that CFTR modulators, vaccination against *P. aeruginosa* and cohort segregation, with or without additional infection control strategies, are

effective in the prevention of primary infection. It should be noted that current infection prevention and control guidelines recommend that CF centres go beyond cohort segregation and prevent any contact between people with CF, regardless of their infection status (174).

Since chronic *P. aeruginosa* is associated with worse clinical outcomes Chapter 5 considers secondary prevention of *P. aeruginosa*, in an attempt to delay the onset of chronic infection. With colleagues, I conducted a Cochrane Systematic Review, looking for interventions to prevent or delay recurrence of *P. aeruginosa* infection after successful eradication of initial infection. Only one study met the inclusion criteria, included only child participants and did not address the question directly. However, cycled antibiotic therapy, following initial eradication, does appear to delay recurrence of *P. aeruginosa* infection. Further work to understand the balance between the efficacy of this regimen and the treatment burden it imposes is required.

Once chronic *P. aeruginosa* infection is established additional treatments are required to maintain clinical stability. This puts patients at risk of side effects which may also be detrimental to their long-term wellbeing. Aminoglycoside antibiotics, used in inhaled form or intravenously, have excellent activity against *P. aeruginosa* but present a risk of ototoxicity and subsequent hearing loss. In Chapters 6 and 7 I described the assessment of a novel hearing test, the high frequency digit triplet (HFDT) test, as a potential screening test for hearing loss. I identified hearing loss in a quarter of adult and adolescent patients in the standard hearing frequencies, with up to 80% of this population

having loss in the extended high frequencies. Whilst the HFDT was insufficiently sensitive to be useful as a screening test, I was able to demonstrate the feasibility of hearing testing within CF clinics, and at the bedside, in well and unwell adults and adolescents, and in children.

These findings are discussed in more detail in the sections below.

8.1.1 Fear, knowledge and infection prevention

People with CF, and their families have a negative emotional response to *P. aeruginosa* acquisition, fearing the consequences of declining health and increased treatment burden. Anticipation of infection, without sufficient understanding of how to prevent it, engenders a loss of control (187). In turn this leads to the development of maladaptive practices, in an attempt to stave off infection, with consequent guilt if these methods fail. Families who attempted to prevent any contact with *P. aeruginosa* continually increased their hygienic precautions as new risk situations were identified (188). It is therefore possible that increasing knowledge about potential *P. aeruginosa* sources, without an understanding of evidence-based interventions that actually prevent infection, will only increase stress levels for patients and their families.

Physicians are also unclear as to what preventative measures should be advised (186). UK CF Trust guidance focuses primarily on prevention of cross-infection and early treatment of initial infection to prevent chronic *P. aeruginosa*, with the only environmental precaution recommended being to avoid aerated baths (175). Later guidance from the CF Foundation additionally recommends avoiding construction dust

and organic matter, whether from soil or animal/bird litter (174). More recently an article suggesting advice for patients and parents on strategies to reduce *P. aeruginosa* acquisition has given more extensive guidance based on known potential sources (711). Parents report performing more precautions than those advised (187) therefore a balance must be struck between reasonable preventative strategies and increasing stress.

In an attempt to understand what strategies patients and families could reasonably adopt to reduce the risk of *P. aeruginosa* infection the evidence for *P. aeruginosa* prevention strategies was systematically reviewed (see chapter 4). Unfortunately, while a number of studies have examined potential sources of *P. aeruginosa* in the home, there are no studies looking at interventions to prevent acquisition in the non-healthcare environment.

It is unlikely that these studies will ever be done. Deliberate exposure of people with CF to *P. aeruginosa* is unethical. Any RCT of an intervention would need very large numbers and long follow-up to show a difference, whilst controlling for the confounding effects of differing lifestyle factors would be highly challenging. In addition, the effect of individual circumstances, such as recent illness, on *P. aeruginosa* acquisition has not been resolved.

Prospective observational studies, comparing patient samples to environmental samples, have been attempted, but could not sample enough sites sufficiently frequently to understand the direction of

transmission, even where matching strains were identified (163). To sample sufficient patients and their home environments, sufficiently frequently, to demonstrate environment-to-person transmission is probably impossible (186).

The ubiquitous nature of *P. aeruginosa* in the environment means it is impossible to completely avoid. A balance between sensible precautions and the avoidance of panic requires careful education of patients and families, since better understanding of *P. aeruginosa* infection is associated with fewer precautions and reduced stress (187). In the absence of clear evidence about what precautions are definitively helpful, and in acknowledgment that the perception of risk is individualised (711), perhaps the following messages may be beneficial for patients and families:

- That it is impossible to avoid *P. aeruginosa* completely (711),
- That contact does not automatically equate to infection (188),
- That early identification and treatment of *P. aeruginosa* infection gives the best chance of eradication (663), and
- That, where chronic infection does develop, this does not equate to an immediate, inexorable decline (218, 226).

In addition, a frank discussion about hygienic precautions, their benefits and drawbacks, is important to allow families to make their own decisions. In the wake of initial *P. aeruginosa* infection many parents report insufficient provision of information from their healthcare teams (712, 713). In the absence of clear information from clinicians many

parents will turn to online sources for information. A myriad of potential precautions are widely reported in CF forums (personal communication, Oliver Rayner) and guilt following an episode of infection may drive the incorporation of additional, intrusive behaviours into family routines (188, 712, 713).

In an attempt to reduce parental stress and guilt, while reducing the number of unproven hygienic measures performed, Ullrich *et al*. recommended families be given clear simple advice, while stressing the responsibility of the healthcare team in *P. aeruginosa* prevention, through early identification and eradication therapy (187, 188).

8.1.2 Treatment of infection and therapeutic burden

Eradication of *P. aeruginosa* after initial isolation is more effective than no treatment at clearing infection, at least in the short-term (714). No clinical deterioration was found in patients "free" of *P. aeruginosa* after initial infection (191) and, in children, only chronic *P. aeruginosa* infection was associated with a reduction in ppFEV$_1$ (201). However clinical improvement after eradication could not be confirmed in a Cochrane Systematic Review of eradication (714) and re-emergence of the same strain of *P. aeruginosa* in the upper or lower respiratory tract after presumed successful eradication may occur in almost half of all patients where infection recurs (715).

A Cochrane Systematic Review was therefore conducted to examine strategies which might prevent, or at least delay, recurrence of *P. aeruginosa* infection after successful eradication (see Chapter 5). This

found moderate evidence to suggest that cycled antibiotics, given after successful eradication, delay time to next *P. aeruginosa* isolation. These conclusions are however, limited by a number of factors. Primarily, only one study was found that met the inclusion criteria for the systematic review (204). Additionally, this study was designed principally as an eradication study and was therefore not powered to answer the question of secondary *P. aeruginosa* prevention.

Cycled antibiotic therapy, aiming to prolong time to recurrent *P. aeruginosa* infection (204), will lead to an increased treatment burden. Increased treatment burden was described by patients and their families as one of the negative consequences of *P. aeruginosa* infection (see section 3.3.3.2). Nebulised treatment is time-consuming and inconvenient, and whilst newer devices have reduced the time taken for the nebuliser to run (716), preparation, cleaning and sterilisation remain onerous (717).

It is important to note therefore, that whilst strategies to mitigate *P. aeruginosa*-induced harm may be beneficial on one hand, they may have additional, less desirable consequences. This indirect harm also requires mitigation. Patients with chronic *P. aeruginosa* infection require ongoing treatment to reduce continuing clinical deterioration and treat acute exacerbations. Increasing exposure to such medications however leads to an increased risk of medication side effects (273).

Aminoglycoside antibiotics, used in both chronic suppression of *P. aeruginosa* infection, and acutely to treat a pulmonary exacerbation, have recognised complications in the form of nephro- and ototoxicity (273). Monitoring of blood tests for renal function, particularly during periods of intravenous treatment, whilst not perfect (718) is reasonably simple to perform at the bedside. However, monitoring of ototoxicity requires more advanced equipment and training, even where point-of-care testing is utilised. Recommendations to consider an annual PTA in patients with recurrent exposure to IV treatments (625) are a long way from the advice from the American Academy of Audiology, which suggests a baseline PTA, weekly or biweekly testing during administration and a further PTA several months after the end of the course (303). Once again, managing this potential complication, with frequent audiograms, brings harm, in the form of additional appointments with the social, academic and workplace considerations these involve (719).

Web-based audiometry, done at home, has a number of potential benefits for people with CF, including reduced impact on home, school and work life, less travel time, reduced travel and parking costs, and no infection control considerations. Theoretically testing frequency could be higher than if hospital PTA is required. Telephone and web-based speech-in-noise tests have been validated for home use for hearing screening (406, 681, 720). However, when home web-based audiometry was trialled in a group of CF patients the results, though highly specific were insufficiently sensitive to use for ototoxicity

screening (359). The authors found that the quality of home headphones was insufficient to present high frequency tones and recommended that, until better earphone options were available, tablet-based audiometry within the CF clinic gave the best balance between an effective monitoring regimen and reducing treatment burden.

8.1.3 Reduction in harm caused by established *P. aeruginosa* infection

The work presented in chapters 6 and 7 has shown that such testing is feasible, in both well and unwell adult and adolescent populations, and in children. The HFDT test described, as a speech-in-noise test, provides evidence of clinically significant hearing impairment where this is present. Unfortunately, it is insufficiently sensitive to be used as a screening tool.

Tablet-based audiometry in clinic or at the bedside has good sensitivity and specificity in identifying hearing loss, including in the extended high frequencies (359), and can be administered by a non-audiologist (359, 704). New guidelines for hearing screening in CF recommend asking about symptoms at every clinical encounter, with audiological and vestibular testing at IV aminoglycoside initiation and follow-up, and yearly monitoring in all patients (707). These suggestions will be invaluable in detecting early hearing loss and facilitating any necessary management but are necessarily associated with increased treatment burden, even where testing is performed during a (prolonged) outpatient appointment.

As people with CF live longer the impact of ototoxicity will increasingly intersect with presbyacusis. Prevention of ototoxic hearing loss in younger patients becomes more important to protect functional hearing in old age. A balance therefore must be struck between the risks of hearing loss and its attendant social consequences (347, 351), and the impact of additional testing and appointments, which in turn have more immediate, potentially negative social and financial consequences. It is likely that this balance will be different in each individual and will have to be managed accordingly (721).

8.2 *P. aeruginosa* and developments in CF care

8.2.1 CFTR modulators

The impact of CFTR modulators on *P. aeruginosa* in CF is likely to be large. As shown in chapter 4, CFTR modulation may reduce the acquisition of *P. aeruginosa* (100, 101). Furthermore, modulators may reduce prevalence of *P. aeruginosa* (722) and may enhance clearance of the organism (99), over and above the reduction in *P. aeruginosa* identification expected due to reduced sputum production (100). As modulators are introduced to ever younger patients the burden of lung disease may be diminished in future generations (723).

CFTR modulation has been shown to reduce the frequency of pulmonary exacerbations (109, 722), and therefore the frequency of exposure to antibiotics will reduce. Paradoxically however, as people with CF live longer, (a trend that is predicted to further improve in the

post-modulator world (724)), the lifetime number of courses of aminoglycosides may increase. Better understanding of the effects of cumulative lifetime dose will be needed. Ethical considerations mean that much of these data will need to be retrospective, with the attendant challenges of data completeness (see section 6.3.7).

Alternatively, the reduction in pulmonary exacerbations may be such that cumulative aminoglycoside doses are not increased, with reduction in ototoxicity an additional benefit of CFTR modulation in the CF population. The health gap then, for those eligible, and those ineligible, for CFTR modulators, will widen further.

In light of the clinical benefits afforded by CFTR modulators exploration of strategies to safely reduce treatment burden is underway. This work is primarily focussed on cessation of nebulised airway clearance medications, DNase and hypertonic saline (725-727). Studies into the discontinuation of long-term inhaled antibiotics, predominantly used by those chronically infected with *P. aeruginosa* and the second most burdensome treatment in a recent survey (728), are not currently in progress (729, 730).

8.2.2 Virtual consultations and the COVID-19 pandemic

The impact of CFTR modulator roll-out on the health of people with CF was potentially confounded by the COVID-19 pandemic, which occurred almost simultaneously with widespread use of elexacaftor-tezacaftor-ivacaftor (ETI). The rate of pulmonary exacerbations decreased during lockdowns, independent of modulator use (731).

This is likely due to a reduction in viral respiratory tract infections facilitated by reduced social mixing. In an attempt to protect potentially vulnerable patients from infection, in addition, CF Centres worldwide switched rapidly from a face-to-face model of care to virtual clinics (732-735).

Exploration of virtual consultations to reduce cross-infection and minimise the impact of repeat clinic attendances on increasingly well patients was underway prior to the COVID-19 pandemic (736, 737). Patients report virtual clinics as more convenient, efficient and cost-effective (738). The effect of virtual clinics on clinical outcomes is less well understood. Some studies have found no change in FEV_1, or even an improvement (735, 739, 740) but these may be confounded by shielding and/or introduction of CFTR modulators. Another study, which compared FEV_1 in the first in-person clinic post-lockdown with the best value prior to the pandemic found a median decline of 5.4% (741). No data are as yet available on the impact of virtual clinics on *P. aeruginosa* acquisition. The benefits of virtual clinics in terms of convenience and possible reduction in cross-infection must be weighed against potential clinical consequences, with more work needed to understand this balance.

8.2.3 Social media and reduction of social isolation

The level of evidence for cohort segregation was found to be low or very low in this and another systematic review of infection prevention and control (546). However, it is unlikely that the recommendation that

people with cystic fibrosis should not mix will change due to the large body of evidence supporting the existence of patient-to-patient transmission. Indeed, although the 2013 CF IP&C guidelines do not advise cohort segregation, they strongly advise avoidance of any social interaction, in both healthcare and non-healthcare settings, between people with CF who do not live in the same household, rating the evidence for this recommendation as 1A (highest possible) (174).

Cohort segregation was reported to be well accepted by patients and families, where this outcome was described, in the studies included in Chapter 4. Segregation however has been criticised by both patients (742) and healthcare professionals (743). Studies have found that while patients and parents are overall in favour of segregation as a method to reduce *P. aeruginosa* acquisition, concerns have been raised over the impact on social interaction (744), loss of peer support and increased social isolation (719, 745).

Cohort segregation relies on historical information; it is only as accurate as the most recent respiratory culture (743), which may only identify 58% of the bacteria present, even when sputum is sampled (72). Individual isolation is recommended by national and international CF infection control guidelines (174, 412) as it has been associated with a reduction in chronic *P. aeruginosa* acquisition, though there is no data on the effect on initial infection (746).

Cohort segregation and advice to limit interpersonal contact, may have a negative impact on people with CF (747). Young people with chronic

health conditions seek out support from those with the same condition (748). In CF the loss of contact with peers places an additional burden over and above that imposed by other chronic diseases (749). Peer support has a protective effect, normalising experiences and reducing social isolation (747). It has also been shown to improve physical and mental wellbeing (749). Online contact through social media may mitigate some of this lost interaction (750), providing an opportunity for connection which is beneficial to reduce social isolation (747). This contact may be particularly important at the time of an acute exacerbation (748).

Perkins *et al.* found that patients expressed desire for CF-specific forums in which to access information and support (750). Support groups are empowering for patients and parents, fulfilling different needs for each group (751).

CF Centres are turning to social media in an attempt to connect with patients, though content about research opportunities and personal stories had better engagement than health promotion posts (752). Whilst concerns have been raised about potential negative uses, including inappropriate gossip and bullying (753), potential positive uses include information provision and obtaining feedback (752).

8.2.4 Telehealth and remote monitoring

Advances in CF care have improved life expectancy but increased treatment burden (736), particularly in those chronically infected with *P. aeruginosa* (717). Increased treatment burden following *P. aeruginosa*

infection is associated with social and psychological burdens for the individual, their family and wider social circumstances (712). Admissions to hospital, increased time spent on treatment and additional hospital appointments can impact work attendance for the patient or family members, which may have negative financial consequences (719).

Accordingly, much work has been done to evaluate the potential for digital technology to reduce treatment burden. Smartphone Apps to track symptoms have been variably successful in reducing admissions and need for IV antibiotics (754, 755) but although users had reduced symptoms than the usual care group, the burden of treatment when using the App was found to be increased (754). Digital solutions have also been used to better understand gastrointestinal symptoms (756), support adherence (757, 758), exercise (759) and well-being (760), but there is little evidence as yet that these strategies provide benefit, and currently they may actually increase treatment burden (761).

8.3 Definitions

Throughout this thesis the importance of definitions has been highlighted. What is *P. aeruginosa* acquisition? When can *P. aeruginosa* be said to have been eradicated? What constitutes hearing loss and should it be classified by age?

As discussed in chapter 1 (see section 1.2.2), even diagnosis of *P. aeruginosa* infection may be challenging and is dependent on the methods used (625). Variations in the sensitivity of different types of

sampling may mean that early infection is missed, particularly in young children for whom upper airway cultures predominate (192). Sampling frequency further affects the chance of a positive culture (190, 191). Even with the gold-standard test, broncho-alveolar lavage (BAL) culture, there are discrepancies between the pathogens identified and those present in simultaneous sputum samples (762). In addition, there is variation in the density of *P. aeruginosa* CFUs at which infection is defined (415, 556).

Identification of cross-infection is similarly dependent on the microbiological techniques used (see section 1.2.2.2). Improving strain resolution with the advance of genotypic techniques has suggested a greater role for cross infection than was previously suspected (160).

Likewise, there is no internationally agreed definition of *P. aeruginosa* eradication. The Leeds criteria describe a state of, “free” from *P. aeruginosa* infection, in which a patient has no positive respiratory samples for 12 months. In the context of a clinical trial this is a long time to wait after eradication is completed, and a long time to wait to start additional treatment to prevent further infection. Trials of eradication have used variable definitions (714), the simplest being single negative cultures at completion of eradication treatment (206, 415, 419), but also including multiple negative cultures (416), time to next *P. aeruginosa* positive culture (204, 418) to freedom from *P. aeruginosa* infection three months after commencement of eradication therapy and remaining free for another 12 months (209).

The single study included in the Cochrane review detailed in Chapter 5 did not specify a definition of eradication after the initial course of treatment, and indeed the participants could have undergone at least one such course prior to enrolment. A pragmatic definition of *P. aeruginosa* eradication as a negative culture at completion of initial eradication treatment was used, however this definition does not differentiate between suppression of *P. aeruginosa* and true eradication. Whilst a sensitivity analysis showed no effect of using this definition, when compared with a negative culture at the following clinic visit, in another study considered for inclusion in the Cochrane review, the authors felt that this time point was unreliable to determine eradication status and thus this study was excluded (205).

A universal definition of chronic *P. aeruginosa* infection is similarly lacking (190) (see Chapter 1, section 1.2.2.3). Once again frequency of culture affects some definitions (191), whilst others require assessment of precipitating antibodies in addition to sputum samples (714).

A number of definitions for pulmonary exacerbations have been proposed (67, 68, 418). A formal definition of pulmonary exacerbation was initially used to identify unwell participants in chapter 7 (67). However, this was found to be insufficiently sensitive and led to the exclusion of a number of participants from the unwell study arm. Pragmatically it was decided that a simpler definition, physician-defined need for IV antibiotics, more closely reflected the clinical picture.

Physician defined need for IV antibiotics has been specified in a number of trials (79, 763).

Multiple definitions of hearing loss, as described in sections 6.4.1 and 7.4.1, affect the assessment of prevalence of hearing loss. Notably the initially described high prevalence was reduced after the introduction of less stringent definitions. More recently studies have reverted to more inclusive definitions, encompassing the extended high frequencies as well as standard PTA (359, 670). High frequency hearing is known to decline with age but age-related normal ranges have not been used in CF research, though one study got around this by excluding frequencies above 12.5kHz due to their adult population (359).

Lack of consistent definitions matters. In a paper examining disability provision, the Australian Institute of Health and Welfare states,

> *"The use of common terms and definitions provides individuals with a basis for common understanding. In this way, communication is assisted, transparency in social programs is improved and needs are better met through accurate identification and understanding of what people require." p.1, section 1.2* (764).

In the absence of common definitions for the terms above progress in reducing the harms of *P. aeruginosa* infection is impaired. There may be inconsistencies in the classification of patients' infection status, potentially compromising cohort segregation. Results of clinical trials cannot be accurately

compared, reducing the impact of multiple studies. Ultimately this may lead to inaccurate conclusions and flawed policy recommendations.

8.4 Further research

Ongoing research may provide additional evidence about some of the strategies to mitigate harm caused by *P. aeruginosa* in CF reported in this thesis. CF START will provide definitive evidence about the effect of anti-staphylococcal prophylaxis on *P. aeruginosa* acquisition (599).

Studies of treatment cessation in people established on CFTR modulators (726, 727) will be invaluable in understanding whether these drugs allow reduction in the burden of other CF treatment, a question that remains in the top 10 research priorities identified in CF (765). If these trials show that cessation of long-term therapy is possible work to look at stopping other medication will follow.

The OPTIMIZE study has now reported and individual patient data can now be requested to allow inclusion in the Cochrane review of strategies to prevent recurrence of *P. aeruginosa* (418). Oral azithromycin therapy three-times a week is a much less burdensome treatment than nebulised therapy, thus, were this to be effective in secondary prevention, it is likely that it would be preferred to cycled inhaled tobramycin by patients after initial eradication.

Further information about sources of *P. aeruginosa* and infection prevention in the home is likely to still be of interest to parents of people

with CF in view of the level of distress this issue causes. However as mentioned above it is unlikely that further trials to improve the evidence base, will be possible.

In terms of *P. aeruginosa* acquisition ongoing data collection about the impact of CFTR modulators on initial infection is of interest; RCT data are unlikely, since it is unethical to discontinue proven treatment, however this outcome may be of interest in studies of novel modulators and in younger children. It is likely that a long duration of follow-up will be needed to show any difference. Longitudinal studies of incidence and prevalence, perhaps using registry data, may be possible but will be confounded by the current, ongoing reduction in *P. aeruginosa* prevalence (228, 575).

Since chronic infection has probably the more serious effect on long-term health in people with CF a systematic review looking at prevention of this could be considered, though Cochrane reviews are already available examining a number of likely strategies (605, 714).

In terms of hearing testing an evaluation of the efficacy and feasibility of the new guidelines for ototoxicity monitoring (707) will be of use to reduce the side effects of aminoglycosides. Reduction of side effects from antibiotics remains in the updated top 10 CF research priorities and has moved up from the 9th priority in 2018 to the 5th priority in 2023 (765). Additionally, evaluation of ototoxicity and hearing loss supports a new research priority identified in 2023, “How to we manage an ageing population with CF?” (4th priority) (765).

All studies of *P. aeruginosa* in CF will benefit from answering the newly identified 2nd research priority, “What is the best way to diagnose lung infection when there is no sputum, e.g., children and those on modulators?” (765). Answering this question may subsequently support internationally approved definitions of *P. aeruginosa* acquisition, eradication and chronic infection.

8.5 Conclusions

P. aeruginosa causes harm to patients with CF psychologically, physically and as a side effect of treatment. Mitigation of this harm is possible but may be associated with additional treatment costs. Quality of life in CF is increasingly important as longevity increases (736). As people with CF are living longer, fuller lives the perceived burden of treatment is increasing (749).

There is a knowledge gap regarding sources of *P. aeruginosa* acquisition. Whilst studies to answer this question may be impractical and unethical, this deficit is detrimental to the mental health of patients and families. Education of the CF community regarding proven prevention strategies, high risk scenarios and other potential precautions must convey the balance between potential for harm in preventative measures, with the risks of *P. aeruginosa* infection.

Definitions are important. Without consistency in definitions research and subsequently clinical guidance is weakened. Work on establishing internationally agreed definitions around *P. aeruginosa* infection in CF is crucial.

The prevalence of *P. aeruginosa* infection is falling but it remains a worry to patients and parents. Hopefully in the long-term the burden caused by *P. aeruginosa* in people with CF will decrease but in the meantime work to reduce the harm caused by *P. aeruginosa* in CF must not be forgotten despite the overwhelming changes in the face of CF care.

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Appendix A: Chapter 4 search strategies

MEDLINE

1. cystic fibrosis.mp. or exp Cystic Fibrosis/
2. Infection Control/ or Cross Infection/ or infection prevention.mp.
3. Cross Infection/ or nosocomial.mp.
4. environment*.mp.
5. source.mp. or Bacteria/
6. 2 or 3 or 4 or 5
7. pseudomonas aeruginosa.mp. or exp Pseudomonas aeruginosa/
8. PSEUDOMONAS/ or pseudomonas.mp.
9. pseudomonas aeruginosa.tw.
10. Pseudomonas Infections/ or P aeruginosa.mp.
11. mixed infection.mp. or Coinfection/
12. gram negative bacteria.mp. or Gram-Negative Bacteria/
13. 7 or 8 or 9 or 10 or 11 or 12
14. 1 and 6 and 13

EMBASE

1. cystic fibrosis.mp. or exp cystic fibrosis/
2. infection prevention.mp. or exp infection prevention/
3. cross infection.mp. or cross infection/
4. hospital infection/ or nosociomal.mp.
5. environment*
6. source
7. infection/ or Pseudomonas infection/ or infection prevention/ or infection risk/ or respiratory tract infection/ or lung infection/ or cross infection/ or Gram-negative infection/ or healthcare associated infection/ or infection control/ or device infection/ or hospital infection/ or mixed infection/
8. pseudomonas aeruginosa.mp. or Pseudomonas aeruginosa/
9. P aeruginosa.mp. or Pseudomonas aeruginosa/
10. 2 or 3 or 4 or 5 or 6
11. 7 or 8 or 9
10. 1 and 10 and 11

CINAHL

#S3 (MH (infection control or infection prevention) OR TX pseudomonas aeruginosa OR TX mixed infection OR TX gram negative bacteria) AND (S1 AND S2)
#S2 MH (infection control or infection prevention) OR TX pseudomonas aeruginosa OR TX mixed infection OR TX gram negative bacteria
#S1 MH cystic fibrosis

PubMed

cystic fibrosis AND (infection prevention OR infection control OR cross-infection OR nosocomial OR environment OR source) AND (pseudomonas OR pseudomonas aeruginosa OR mixed infection OR gram-negative bacteria)

Appendix B: Chapter 4 excluded studies

Reason for Exclusion	References
Wrong question	Brown 2019 (766) Brown 2019 (767) Sosinski 2022 (768)
Wrong population	
PA positive at baseline	Dalzell 1990a (769) Johansen 2008 (770) Kollberg 2003 (627) Luna 2013 (771) Nilsson 2007 (772) Nilsson 2008 (773) Nolan 1982 (774) van der Doef 2009 (775)
Mixed CF and non-CF	Pennington 1975 (776) Unstead 2006 (777)
Non-patient study	Cavallo 2022 (778)
Wrong outcome	
Culture rates, not incidence reported	Bonestroo 2010 (779) De Biase 2020 (780) Heltshe 2015 (99) Kawala 2021 (595) Millar 2018 (781) Savant 2014 (782)

	Sharma 2016 (783) Sims 2007 (613) Stutman 2002 (597)
Prevalence only	Festini 2003 (618) Griffiths 2005 (619) Ledder 2015 (784) Pedersen 1986 (785) Sagel 2021 (786) Sims 2005a (787) Weber 2017 (788) Wenjie 2016 (789)
Development of chronic PA only	Hoiby 1989a (615) Hoiby 1989b (617) Langford 1984 (790) Pedersen 1987 (616) Van Mansfeld 2016 (746) Zuercher 2006 (791)
Does not report novel acquisition	Connett 2015 (650) Farrell 2005 (792) Sims 2005b (793)
Compliance measures only	Johnson 2018 (794) Yilmaz Yegit 2021 (795)
No intervention	
Epidemiological study	Armstrong 2003 (502) Festini 2010 (511)

	Gilchrist 2011 (796) Grothues 1988 (177) Lebecque 2006 (600) Monge-Espinosa 2022 (797) Padman 2008 (798) Schluter 2022 (799) Williams 2010 (800) Wang 2011 (614)
Comment/letter/review	Bellanti 1997 (801) Denton 1996 (802) Jones 2002 (803) Wenstrom 2007 (804)
No comparator	
All participants received intervention	Alanin 2016 (805) Cryz 1994 (806) Dalzell 1990b (807) Davidson 1995 (808) De Winter-de Groot 2013 (809) Guimbello 2021 (810) Schaad 1991 (811)
No data prior to intervention	Ashish 2009 (812) Ashish 2013 (508) Johansson 2014 (813)

	Nichols 2023 (97) Rimbaldo 2022 (741) Schnell 2023 (98) Wiehlmann 2012 (814) Yilmaz Yegit 2020 (815)

Appendix C: Chapter 5 search strategy

Database/Resource	Strategy
ISRCTN registry	[Advanced Search] TEXT SEARCH: pseudomonas OR aeruginosa OR infection CONDITION: cystic fibrosis
Clinicaltrials.gov	[Advanced Search] Search 1: CONDITION/ DISEASE: pseudomonas OR aeruginosa OTHER TERMS: eradicate OR eradication OR eradicating OR prevent OR prevention OR preventing OR preventative OR reoccurrence OR recur OR recurrent OR recurrence OR reoccur STUDY TYPE: Interventional Studies Search 2: CONDITION/ DISEASE: cystic fibrosis AND infection OTHER TERMS: eradicate OR eradication OR eradicating OR prevent OR prevention OR

	preventing OR preventative OR reoccurrence OR recur OR recurrent OR recurrence OR reoccur STUDY TYPE: Interventional Studies
WHO ICTRP	[Advanced Search] TITLE: eradicate OR eradication OR eradicating OR prevent OR prevention OR preventing OR preventative OR reoccurrence OR recur OR recurrent OR recurrence OR reoccur AND CONDITION: cystic fibrosis RECRUITMENT STATUS: all

Appendix D: Publications arising from this PhD.

Palser SC, Rayner OC, Leighton PA, Smyth AR. Perception of first respiratory infection with *Pseudomonas aeruginosa* by people with cystic fibrosis and those close to them: an online qualitative study. BMJ Open. 2016;6(12):e012303

Palser S, Smith S, Nash EF, et al. Treatments for preventing recurrence of infection with Pseudomonas aeruginosa in people with cystic fibrosis. Cochrane Database of Systematic Reviews. 2019(12). Cd012300.

Rowbotham NJ, **Palser SC,** Smith SJ, et al. Infection prevention and control in cystic fibrosis: a systematic review of interventions. Expert review of respiratory medicine. 2019;13(5):425-34.

Appendix E: Presentations arising from this PhD.

Palser SC, Kaziro J, Smyth AR. P062 Surveillance for ototoxicity in children with *Mycobacterium abscessus* lung disease in a UK cystic fibrosis centre. Journal of Cystic Fibrosis 2018;17(Suppl) S76.

Palser SC, MacKinnon RC, Clarke JR, Dewar J, Nash EF, Anwar S, Elliot Z, Mehta RL, Ferguson MA, Smyth AR. Screening for early hearing loss – a novel approach. Pediatric Pulmonology 2017; 52(S47) S363.

Jones P, **Palser SC**, Prayle AP, Hurley MN, Smyth AR. WS20.1 Secular trends in *Pseudomonas aeruginosa* acquisition in the United Kingdom: a registry study. Journal of Cystic Fibrosis 2016; 15(Suppl) S31

Palser SC, Leighton P, Rayner OC, Smyth AR. 287 Perception of first infection with *Pseudomonas aeruginosa* by people with CF, their families and close friends. Journal of Cystic Fibrosis 2015; 14(Suppl) S131.