

## **Early respiratory bacterial detection and anti-staphylococcal antibiotic prophylaxis in young children with cystic fibrosis**

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### **Contributors**

MH conceived the study. All authors were involved in the design of the study. MH & TM analysed the data. MH wrote the first draft. All authors commented on this draft and contributed to subsequent revisions. All authors agree with the final version.

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### **Ethics approval**

The Registry committees of the UK CF Trust Registry and the U.S. Cystic Fibrosis Foundation approved the requests for data. The Institutional Review Board at the University of Nottingham Medical School approved the protocol.

### **Running head – Anti-staphylococcal antibiotic prophylaxis in CF**

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### **At a glance commentary:**

**Scientific Knowledge on the Subject.** Our understanding of the effects of anti-staphylococcal antibiotic prophylaxis is limited to 4 randomised trials examining the utility of various antibiotics. In one study more children in the treatment group isolated *Pseudomonas aeruginosa*.

**What This Study Adds to the Field.** This registry study describes the ‘real world’ first detection of *S. aureus* and *P. aeruginosa* in the UK (where antibiotic prophylaxis is recommended), and US (where it is not used), and provides additional data regarding the benefits and risks of *S. aureus* prophylaxis in young children with CF. Risks of first detection of *S. aureus* and *P. aeruginosa* are greater in the US than the UK. In the UK, risk of first detection of *S. aureus* is not reduced among those receiving flucloxacillin prophylaxis, while the risk of first detection of *P. aeruginosa* is more than twice as great among those receiving flucloxacillin prophylaxis than among those receiving no prophylaxis.

**Online supplement.** This article has an online data supplement, which is accessible from this issue’s table of content online at [www.atsjournals.org](http://www.atsjournals.org)

## **ABSTRACT**

### **Rationale**

Consensus is lacking regarding anti-staphylococcal antibiotic prophylaxis use for young children with cystic fibrosis. Prophylaxis is recommended in the UK, but recommended against in the US.

### **Objectives**

To test the hypothesis that anti-staphylococcal antibiotic prophylaxis is associated with a decreased risk of *Staphylococcus aureus* acquisition, but no increased risk of *Pseudomonas aeruginosa* acquisition.

### **Methods**

We undertook a longitudinal observational study of children with cystic fibrosis who were recruited from birth (or their first registry entry in the period) and followed until the age of 4 years (1500 days) using UK CF Trust and US CF Foundation Registries, 2000-2009. Children were excluded if they had a culture positive for *S.aureus* or *P.aeruginosa*, or were receiving inhaled antibiotics, at first encounter. Time to first *S.aureus* and *P.aeruginosa* detection in the UK/US cohorts were compared using a Cox proportional hazards model. A UK-based analysis compared the same for those receiving flucloxacillin with those who received no prophylaxis. We included the following covariates: sex, age at registry entry, Dornase alfa use, genotype and center size.

### **Main results**

The primary analysis consisted 1074 UK and 3677 US children. The risk of first detection was

greater in US compared to UK for *S.aureus* (hazard ratio (HR) 5.79; 95% CI: 4.85, 6.90;  $p<0.001$ ) and *P.aeruginosa* (HR 1.92; 95% CI: 1.65, 2.24;  $p<0.001$ ). The UK analysis compared 278 children receiving flucloxacillin and 306 receiving no prophylaxis. Flucloxacillin was not associated with a reduced risk of *S.aureus* (HR 1.22; 95% CI: 0.74, 2.0;  $p=0.43$ ), but was associated with an increased risk of *P.aeruginosa* (HR 2.53; 95% CI: 1.71, 3.74;  $p<0.001$ ) detection. None of the covariates significantly affected the risk estimate in either analysis.

### **Conclusions**

Risk of first detection of *S.aureus* and *P.aeruginosa* was greater in US compared to UK. In the UK, the risk of first *P.aeruginosa* detection was increased among those receiving flucloxacillin compared to those who received no prophylaxis. These observational findings should be examined in randomised controlled trials.

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### **Keywords:**

Cystic fibrosis; Children; Antibiotics; Prophylaxis; Infection;

## INTRODUCTION

The hallmark of cystic fibrosis (CF) lung disease is increased susceptibility to chronic endobronchial infection.(1) *Staphylococcus aureus* is the most common respiratory pathogen in infants and young children with CF,(2) the detection of which is independently associated with lower respiratory tract inflammation.(3) In the UK prophylactic antibiotics are administered with the aim of preventing infection with *S. aureus*.(4) U.S. registry data, for young children with CF, show a prevalence of 60-70% for *S. aureus*, and a prevalence of around 20% for *P. aeruginosa*.(5) In the U.K., comparable registry figures are 14% for *S. aureus* and 21% for *P. aeruginosa*.(6)

Internationally, the issue of antibiotic prophylaxis for *S. aureus* is controversial.(7-9) The evidence supporting the use of anti-staphylococcal therapy is summarized in a Cochrane review which concluded that, while prophylaxis appeared to reduce the detection of *S. aureus*, no effect was observed on clinical status and the significance of the increased rate of *P. aeruginosa* detection seen in one trial was uncertain.(10) Nevertheless, the Cochrane review(10) and the UK CF Trust working group(4) recommend the use of *S. aureus* prophylaxis until 3 years of age. In contrast, concerns regarding the possibility of the emergence of *P. aeruginosa* and uncertainty regarding clinical benefit prompted the US Cystic Fibrosis Foundation to recommend against prophylaxis.(9) As a result, *S. aureus* prophylaxis is in general not practiced in the US.

Our objective was to test the hypothesis that anti-staphylococcal antibiotic prophylaxis confers a positive microbiologic outcome for children with CF (prolongation of the time to first *S. aureus* detection), without an increase in microbiologic complications (reduced time to first detection of *P. aeruginosa*).

## **METHODS**

We aimed to identify the first occasion when *S. aureus*, *P. aeruginosa* and MRSA was detected for each child and describe the time to first infection for each cohort.

### The Registries

We retrieved data for the years 2000-2009 inclusive from the UK Cystic Fibrosis Trust Registry and US Cystic Fibrosis Foundation National Patient Registry. In the US Registry, data from each clinical encounter are entered into the Registry (Figure S1). The UK Registry however documents annual data. As a result clinical encounters of the year are reviewed and summarised at the annual review, traditionally in the child's birth month. It is the date of the annual review and the detail of the annual summary that is recorded on the Registry.

In order to make the US Registry data as equivalent as possible to the UK for the primary (UK/US) analysis, we annualised the US data by condensing the encounter-level data to a single record per calendar year. This was achieved by identifying the encounter nearest to the child's birthday. For the variables of interest, this entry summarised the activity in the previous year. This is described in more detail in the online supplement.

### Study population

Our cohorts comprised children with a diagnosis of CF enrolled in the registries before the age of 1500 days (approximately 4 years). We chose 4 years to maximize the time for children to have a bacterium isolated from their respiratory samples, 1 year beyond the time period that UK guidelines recommend anti-staphylococcal antibiotic prophylaxis be used. Routine practice in the UK is for children to have a respiratory sample taken at each clinical encounter (4-6 times a year) and in the US samples taken quarterly. We excluded children who isolated

*S. aureus* (methicillin-susceptible or –resistant) and *P. aeruginosa* at first registry encounter. We excluded those receiving inhaled tobramycin or colistin at first registry encounter, as these children were likely to have already acquired *P. aeruginosa*. We also excluded those children with only one registry entry.

We unexpectedly identified a cohort of children in the UK that were documented as not receiving antibiotic prophylaxis. As a result we were able to undertake two analyses.

### Analyses

The primary analysis evaluated the time to first detection of infection in children with CF in the UK (*S. aureus* prophylaxis recommended until three years of age) and US (*S. aureus* prophylaxis not practiced in the first three years of life). We were also able to undertake a secondary analysis evaluating time to first infection with *S. aureus* and *P. aeruginosa* among children in the UK recorded as receiving flucloxacillin prophylaxis vs. no prophylaxis. There were a number of children in the UK registry recorded as receiving a number of different oral antibiotics given over a prolonged time, making it unclear whether these were prophylactic antibiotics. We therefore limited the analysis of prophylactic antibiotics in the UK to children in whom the same chronic antibiotic - or no antibiotic - was recorded over two successive years. Due to the small number of children who consistently received an antibiotic other than flucloxacillin these children were also excluded. Thus the final comparison was between those who received flucloxacillin or no prophylaxis.

### Analytic methods

For the survival analyses, failure time was defined as time to first detection of *P. aeruginosa* or *S. aureus*, respectively. Children not acquiring these organisms during follow up were

censored at the last data entry prior to the age of 1500 days, or end of the study (31 December 2009) for those not reaching the age of 1500 days. We determined if country (US/UK) or the use of antibiotic prophylaxis (for the UK-based analysis) was associated with the time to first detection using a Cox proportional hazards regression model, after confirming the proportional hazards assumptions were not violated based on Schoenfeld residuals. Potential confounding factors (gender, age at registry entry, Dornase alfa use, genotype (homozygous Phe508del, other) and center size) were included if they individually resulted in a 10% or greater change to the estimate.(11) Kaplan-Meier plots were constructed to illustrate the survival analysis.

We undertook several sensitivity analyses in order to detect any effects of decisions made in the protocol upon the final results, these are described in the online supplement. The STROBE guidelines were used for reporting(12). All data was analysed with Stata SE12 (College Station, Texas, USA). The study received no specific funding. MH was funded by a Wellcome Trust Research Training Fellowship (WT092295MA) and latterly a NIHR Academic Clinical Lectureship.

The protocol is available online as detailed in the online supplement.

## **RESULTS**

### **Primary analysis cohort derivation and characteristics**

For the years 2000-2009, there were 2325 individuals in the UK registry and 11002 individuals in the US registry younger than 1500 days at registry entry. After excluding children who were receiving inhaled tobramycin or colistin at the first annualised registry entry (n=474 in the UK, n=1589 in the US) those in whom *S. aureus* or *P. aeruginosa* was detected at their first entry



(n=272 in the UK, n=5104 in the US) and those with only one registry entry (n=505 in the UK, n=745 in the US), the final study cohort consisted of 1074 UK children and 3564 US children (Figure 1). As shown in Table 1, the characteristics of children included in and excluded from the final cohorts as well as those of the UK and US cohorts were similar with the exception of a much higher prevalence of Dornase alfa use in the US.

### **Primary analysis – comparison of detection of bacteria between UK and US**

The risk of first detection of *S. aureus* (MSSA) was significantly greater in the US cohort than the UK cohort (hazard ratio (HR) 5.79; 95% CI 4.86, 6.90,  $p < 0.001$ ) (Figure 2). Similarly, the risks of first detection of *P. aeruginosa* (HR 1.92; 95% CI 1.65, 2.23,  $p < 0.001$ ) (Figure 2) and MRSA (HR 5.66; 95% CI 3.35, 9.57,  $p < 0.001$ ) (Figure 2) were significantly greater in the US than the UK. None of the model estimates was changed by 10% or more following inclusion of sex, CF genotype, Dornase alfa or age at registry entry as covariates.

In order to determine if the method of cohort selection exerted a significant effect upon the results, a sensitivity analysis was conducted which included all children younger than 1500 days. Another sensitivity analysis determined if the direction of effect changed over the time period of the study by comparing the periods 2000-2004 and 2005-2009. For both sensitivity analyses the estimates of the hazard ratios were similar to the original analyses (see online supplement).

### **Secondary analysis– UK based comparison of flucloxacillin versus no prophylaxis**

Of all the 2325 children in the UK registry, 1696 children were documented to have received a consistent regimen (either a consistent antibiotic class or no chronic antibiotics). Of the 1074 children included in the UK cohort of the UK/US analysis, 470 were excluded from this

secondary analysis due to inconsistent antibiotic prophylaxis (n=442) or consistent use of prophylaxis with an antibiotic other than flucloxacillin (n=28). We included 604 children, with 2 or more years of data, in the analysis, of whom 326 received flucloxacillin and 278 received no prophylaxis (Figure 1).

Distribution of prophylactic antibiotic use by center was examined and (Figure S2) demonstrated a spectrum of use across centers. The characteristics of the UK cohort, comparing those included to those excluded from analysis, is shown in Table 2.

### **Time to first detection of *S. aureus* and *P. aeruginosa***

Sixty-four children experienced their first *S. aureus* culture detection during 1023.9 person years at risk. Flucloxacillin use was not associated with risk of first detection of *S. aureus* (HR 1.22; 95% CI 0.74, 2.0,  $p=0.43$ ). One hundred and thirteen children had their first *P. aeruginosa* detection during 970.7 person years at risk, with those receiving flucloxacillin having a significantly increased hazard (HR 2.53; 95% CI 1.71, 3.74,  $p<0.001$ ) compared to those receiving no prophylaxis. There was no association detected between prophylaxis use and detection of MRSA (HR 1.57; 95% CI 0.1, 25.2,  $p=0.75$ ). Inclusion of sex, age at entry to the registry, Dornase alfa use, genotype and center size as covariates did not significantly affect the risk estimates for any of the models.

### **Sensitivity analysis**

To determine if the results of the UK-based analysis were similar in those with the most complete data, in those children up until the age of 3 (as per UK guidance regarding duration of prophylaxis) and in order to consider if time trends exerted an effect over the duration of

the study, further sensitivity analyses were conducted, the results of which were not significantly different than the original results (presented in the online supplement).

## **DISCUSSION**

This study is the first observational study to examine the effect of *S. aureus* antibiotic prophylaxis in infants with CF on microbiologic outcomes using 'real-world' data and furthers the debate regarding its risks and benefits. In this retrospective study describing the first detection of *S. aureus* and *P. aeruginosa* in the UK and US we found that the risk of first detection of both organisms is significantly increased in the US compared to the UK. Unexpectedly, we discovered a cohort of children in the UK that were not documented to be receiving anti-staphylococcal antibiotic prophylaxis. We therefore undertook an analysis of a cohort of children in the UK who either consistently received flucloxacillin or received no prophylaxis. We found that flucloxacillin use does not appear to be associated with a reduced risk of first detection of *S. aureus*. However flucloxacillin use does appear to be associated with an increased risk of first detection of *P. aeruginosa*.

Our findings differ from the conclusions of a Cochrane review which found that anti-staphylococcal prophylaxis resulted in a reduction in the proportion of children isolating *S. aureus*.(10) The Cochrane review considers only randomised controlled trials, which may in part explain this difference. Furthermore, only two of the included studies involved flucloxacillin and both of these were open label studies comparing continuous flucloxacillin prophylaxis with 'as-required' arms instead of placebo. The only double-blind randomised trial of antibiotic prophylaxis used cephalexin and observed a delay in detection of *S. aureus* but an increase in detection of *P. aeruginosa*, (13), an observation that is consistent with our

data. An Australian observational study utilizing bronchoalveolar lavage-based microbiological sampling found that co-amoxiclav (amoxicillin-clavulanate) antibiotic prophylaxis use was not associated with either detection of *P. aeruginosa* or *S. aureus*(14), though there was a non-significant excess of *P. aeruginosa* isolates in the prophylaxis group. It is possible that the Australian study did not have sufficient power to detect a significant difference.

There is a contradiction that lies within these data – in the US (where antibiotic prophylaxis is not administered) the risk of first detection of *P. aeruginosa* is greater than in the UK. Yet when examining the UK data we observe that those administered prophylaxis have a reduced time to first *P. aeruginosa* infection compared with those not given prophylaxis. It is likely that the observed differences in risk of detection of *P. aeruginosa*, *S. aureus* and MRSA between the US and UK are due to differences in ecological conditions in the two countries (e.g, very different rates of microbial colonization in the general and CF populations and major differences in the healthcare systems), rather than any association with staphylococcal prophylaxis. In fact, the finding of a significantly earlier age of first detection of *S. aureus* in the US compared to the UK is not surprising given the previously identified 3–fold greater annual prevalence of MSSA and an 8-fold greater annual prevalence of MRSA in US CF centers compared to the UK.(15) The nasopharyngeal carriage of *S. aureus* among healthy children in the UK is unknown whereas such carriage has been reported to be as high as 48% in the US(16) and 36% in the Netherlands.(17)

It is known that more sampling opportunities provide a greater chance of isolating an organism should it be present. The European Cystic Fibrosis Society and UK CF Trust standards of care suggest that patients should be seen every 1-3 months,(7, 8) however while this may be commonplace for young children, anecdotally the true frequency of visits is likely to lie at

the upper end of the range. In the US dataset, where data from every clinic visit are documented, the median gap between visits for children under 4 years (1500 days) old was 53 days (IQR 28, 89 days). The more frequent sampling in the US could have contributed to the observed higher detection rates, but again are unlikely to explain the entire effect.

Due to the differences in the way data are recorded in the two registries, namely that the US data consists of encounter-based data whereas the UK data consists of an annual summary of the previous year, an immortal time bias (a period of time where it is not possible to detect a bacterial isolate) could exist. We annualised the US data to minimize this source of bias. The size of the observed effect, in combination with the significant differences between prevalence of infection in the two countries, suggest that immortal time bias is unlikely to account for all the observed differences.

One potential explanation for the increased risk of *P. aeruginosa* infection among those receiving flucloxacillin in the UK analysis is reverse causation – that patients were actually receiving *treatment* of persistent symptoms rather than prophylaxis. If that were the case, these individuals would have been sicker, with more structural lung disease, and so consequently at increased risk of *P. aeruginosa* infection. The fact that *S. aureus* detection rates were similar between these two groups argues against reverse causation to some degree.

In the earlier years of the UK Registry a substantial proportion of individuals had incomplete data (29% in 2001). However the proportion with incomplete data has steadily decreased over time (11% incomplete in 2014). Our sensitivity analyses suggest that changes in the completeness of data did not have a significant effect upon our findings. The time period of this study (2000 – 2009) includes a period when newborn screening was not widely

implemented in either the UK or the US. Furthermore, microbiological laboratory techniques may have changed. These factors may limit the generalisability of our findings.

The polymicrobial nature of CF lung infection is increasingly appreciated.(18) Those with good lung function host greater lung microbial diversity compared to their counterparts with poorer lung function or who experience frequent exacerbations.(19, 20) The effect of prophylactic antibiotics upon this complex ecosystem is unknown. This question is particularly significant given that the effect of intravenous antibiotics upon the microbiome appears to be limited in terms of quantitative microbiology, but significant in terms of bacterial diversity.(20) (21) It may be that chronic exposure to prophylactic antibiotics disrupts the fecal and/or respiratory microbiome, providing a favourable ecosystem for opportunistic bacteria like *P. aeruginosa*.

The large numbers of children from the two countries providing data to the registries is a significant strength. One important limitation of our study is that it relies on oropharyngeal and cough swabs, which are known to have relatively low sensitivity and specificity for lower airway infection, (22) (23) particularly as *S. aureus* colonisation of the upper airways of healthy children is common.(16) Thus, our results describing upper airway cultures may not be generalizable to lower airway infection. We also do not have adherence data in either registry.

Given these limitations, these data should be interpreted with caution. Nevertheless, these results will be concerning to those who endeavour to postpone the age at which infection with either *S. aureus* or *P. aeruginosa* is first acquired. Infection with *S. aureus* in the 1960s and 1970s had a devastating effect in young children. However the improved management, standards of living, nutrition and subsequent survival of children with CF is such that the spectrum of disease seen in this earlier era does not reflect the current situation.(24) This

might mean that the balance of risks and benefits of staphylococcal prophylaxis has changed - the tenet of 'first do no harm' appears to be apt.

A randomised controlled trial of antistaphylococcal prophylaxis in the UK has commenced (<http://www.nets.nihr.ac.uk/projects/hta/142223>) which will address the biases of the previous studies. It will also be important in the future to determine the effect of such antibiotic administration upon the flora of the lungs of young children with CF in order to explain the findings of the RCT in microbiological terms.

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## Figure legends

Figure 1: Illustration of derivation of cohorts for a) UK vs. US and b) UK flucloxacillin vs. no prophylaxis comparisons.

Figure 2: Kaplan-Meier plot of time to first acquisition of *S. aureus*, *P. aeruginosa* and MRSA in UK and US truncated at 3 years

Figure 3: Kaplan Meier plot of time to first acquisition of *S. aureus* and *P. aeruginosa* by prophylaxis use in UK truncated at 3 years

Figure S1: Illustration of clinical encounters and data entry. Clinical encounters (gold dots) are summarised at the annual review (black dot) and entered onto the Registry. The first bacterial detection (hollow diamond) is entered onto the Registry at the annual review (black dot and solid diamond). Child D is excluded from the study as they were receiving inhaled colistin suggesting a previous infection. Child E remains infection-free and is censored at the end of the study period as they do not reach the exit age of just over 4 years (1500 days).

Figure S2: Distribution of antibiotic prophylaxis use in UK by center

## Tables

Table 1: Baseline characteristics of UK and US registries before and after cohort selection included in UK/US analysis (% , IQR) for children up to just over 4 years (1500 days).

	All children aged younger than 1500 days at registry entry (n (%) or median (IQR))		Included in final cohort (n (%) or median (IQR))	
	UK	US	UK	US
n	2325	11002	1074	3564
Male	1169 (50.3%)	5512 (50.1)	562 (52.3%)	1806 (49.3%)
Female	1156 (49.7%)	5490 (49.9)	512 (47.7%)	1758 (50.7%)
Age at entry (days)	475 (351, 975)	352 (319, 717)	381 (225, 691)	354 (322, 718)
<b>Diagnosed by screening/PNS</b>	815 (35.1%)	3550 (32.3%)	417 (38.8%)	955 (26.8%)
BMI z-score	0.4 (-0.3, 1.1)	0.3 (-0.5, 0.9)	0.36 (-0.5, 1.1)	0.26 (-0.5, 1.0)
Dornase alfa (ever in first 1500 days)	158 (6.8%)	4157 (37.8%)	71 (6.6%)	1171 (32.9%)
Homozygous Phe508del *	1271 (54.7%)	5094 (46.3%)	588 (54.8%)	1665(46.7%)

\*percentage of total including those with missing or unknown genotype data. After annualisation of US data. PNS – prenatal screening.

Table 2: Baseline characteristics of children included in and excluded from UK comparison

(N (%) or median (IQR))

	No prophylaxis	Flucloxacillin prophylaxis	Between included groups, p=	Excluded	Included vs. excluded, p=
<b>n</b>	278	326	-	<b>1721</b>	-
<b>Male</b>	159 (57.0)	167 (50.9)	<b>0.16</b>	843 (49.0)	<b>0.04</b>
<b>Age at entry, days</b>	410 (277, 773)	383.5 (270, 699)	<b>0.06</b>	547 (359, 1085)	<b>&lt;0.001</b>
<b>BMI z-score</b>	0.46 (-0.47, 1.20)	0.37 (-0.46, 1.08)	<b>0.47</b>	0.44 (-0.29, 1.15)	<b>0.52</b>
<b>Dornase alfa (ever in first 1500 days)</b>	18 (6.5%)	21 (6.4%)	<b>1.0</b>	119 (6.9%)	<b>0.78</b>
<b>df508/df508*</b>	151 (54.3%)	179 (54.9%)	<b>0.94</b>	941 (54.7%)	<b>1.0</b>

\*percentage of total including those with missing or unknown genotype data.

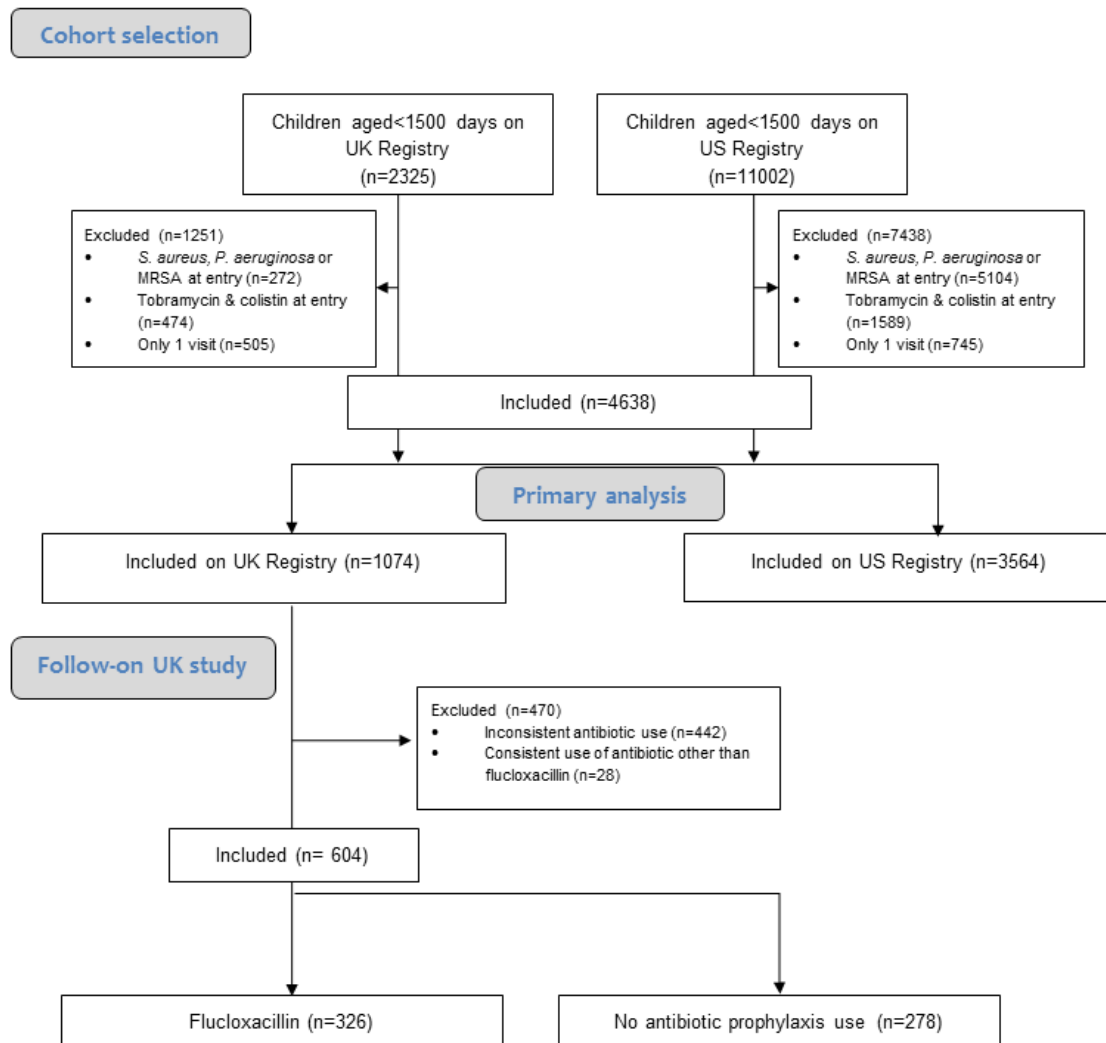
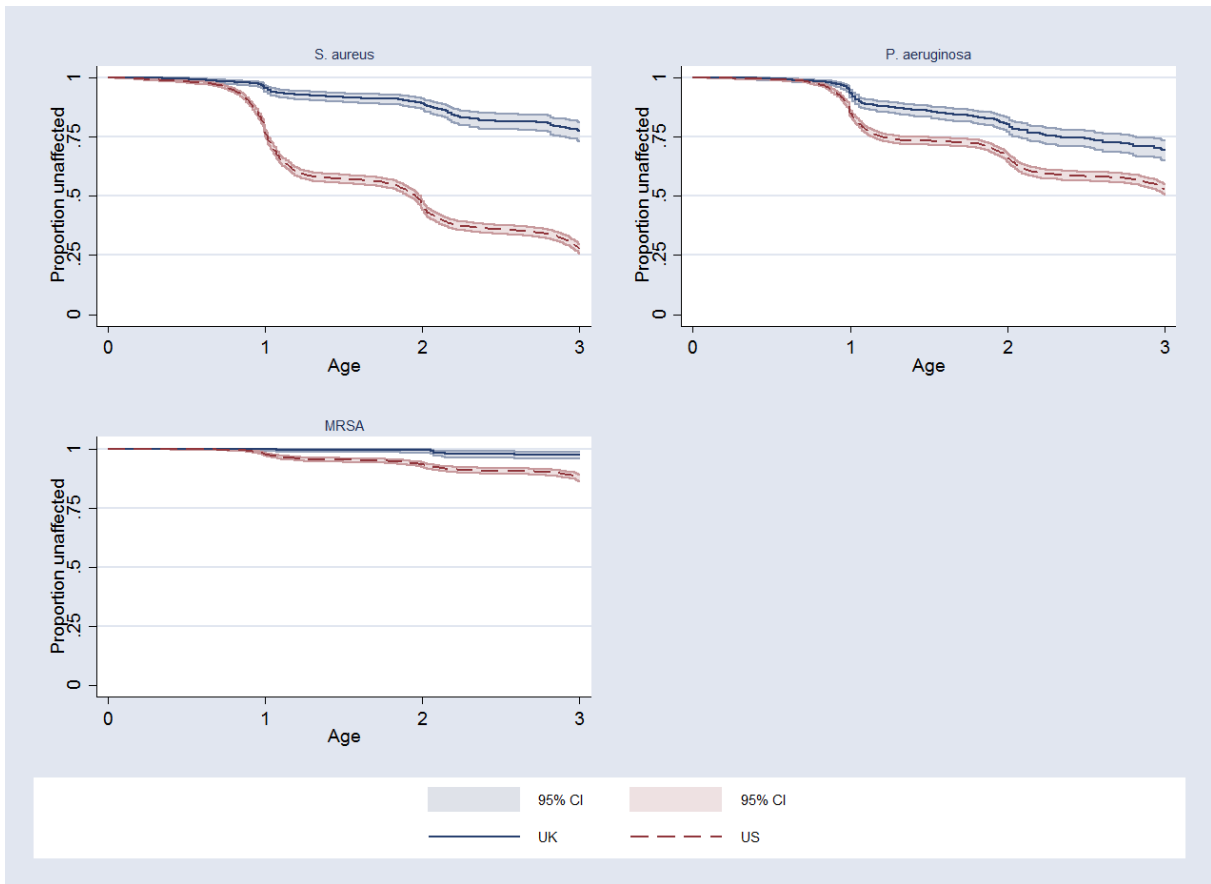
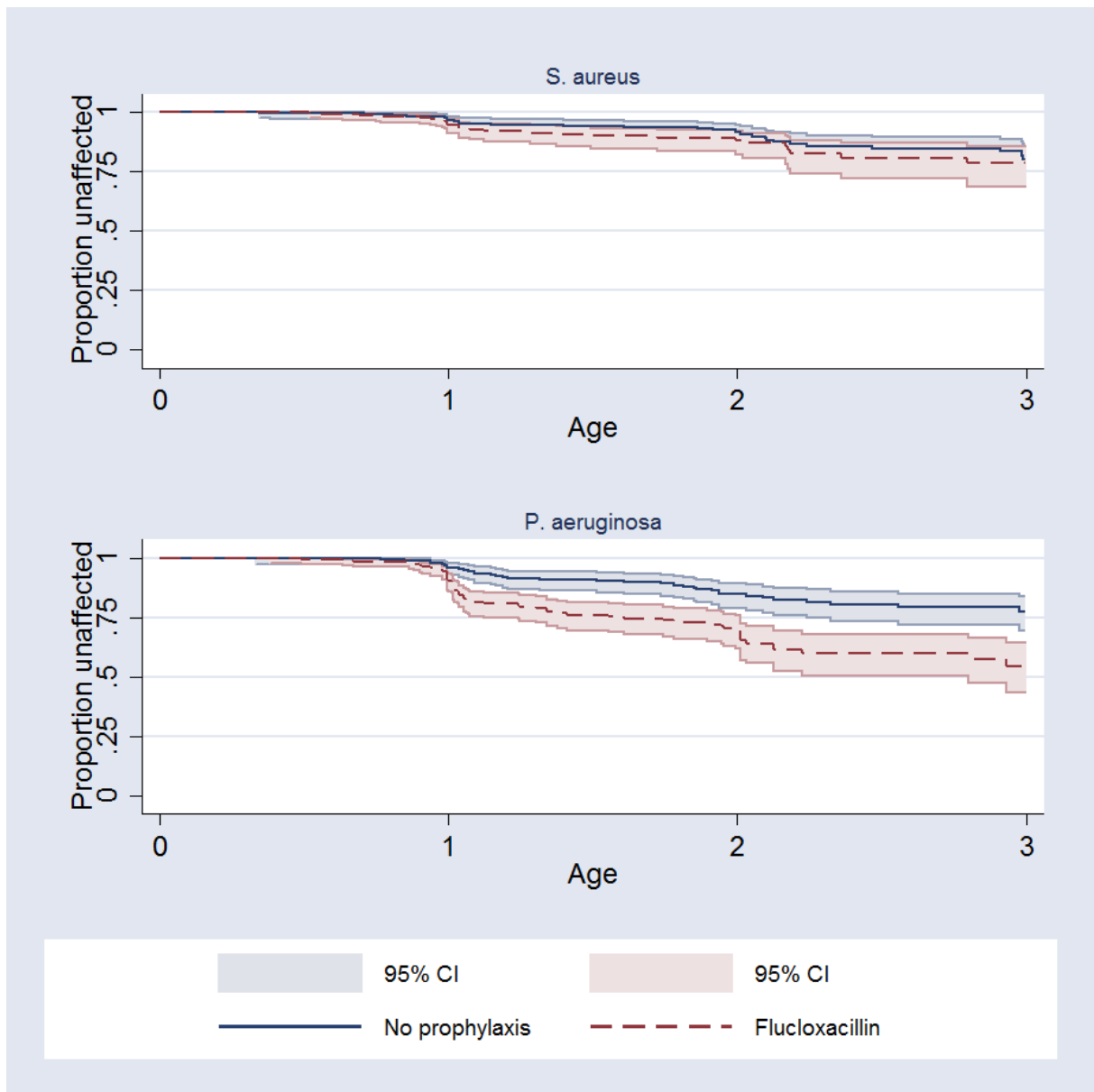


Figure 1: Illustration of derivation of cohorts for a) UK vs. US and b) UK flucloxacillin vs. no prophylaxis comparisons.



Kaplan-Meier plot of time to first acquisition of *S. aureus*, *P. aeruginosa* and MRSA in UK and US truncated at 3 years



Kaplan Meier plot of time to first acquisition of *S. aureus* and *P. aeruginosa* by prophylaxis use in UK truncated at 3 years

## **ONLINE DATA SUPPLEMENT**

**Early respiratory bacterial detection in young children with cystic fibrosis in the US and UK and association with anti-staphylococcal antibiotic prophylaxis use in the UK**

**Matthew N Hurley, Andrew Fogarty, Tricia M McKeever, Chris CH Goss, Margaret Rosenfeld, Alan R Smyth**



## Further methods

### *Outcome and annualisation*

The US Registry details encounter-based data and so data from each clinical encounter is documented (gold dots on the illustration; Figure S1). The UK Registry however documents annual data. As a result clinical encounters of the year are reviewed and summarised at the annual review (black dots), traditionally in the child's birth month. It is the date of the annual review that is recorded on the Registry.

As in the illustration below (Figure S1), in the UK Registry encounters (gold dots) do not appear on the Registry, instead they are summarised annually at the annual assessment (black dots). For example, child A & B were identified through newborn screening and diagnosed soon after. They were present throughout the study period. Child A had a positive isolate at 3 years 3 months of age (hollow red diamond). This was captured at the annual assessment and recorded on the Registry (black dot at 1460 days). Child A exits the study at that point. The time of 'failure' for the time-to-event analysis is the date of the annual assessment (solid red diamond).

Child B had a positive isolate at 1 year 5 months of age (hollow red diamond) and this was captured at the following annual assessment and recorded on the Registry (black dot at 720 days). The time of 'failure' for the time-to-event analysis is the date of the annual assessment (solid red diamond).

Child C enters the study at the age of 18 months as they were born before commencement of the study period (left censoring). Child C had a positive isolate at 3 years 3 months of age (hollow red diamond). This was summarised at the following annual assessment and recorded

on the Registry (black dot at 1460 days). The time of 'failure' for the time-to-event analysis is the date of the annual assessment (solid red diamond). Child D's data are also left censored however there is an indication that previous infection may have been encountered as the child was receiving inhaled colistin at first entry, and so was excluded from the study.

Child E does not have a positive isolate however does not reach their 4<sup>th</sup> birthday as the study period ends and so their entry is right censored.

In contrast, as the US data documents each encounter the data is more continuous and so there are Registry data entries for each dot. In order to replicate the structure of the UK Registry, the US data were 'annualised'. This was achieved by identifying the encounter nearest to the child's birthday. For the variables of interest, this entry represented the activity in the previous year. Just as in the UK Registry, if a child encountered an isolate in the previous year, as is the case with Child B at 18 months, this is recorded at the birthday encounter (at 720 days).

#### Culture methods

Almost all respiratory cultures were obtained from cough swabs or oropharyngeal swabs, as young children can rarely expectorate and bronchoalveolar lavage is not routinely performed in the US or UK.

We undertook several sensitivity analyses in order to detect any effect of decisions made in the protocol upon the final results. First, for the US/UK comparison, we repeated it for all children <1500 days at study entry regardless of whether they met the exclusion criteria. To evaluate the effect of incomplete data collection in the early years of the UK Registry, we conducted a sensitivity analysis for both the UK/US comparison and the within-UK

comparison comparing the time periods 2000-04 and 2005-09 (table 3). For the UK evaluation of prophylactic antibiotics, we repeated the analysis including the children receiving an inconsistent antibiotic regimen in the prophylaxis group. Further sensitivity analyses were conducted for the UK-based analysis limiting the cohort to those with three or more consistent visits with a respiratory culture result recorded and those aged younger than 3 years. In the sensitivity analyses, characteristics of the included and excluded samples were compared using Pearson  $\chi^2$  test, Fisher exact and the Mann-Whitney U test as appropriate.

### **Protocol**

The study protocol is available at <http://eprints.nottingham.ac.uk/37205/>

## Further results

### UK vs. US analysis

#### *Sensitivity analysis*

In order to determine if the selection of the included cohorts had a significant effect on the results, the analysis was repeated using the entire unselected cohort of children <1500 days of age (2325 UK children and 11002 US children). The risk of first acquisition of *S. aureus* for the US vs. the UK was HR 5.47; 95% CI 4.70, 6.38,  $p < 0.001$ ; *P. aeruginosa*, HR 1.82; 95% CI 1.60, 2.07,  $p < 0.001$ ; and MRSA HR 6.67; 95% CI 4.27, 10.41,  $p < 0.001$ , similar to the primary analysis. A second sensitivity analysis investigated whether there were significant time trends between the beginning and end of the period demonstrating that the same results held (table E1). In this analysis we saw the same trends as observed in the original analysis, albeit with larger confidence intervals.

## UK (Flucloxacillin vs. No prophylaxis) analysis

### *Centre-based analysis of variation in practice*

Figure S2 illustrates the variation in practice across individual centres.

### *Sensitivity analysis*

To determine if the results of the UK-based analysis held in those with most complete data (those with three or more consistent visits with a respiratory culture recorded) we conducted a sensitivity analysis in this group (n=288). There was still no statistically significant difference in the risk of first acquisition of *S. aureus* associated with flucloxacillin use (HR 1.27; 95% CI 0.71, 7.19, p=0.429), while the finding of a statistically significant increased risk of first acquisition of *P. aeruginosa* persisted (HR 2.17; 95% CI 1.39, 3.38, p=0.001).

In a second sensitivity analysis, considering time at risk only up to the age of 3 years (n=446), the risk of first acquisition of *S. aureus* is also not statistically significantly different between the two groups (HR 1.55; 95% CI 0.72, 3.33, p=0.26) while the finding of a statistically significantly increased risk of first acquisition of *P. aeruginosa* in the flucloxacillin group persisted (HR 2.94; 95% CI 1.78, 4.85, p<0.001). A third sensitivity analysis investigated whether there were significant time trends between the beginning and end of the period demonstrating that the same results held, although the increased risk of *P. aeruginosa* in the later period was no longer significant (table E2).

We detected significant differences in the UK/US analysis and in the *P. aeruginosa* analysis of the UK data. The post-hoc power of the UK flucloxacillin/none analysis (*S. aureus*) comparison (for which there was no difference) is 67% based on an estimated power for two-sample comparison of survivor functions - Log-rank test, Freedman method.

UK/US analysis	Failures (n)	All years (HR, 95% CI)	Failures (n)	2000-2004 (HR, 95% CI)	Failures (n)	2005-2009 (HR, 95% CI)
<i>S. aureus</i>	2232	5.79 (4.85, 6.90)	1561	4.73 (3.90, 5.75)	671	11.03 (7.2, 16.89)
<i>P. aeruginosa</i>	1495	1.92 (1.65, 2.24)	1088	1.72 (1.45, 2.04)	407	2.80 (2.02, 3.87)

Table E1: Sensitivity analyses of risk of first acquisition by epoch

Flucloxacillin / No prophylaxis	Failures (n)	All years (HR, 95% CI)	Failures (n)	2000-2004 (HR, 95% CI)	Failures (n)	2005-2009 (HR, 95% CI)
<i>S. aureus</i>	64	1.22 (0.74, 2.0)	54	1.29 (0.76, 2.21)	10	1.16 (0.30, 4.48)
<i>P.aeruginosa</i>	113	2.53 (1.71, 3.74)	91	2.73 (1.78, 4.18)	22	2.03 (0.73, 5.68)

Table E2: Sensitivity analyses of risk of first acquisition by epoch

### Supplemental Figure Legends:

**Figure S1:** Illustration of clinical encounters and data entry. Clinical encounters (gold dots) are summarised at the annual review (black dot) and entered onto the Registry. The first bacterial detection (hollow diamond) is entered onto the Registry at the annual review (black dot and solid diamond). Child D is excluded from the study as they were receiving inhaled colistin suggesting a previous infection. Child E remains infection-free and is censored at the end of the study period as they do not reach the exit age of just over 4 years (1500 days).

**Figure S2:** Distribution of antibiotic prophylaxis use in UK by center

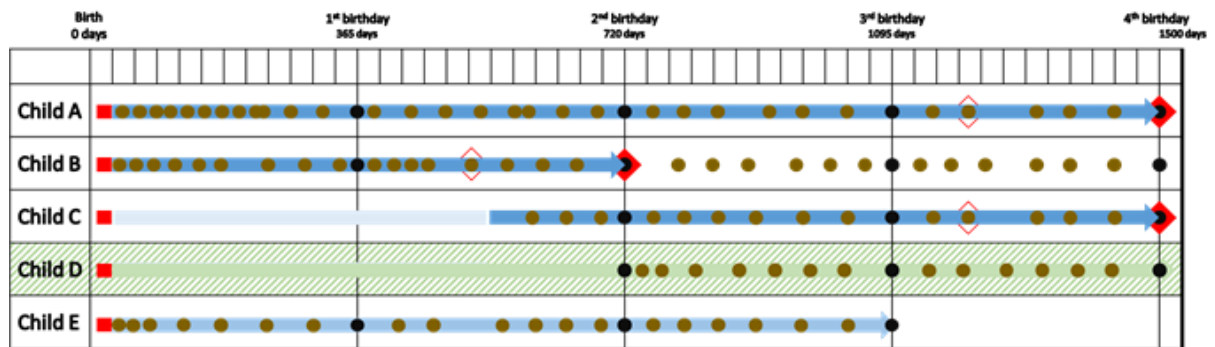
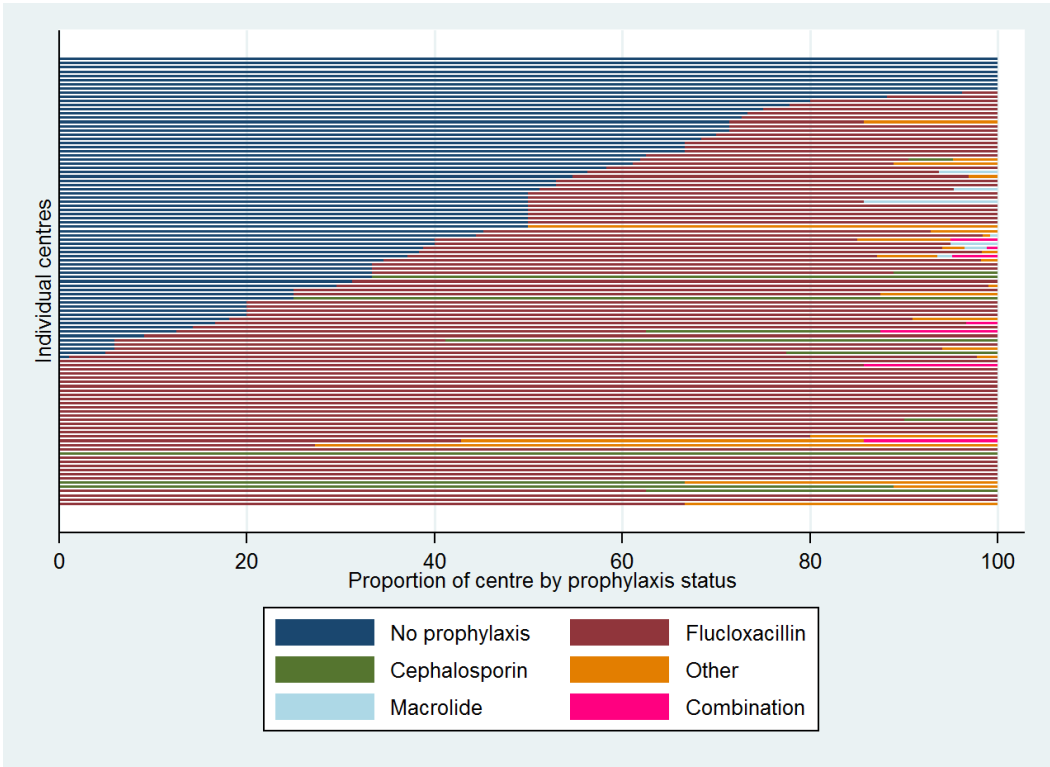


Illustration of clinical encounters and data entry. Clinical encounters (gold dots) are summarised at the annual review (black dot) and entered onto the Registry. The first bacterial detection (hollow diamond) is entered onto the Registry at the annual review (black dot and solid diamond). Child D is excluded from the study as they were receiving inhaled colistin suggesting a previous infection. Child E remains infection-free and is censored at the end of the study period as they do not reach the exit age of just over 4 years (1500 days).



Distribution of antibiotic prophylaxis use in UK by center