

**Prophylactic azithromycin in protracted bacterial bronchitis: parental experience and impact on bacterial resistance and the microbiome.**

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A thesis submitted in fulfilment of the requirements for the degree of Doctor of Medicine

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Infection, Immunity and Cardiovascular Disease

June 2022

# Summary

**Introduction**

Protracted bacterial bronchitis (PBB) commonly underlies childhood chronic wet cough. Azithromycin prophylaxis is increasingly used to reduce exacerbations. We aimed to explore parental experience of having a child prescribed long-term antibiotics and investigate the impact of azithromycin prophylaxis on antimicrobial resistance and the nasopharyngeal microbiome.

**Methods**

Fifteen parents of children prescribed prophylactic antibiotics for respiratory infections were interviewed. Thematic analysis was carried out.

Antimicrobial resistance and the microbiome were investigated by deep nasopharyngeal swabbing of children with PBB who were prescribed prophylactic azithromycin or not on clinical grounds. Protocols were optimised in an initial feasibility study. In total, 50 children were enrolled - 25 were planned to take azithromycin over the winter months and 25 were not. Serial nasopharyngeal swabs were collected over the study periods (12-20 months). Bacterial isolates were tested for antibiotic resistance and resistant isolates sequenced. 16s PCR was performed on a subset of samples.

**Results**

From parental interviews key themes emerged, and a behavioural model to explain the parental experience developed. Parents desire their children to be well now, with antimicrobial resistance regarded as a possible future problem.

Higher than expected rates of azithromycin resistance were found in both PBB groups, although the resistance was mostly low level. Resistance was mostly driven by azithromycin-resistant *S. pneumoniae,* yet these isolates were predominantly erythromycin susceptible. There was a trend towards fewer resistant bacteria in the azithromycin group, IRR 0.63 (95%CI 0.37-1.06), p=0.083.

Alpha diversity was reduced in swabs from children taking azithromycin at the time of swab collection, p=0.02. Beta diversity was significantly different between the 2 groups at the study end; R2=0.14, p=0.0049.

**Conclusions**

A holistic approach should be taken when prescribing long-term antibiotics. Discrepancies between erythromycin and azithromycin susceptibility require further investigation. Azithromycin for PBB did not promote antimicrobial resistance over the course of the study but did perturb the microbiome.

# **Acknowledgments**

Without the support, advice and assistance of a great many people and their belief in me that this thesis could be submitted, I may not have reached the final stage.

Firstly, to my supervisor Professor Condliffe who has guided me along this journey. Her great experience, advice and encouragement has motivated me to continue through the ups and downs of these studies confounded by a pandemic! I am extremely grateful for her support. I am also incredibly grateful to Professor Lee whose expertise, thought provoking discussions and patience enabled me to carry out the qualitative research.

My thanks must extend to my clinical supervisors, Dr Shackley who changed the direction of my career with her enthusiasm and great ideas and Dr Ugonna whose support and pragmatism enabled me to carry out the projects. The respiratory nurses shared in the pain of recruitment and without their help and kindness, we may have stalled at the very beginning. The families and children who took part in this project require special thanks as they persisted with nasal swabs and interviews and made the studies possible.

Dr Darton and Dr Suligoy, I would like to thank for supporting me up a steep learning curve through antimicrobial resistance and sequencing interpretation. Rebecca Hull’s introduction to working in a laboratory has been incredibly valuable and her assistance with troubleshooting protocol issues has been amazing. Rachelle Macham and the microbiology laboratory team at STH have been incredibly helpful with culturing and susceptibility testing the bacterial isolates.

My thanks go also to the team at the Centre for Inflammation Research, University of Edinburgh, headed by Professor Bogaert who kindly collaborated with the project. I am very grateful to Paula Lusarreta Parga who processed all the samples for sequencing. Dr Ruiz Rodriguez and Justyna Binkowska’s advice was excellent, and they equipped me with the skills I needed to get me through the process of analysing the microbiome. I am extremely grateful to Professor Bishop who has championed the Bassetlaw fellowship programme, enabling me to have the opportunity to further my interest in paediatric research. Samya Armoush’s expertise have been incredible with registering the studies and helping apply for ethical approval. Further thanks go to the charity ANTRUK, the Sir Halley Stewart Trust and the Florey institute, whose funding enabled us to carry out these projects.

I must now thank my family who have all been through this with me. Their endless belief, support and sacrifices have been invaluable throughout this process. My wife Sam, has taken one for the team, kept life going enabling me the time to complete this and believed that we would all get there in the end. Thank you.

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# Abbreviations

AMR Antimicrobial resistance

ASV Amplicon sequence variant

BAL Bronchoalveolar lavage

BTS British Thoracic Society

CF Cystic fibrosis

CFTR Cystic fibrosis transmembrane regulator

CI Confidence interval

COPD Chronic obstructive pulmonary disease

DNA Deoxyribonucleic acid

DNPS Deep nasopharyngeal swab

EUCAST European Committee on Antimicrobial Susceptibility Testing

FEV1 Forced expiratory volume in 1 second

GP General Practitioner

*H. influenzae* *Haemophilus influenzae*

HIV Human immunodeficiency virus

HRCT High resolution computed tomography

IL Interleukin

IRR Incidence rate ratio

LCQ Leicester cough questionnaire

LRTI Lower respiratory tract infection

*M. abscessus* *Mycobacterium abscessus*

*M. catarrhalis Moraxella catarrhalis*

MIC Minimum inhibitory concentration

NBR Negative binomial regression

NCBI National Centre for Biotechnology Information

*N. gonorrhoea Neisseria gonorrhoea*

NICE National Institute for Health and Care Excellence

OTU Operational taxonomic unit

*P. aeruginosa Pseudomonas aeruginosa*

PBB Protracted bacterial bronchitis

PBP Penicillin-binding protein

PCV7 Pneumococcal conjugate vaccine – heptavalent

QTc Corrected QT interval (electrocardiogram)

RCT Randomised controlled trial

REC Research ethics committee

RNA Ribonucleic acid

RR Relative risk

*S. aureus Staphylococcus aureus*

SCFA Short chain fatty acids

SCH Sheffield Children’s NHS Foundation Trust

*S. pneumoniae Streptococcus pneumoniae*

STH Sheffield Teaching Hospitals NHS Foundation Trust

UK United Kingdom

URTI Upper respiratory tract infection

UTI Urinary tract infection

16S rRNA 16S ribosomal ribonucleic acid

# Glossary

* **Minimum inhibitory concentration**: The lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism
* **EUCAST susceptibilities categories:**
  + Susceptible: High likelihood of therapeutic success using a standard dosing regimen of the agent.
  + Intermediate: Susceptible, increased exposure. High likelihood of therapeutic success because exposure to the agent is increased by adjusting the dosing regimen or by its concentration at the site of infection.
  + Resistant: High likelihood of therapeutic failure even when there is increased exposure
* **Incident rate ratio:** A measure used to compare the incident rates of events occurring at any given time point. A value of 1 indicates no difference, a value of > 1 demonstrates increased risk of the outcome in the exposed group whilst a value of <1 indicates a reduced risk of the exposure in the exposed group.
* **Microbiota:** The collective microorganisms within a defined area which may include bacteria, viruses, fungi and protozoa.
* **Microbiome:** The collective genomes from all the microorganisms found in a particular area
* **Metagenomics:** The study of collective genomes of a mixed community of microorganisms.
* **Protracted Bacterial Bronchitis:** A history of chronic (≥ 4 weeks) wet cough, response to 2 weeks of appropriate antimicrobial therapy and an absence of clinical indicators suggesting an alternative cause for the cough (Marchant et al., 2006; Shields MD, 2008)
* **Bronchiectasis**: Bronchiectasis is defined in terms of “irreversible dilatation of peripheral airways” as diagnosed by chest- high resolution computerised tomography (c-HRCT) (Chang AB, 2008). Symptoms include prolonged (>12 weeks) wet cough, exertional dyspnoea, asthma-like symptoms and recurrent chest infections. Clinical signs, which are often delayed, may include growth failure, digital clubbing, chest wall deformity, hyperinflation and adventitial sounds (‘wet’ crackles) on chest auscultation.
* **Alpha diversity:** Measures of the structure of an ecological community with regards to richness (number of taxonomic groups), evenness (distribution of abundances of the groups) or both (Willis, 2019a).
* **Beta diversity:** Measures of the variation in species composition among communities (Ricotta, 2017).
* **OTU clustering:** Operational Taxonomic Unit clustering is a metagenomic analysis approach that categorises bacteria based on sequence similarity, whereby each cluster represents a taxonomic unit of species or genus.
* **ASV analysis:** Amplicon Sequence Variant analysis is a metagenomic approach that determines the exact sequences recovered from high throughput gene marker (often 16s rRNA). Rather than clustering, an error model is used to determine the probability of sequence errors. ASVs infer true biological origin as opposed to a clustering approach

# **Chapter 1. Introduction**

## Chronic cough in children

Chronic cough in children is very common, with a prevalence of around 13% in one multi-centre study of 7-11 year olds (Leonardi et al., 2002). It causes significant worry, stress and sleepless nights for parents and can affect their reporting of the burden of illness (Marchant et al., 2008). Of 190 children referred to a tertiary centre in Australia, >80% had sought medical advice on 5 or more occasions over the past 12-months making it a very common presenting complaint (Marchant et al., 2008). The commonest causes in children include asthma, protracted bacterial bronchitis (PBB), gastro-oesophageal reflux disease and tuberculosis, depending on the population studied.

Guidelines for the investigation and management of chronic cough in children have recently been published by the European Respiratory Society (Morice et al., 2020). These state that, following a detailed history and examination for signs and symptoms of specific disease, a chest radiograph and spirometry (if older than 5 years) should be performed. If no specific pointers of disease are identified, the cough should be categorised as either wet or dry. If wet, a sputum sample should be obtained if possible, and antibiotics started to treat for possible protracted bacterial bronchitis.

## Protracted bacterial bronchitis

Protracted bacterial bronchitis (PBB) is characterised by an isolated wet cough, growth of respiratory pathogens (which may require bronchoalveolar lavage in children, hence not always proven) and response to 2-4 weeks of appropriate oral antibiotics. It is most often described in pre-school children although it may be diagnosed in those over 12-years, and has been identified as the commonest cause of chronic cough in younger children referred to paediatric hospital specialists, accounting for up to 40% of cases (Marchant et al., 2006). PBB may be defined by one of three sets of criteria; clinical PBB, microbiological PBB and extended PBB (Table 1). A suggested pathway for diagnosis is provided by the European Respiratory Society statement on PBB (Figure 1‑1).

**Table 1:** Diagnostic classification of PBB (Kantar et al., 2017)

|  |  |  |  |
| --- | --- | --- | --- |
| PBB Definition | **Clinical** PBB | **Microbiological** PBB | **Extended** PBB |
| Clinical Criteria | •Chronic wet cough  > 4 weeks  •Absence of signs of other causes  •Cough resolves with 2 weeks of appropriate oral antibiotic treatment | •Chronic wet cough > 4 weeks  •Lower airway infection (isolation of a single bacterial species greater 104 colony-forming units/ml in sputum or BAL)  •Cough resolves with 2 weeks of appropriate oral antibiotic | •PBB clinical or PBB microbiological but cough resolves after 4 weeks of oral antibiotics. |

Diagram

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**Figure 1‑1:** Proposed diagnostic pathway for children with chronic cough

***\*****Specific cough pointers include symptoms of haemoptysis, feeding difficulties, recurrent sinopulmonary infections and signs such as digital clubbing or chest wall deformities. (See appendix 1 for more comprehensive list). If no cough pointers are present and the cough resolves with a trial of an appropriate course of oral antibiotics, a clinical diagnosis of PBB can be made. (Adapted from (Kantar et al., 2017))*

Children are rarely able to produce a sputum sample and the only way of obtaining uncontaminated lower airways samples may be by bronchoscopy, which requires a general anaesthetic in this age group. Due to the risk/benefit balance of invasive sampling, the diagnosis of PBB is often made on clinical grounds. The European Respiratory Statement on PBB recommends a ≥2-week course of oral antibiotics (most widely used antibiotic is amoxicillin-clavulanate), active against typical respiratory bacterial pathogens found in the lower airways of these children (Kantar et al., 2017). Following successful treatment, some children have further pulmonary exacerbations characterised by increased cough, volume of sputum and often associated with new chest signs such as crackles. Limited retrospective studies with a variety of PBB definitions have looked at recurrence rates and report up to 75% have multiple episodes of PBB (Kantar et al., 2017). These recurrent episodes may be due to bacterial or viral causes - without sampling, it is difficult to tell them apart. Factors thought to be involved in driving the pathophysiology of PBB include high bacterial loads and neutrophilic inflammation as detected by bronchoalveolar lavage (BAL). *H. influenzae* is the most common organism found on BAL in PBB, and it is likely that the presence of biofilms contributes to a state of chronic inflammation (Chang et al., 2016). Airway ‘malacia’ is a condition whereby there is laxity of the cartilaginous airway walls allowing for a degree of collapse of the airway. Large airway malacia was reported to be unexpectedly common (44% of PBB cases), predominantly in children less than 1 year of age (Wang et al., 2015); however, a prospective study of 104 children with PBB (Wurzel et al., 2014) found that malacia was not significantly more common in children with PBB than in those undergoing bronchoscopy for other respiratory indications (68% vs. 53% respectively). The role of viruses is unclear, given that similar rates of viruses have been found in BAL of children with PBB and healthy controls (Y. Wang et al., 2019). Figure 1‑2 summarises the pathophysiological features. The natural history of mild disease remains unclear, however, recurrent PBB (>3 episodes per year) has been reported to be predictive of the future diagnosis of bronchiectasis (Goyal et al., 2014; Wurzel et al., 2016), which confers lifelong susceptibility to respiratory infection and increased risk of premature death (Quint et al., 2016). Data from a cohort of 166 children with PBB and 28 controls report an overall 3-fold reduction in PBB exacerbations during the 5-year follow up period. However, 67.5% had ongoing symptoms with 27% having clinician-diagnosed asthma (Ruffles et al 2020). Due to the young age of children within this cohort, the diagnosis of asthma may not have been accurate in all cases (median age during follow up 8 years, IQR 4-20 years). A diagnosis of bronchiectasis was made in 9.6% with significant predictors of bronchiectasis being recurrent episodes of PBB in year one of follow up and the presence of *H. influenzae* on BAL (Ruffles et al., 2021).

Controversy exists regarding the diagnosis and treatment of PBB, with some critics questioning whether the clinical diagnostic criteria are robust enough to distinguish between PBB, a prolonged post-viral cough and other causes (Bidiwala et al., 2015). The concern is that over diagnosis will lead to unnecessary antibiotic use (Bidiwala et al., 2015). Long-term azithromycin is often prescribed in PBB to reduce infection burden and thus the risk of developing bronchiectasis. This may be given as a 6-month prescription to cover the winter months or throughout the year, for several years.

Diagram

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**Figure 1‑2:** The pathophysiology of Protracted Bacterial Bronchitis (PBB)

*Comparison of the bronchioles in health and in children with PBB. Both clinical and pathophysiological factors underlying PBB are described. Clinically PBB is defined as a chronic wet cough (>4weeks), the absence of symptoms or signs of other causes of a wet cough and resolution following 2-4 weeks of oral antibiotics. The underlying pathophysiology involves the presence of respiratory bacterial pathogens in BAL cultures at high bacterial loads (>105 CFU/ mL-1). BAL fluid has demonstrated intense airway neutrophilia and associated inflammatory mediator response with increased levels of pro-inflammatory mediators and cytokines. Bronchoscopy findings have also shown that large airway malacia is common although it is unclear if this is a significant predisposing factor for PBB, whether inflammation leads to secondary airway malacia, or whether it is a non-specific finding in young children with respiratory disease.*

*(Designed using Biorender and* [*http://clipart-library.com/mucus-cliparts.html*](http://clipart-library.com/mucus-cliparts.html)

There are approximately 525 children under review with a primary diagnosis of PBB and of these, approximately 180 receive prophylactic antibiotics (Dr Kelechi Ugonna, personal communication). Although no national guideline exists, the practice of prescribing long-term prophylactic antibiotics is included in the Sheffield Children’s Hospital guideline for the management of PBB and states that azithromycin should be the first line antibiotic of choice for prophylaxis in children experiencing more than three exacerbations of PBB a year (see appendix 2). The use of prophylactic antibiotics has also been adopted by other local paediatric hospitals (Manchester and Nottingham Children’s Hospitals – personal communication from Dr Kelechi Ugonna, paediatric respiratory consultant Sheffield Children’s Hospital) and included in a recent article reviewing the management of PBB (Gilchrist, 2019).

Importantly, my review of the literature did not find any studies investigating either recurrent or long-term macrolide use in children with PBB with regards to efficacy or impact on antimicrobial resistance or microbiota. The recognition of persistence of bacterial respiratory pathogens associated with a chronic cough is not new. The term ‘protracted bacterial bronchitis’ was however first used in 2006 and hence only more recent studies may have used this term. Despite using other search terms, no studies in this field were found.

## Antibiotics

An antibiotic is an antimicrobial substance which can either kill bacteria or limit bacterial growth. Since the discovery of penicillin in 1928 antibiotics have been used to treat or prevent bacterial infections, and a range of different antibiotics have been developed to treat diverse bacterial pathogens. Antibiotics are commonly classified based on their mechanism of action. Those that target the bacterial cell wall (including penicillins, cephalosporins, glycopeptides) or the cell membrane (for example, daptomycin), or interfere with essential bacterial enzymes (such as quinolones, trimethoprim and sulphonamides) are usually bactericidal and thus have the ability to kill bacteria. Protein synthesis inhibitors (macrolides, linezolid and tetracyclines) are usually bacteriostatic and inhibit the growth of bacteria rather than kill them (with the exception of the bactericidal aminoglycosides such as gentamicin and tobramycin).

## Antibiotic resistance

There are major concerns about the spread of antibiotic resistance, which may be promoted by excessive or inappropriate antibiotic use. Antibiotic resistance occurs when bacteria evolve or acquire mechanisms that protect them from the effects of antibiotics; resistant organisms may be selected for by the use of antibiotics. Resistant bacteria are more difficult to treat, requiring higher antibiotic doses, antibiotic combinations or alternative treatments which may prove more toxic. Microbes resistant to multiple antimicrobials are called multidrug resistant. Antibiotic resistance poses a serious threat to global health (WHO, 2020). In 2016, the UK government published its final report on AMR. It was estimated that 10 million lives could be at risk by 2050 if the problem of drug resistance was not addressed (O'Neill, 2014). In order to tackle this problem, ten recommendations were made. These included global public awareness campaigns, reduction of unnecessary use of antimicrobials in both agriculture and health care, improving global surveillance of drug resistance and promoting rapid diagnostic tests (O'Neill, 2014). To address these concerns, antibiotic stewardship programmes and frameworks such as the “One Health” initiative have been instituted to promote the judicious use of antibiotics across a multitude of sectors.

A number of key mechanisms enable bacteria to develop resistance to antibiotics, with antibiotic exposure providing evolutionary pressure to select for resistant organisms. Antibiotic resistance may be acquired through horizontal acquisition of genetic material (via mobile genetic elements, see below) or by mutations within the bacteria’s own DNA. Antibiotic resistance may be conferred by efflux pumps which excrete the antibiotic, inactivation of the antibiotic (e.g. by beta-lactamases), modification of the antibiotic target, and target by-pass whereby new pathways may be created in order to circumvent the originally targeted enzyme (Uluseker et al., 2021), see Figure 1‑3. Some bacterial species may be intrinsically resistant to an antibiotic such as cephalosporin resistance in enterococci, which has been associated with a low binding affinity of cephalosporins for the enterococcal penicillin-binding proteins (PBPs).

Mobile genetic elements promote the accumulation and spread of antibiotic resistance genes in bacterial populations. The three main strategies through which bacteria transfer genetic material are (Munita et al., 2016):

1. Transformation – Extracellular DNA is taken up and incorporated into the bacterial chromosome.
2. Transduction – Genetic material is transferred between bacteria by a virus (bacteriophage).
3. Conjugation – Transfer of mobile genetic elements, such as plasmids and transposons, which involves bacterial cell-to-cell contact.

Diagram

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**Figure 1‑3:** Antibiotic targets and key resistance mechanisms.

Schematic diagram showing the different antibiotic targets and key mechanisms involved in antibiotic resistance**.** Adapted from: (Uluseker et al., 2021). Designed using Biorender.

The mechanisms involved in antibiotic resistance vary with class of antibiotic, and additionally different mechanisms may predominate in particular bacterial species. Macrolide resistance is achieved in 3 main ways; by prevention of the antibiotic to bind to its ribosomal target, through efflux of the antibiotic and via drug inactivation (Leclercq, 2002). This is discussed in more detail in section 1.3. Beta-lactam resistance mechanisms vary between gram-positive and gram-negative bacteria. In gram-positive bacteria, penicillin resistance is mostly achieved by modification of the target penicillin-binding proteins (PBPs) which are involved in the biosynthesis of the cell wall component murein. There are 6 PBPs in *S. pneumoniae* of which 3 are associated with resistance: PBP1a, PBP2x and PBP2b. Resistance occurs through acquisition of mosaic genes. These are genes composed of DNA fragments from different phylogenetic origins, and in this case encode altered PBPs (Boc et al., 2011; Chambers, 1999; Hakenbeck et al., 1999). DNA transfer may occur between bacteria within the same species or different species. A further mechanism is through altered or mosaic forms of the *MurM* gene which modifies the pneumococcal cell wall crosslinking (Filipe et al., 2000; Smith et al., 2001). In gram-negative bacteria, penicillin resistance is achieved through the production of beta-lactamases which destroy the drug. The major beta-lactamase resistance genes *in Escherichia coli* are members of the blaCTX-M, which mainly hydrolyse third generation cephalosporins, and members of the blaTEM group, which hydrolyse penicillin and first generation cephalosporins (Clasen et al., 2019; D'Andrea et al., 2013). These genes are mostly located on plasmids.

Determining antibiotic resistance for individual bacteria species is a complex process. Clinical breakpoints are set which define whether the bacterial species is phenotypically susceptible, intermediate or resistant to an antibiotic (EUCAST, 2019).

* Susceptible: High likelihood of therapeutic success using a standard dosing regimen of

the agent.

* Intermediate: Susceptible, increased exposure. High likelihood of therapeutic success

if exposure to the agent is increased by adjusting the dosing

regimen or by its concentration at the site of infection.

* Resistant: High likelihood of therapeutic failure even when there is increased

exposure.

Breakpoints are set by breakpoint committees and must take into account a number of factors, including the in vitro effect of the antibiotic against the bacterial sample but also the dose of antibiotic that can safely be administered, the pharmacokinetics and dynamics of the drug as well as the resistance mechanism involved. The MIC - minimum inhibitory concentration - is the lowest concentration of an antibiotic required to inhibit bacterial growth. Methods to measure the MIC include disk diffusion, the use of E-test strips and the agar inoculation method (detailed description of methods in section in 2.4.3). If the MIC is less than or equal to the breakpoint, the bacterial isolate is considered susceptible.

In the presence of an appropriately dosed antibiotic, susceptible bacteria are killed. Any resistant bacteria however persist and may outgrow the susceptible strain. This selection pressure allows resistant bacteria to survive and transfer antibiotic resistance genes. Antibiotic concentrations below the MICs for bacteria will select for resistance (Andersson et al., 2012). This has potential implications for those taking long-term antibiotics, in particular if compliance is intermittent.

As such, the individual benefits of taking antibiotics must be weighed against the potential risks of promoting antibiotic resistance, particularly when the exposure is long-term (e.g. for prolonged antibiotic regimes such as for tuberculosis, or in the setting of prophylaxis to prevent infections, see below and section 1.6). This may be both a problem for the individual patient if resistant organisms arise, but also for the wider community in terms of spreading antibiotic resistant bacteria.

## Macrolide resistance

### Macrolide resistance mechanisms

A number of genetically encoded resistance mechanisms induce macrolide resistance (Figure 1‑4). These include modifying the ribosomal target site and reducing the affinity of macrolides to bind, mediated by *erm* (erythromycin resistance methyltransferase) genes, antibiotic efflux pumps (*mef* – macrolide efflux and *msr* - major facilitator superfamily-type genes) and enzymatic catalysed inactivation of macrolides (*ere* – erythromycin esterase and *mph* - macrolide 2′ phosphotransferase genes). Such genes predominate in different bacterial species, however they may be passed between strain, species and genus via horizontal gene transfer mechanisms such as plasmids (Feßler et al., 2018; Leclercq, 2002).

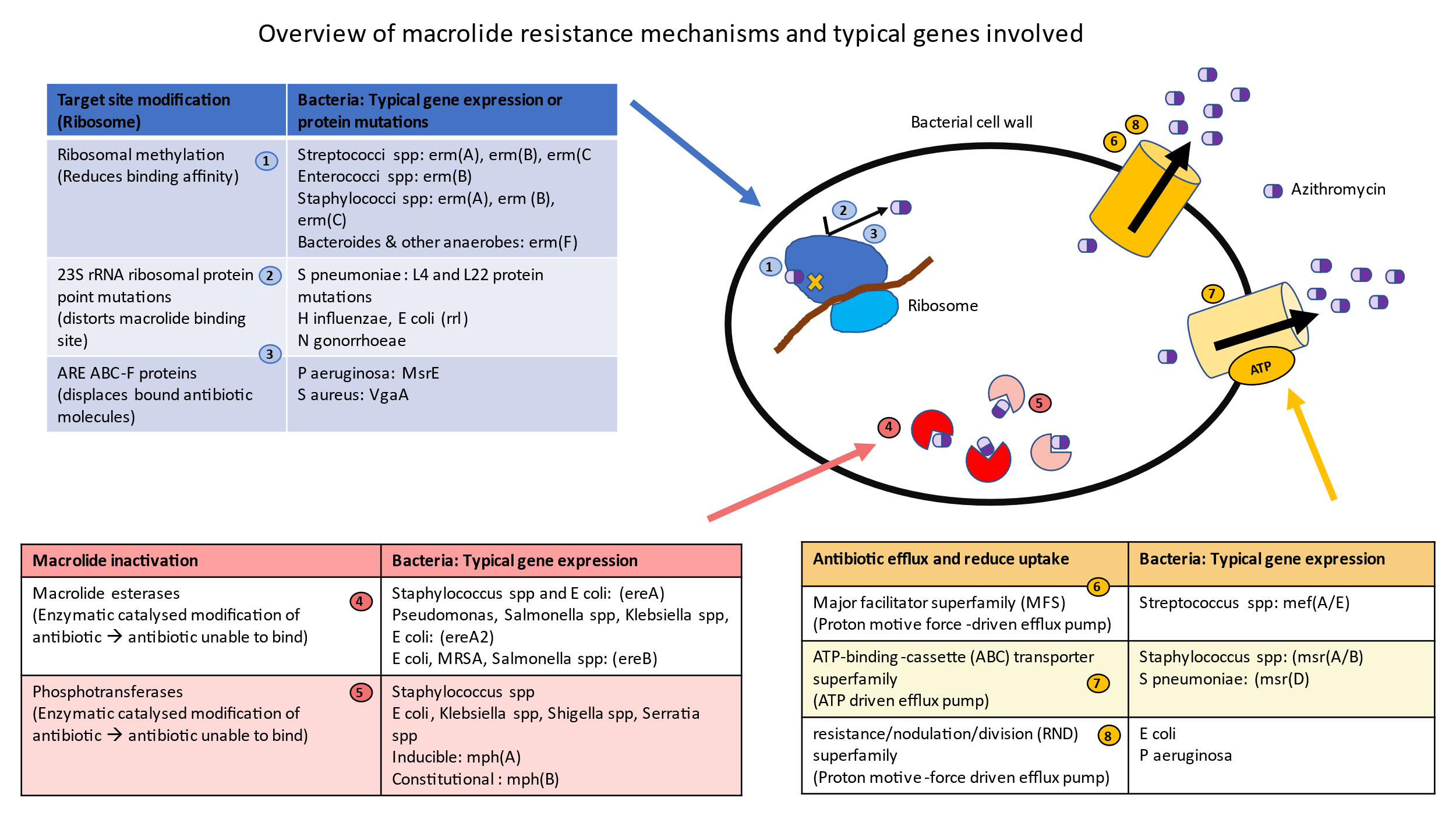
### The magnitude of macrolide resistance

Antimicrobial resistance (AMR) is seen as one of the biggest threats to global public health. Trends in macrolide resistant respiratory pathogens vary worldwide (Andrejko et al., 2021). In the European Union, the population-weighted mean percentage of invasive macrolide resistant *S. pneumoniae* isolates reduced from 16.6% in 2015 to 14.5% in 2019 (ECDC, 2020). Current national percentages of macrolide resistant invasive *S. pneumoniae* reported in European countries in 2019 range from 3.5% (Denmark) to 30.4% (Croatia). For the UK there was a slight decline over the time period 2015-2019 from 6.9% to 5.5% (ECDC, 2020). In the US, 2018-2019, 47% of *S. pneumoniae* from respiratory isolates were resistant to macrolides (Gupta et al., 2021). The highest rates of 95% macrolide resistant *S. pneumoniae* have been reported in isolates from China (C. Y. Wang et al., 2019). Given *S. pneumoniae* is the most common cause of bacterial pneumonia in children, this is concerning, particularly for those allergic to beta lactams where macrolides are used as first line therapy (NICE, 2019; WHO, 2021).

Although concerns have been raised about the emergence of resistant respiratory pathogens with increased long-term macrolide use in respiratory diseases (section 1.5), it should be noted that the effects are systemic. Thus, bacteria elsewhere in the body are also exposed, such as those in the digestive tract, which may develop and harbour resistance. There are also potential implications for other distant pathogens such as *N. gonorrhoeae,* of which there are highly resistant strains emerging (Whittles et al., 2018).

Macrolide resistance poses a threat to the individual, but the impact at a population level also needs to be taken into consideration. Children are known to have higher carriage rates of nasopharyngeal *S. pneumoniae* compared to adults (30-50% vs 5-30% ) (Le Polain de Waroux et al., 2014). Hence treating children with macrolide antibiotics has potential for emerging resistant pathogens to spread. This is especially so in children within families or attending day care centres and schools . The introduction of the childhood pneumococcal vaccine demonstrates the ability of children to act as vectors. Vaccination of children with PCV7 was associated with a significant decrease in adult pneumococcal pneumonia secondary to the vaccine serotypes (Pletz et al., 2016). On the other hand, long-term macrolide use could potentially reduce the likelihood of resistant pathogen spread by improving the cough and thus the transfer by droplet infection. However, this has not been examined as a primary outcome in paediatric respiratory conditions.

In *S. pneumoniae*, the *erm(B)* gene is responsible for most high level resistance and conveys cross-resistance to macrolides, lincosamides (e.g. clindamycin) and streptogramin B (Schroeder et al., 2016). The gene *mef(A)* typically induces lower level macrolide resistance (Cillóniz et al., 2018). Significant geographical variation exists with regards to the distribution of these genes. In European countries *erm(B)* genotypes are more common compared to the USA where *mef(A)* genotypes predominate (Cillóniz et al., 2018).



**Figure 1‑4**: An overview of macrolide resistance mechanisms and the genes involved

Schematic diagram describing the key macrolide resistance mechanisms and typical genes involved. The three main mechanisms include ribosomal target site modification, macrolide inactivation and antibiotic efflux. These may vary with bacterial species. Adapted from (Serisier, 2013)

## Antibiotic prophylaxis

In contrast to the administration of antibiotics to treat an established infection, antibiotic prophylaxis is the administration of antibiotics to either prevent an initial bacterial infection (primary prophylaxis) or to prevent the future recurrence of a bacterial infection (secondary prophylaxis). The duration of prophylaxis ranges from a single dose to lifelong medication. There are generally three main indications for prescribing antibiotic prophylaxis that cover both surgical and medical conditions (Canadian paediatric society, 1999).

1. To prevent infection by a particular pathogen.
2. To protect an infection-prone site.
3. To protect a vulnerable host.

In some cases, more than one indication may apply, e.g. antibiotic prophylaxis may be used to protect a vulnerable site (e.g. the lungs) in patients with inherited or acquired immune defects. An example of this would be the use of prophylactic co-trimoxazole to prevent *Pneumocystis jiroveci* pneumonia (PCP) in those with a low CD4 count. The strength of evidence that supports the use of antibiotic prophylaxis varies widely. At one extreme, there are evidence-based guidelines and recommendations for conditions such as *Pneumocystis jirovecii* prophylaxis in HIV infection and prevention of *S. pneumoniae* infections in sickle cell disease (WHO, 2006; Yawn et al., 2014). However, in other conditions the supporting evidence is less robust. When treatment regimens have been adopted based on limited evidence, it can then be difficult to conduct placebo-controlled studies in this field (Smith, 1999). Table 2-4 give examples within a paediatric setting, of indications in situations (in some cases more than 1 category applies) where prophylaxis is currently recommended, or not. Over recent years however, seemingly established practices have been challenged given the increase in AMR and the need for antimicrobial stewardship. Prophylaxis strategies may be amended when circumstances change, for example the use of co-trimoxazole prophylaxis in Human Immunodeficiency Virus (HIV)-exposed but HIV un-infected infants (a policy initiated when HIV testing of infants was impossible and mothers had poor access to antiretroviral treatment, but subsequently shown to be unnecessary with recent advances in testing and treatment) (Daniels et al., 2019), and stopping co-trimoxazole prophylaxis dependant on geographic area in HIV infected adults (co-trimoxazole was beneficial even in ART-treated patients for those at high risk for severe bacterial infections or malaria) (Anywaine et al., 2018; Daniels et al., 2019).

**Table 2:** Examples of prophylactic antibiotics to prevent infection by a particular pathogen

|  |  |  |  |
| --- | --- | --- | --- |
| Pathogen | Disease prevented | antibiotic | comments |
| Group A streptococcus | Rheumatic fever.  Secondary prophylaxis in those with a history of rheumatic fever or with rheumatic heart disease | Benzathine penicillin G IM 4 weekly  OR  Penicillin V twice daily | Recommendations for duration of prophylaxis vary:  5-10 years after last episode of rheumatic fever or until 21 years of age or until 35/40 years of age or lifelong. Dependent on guideline and stratified risk (Gerber et al., 2009) (RHD Australia), 2012) |
| Streptococcus pneumoniae | Pneumococcal infections in sickle cell disease or asplenia | Penicillin V twice daily | No consensus on when to stop prophylaxis. 2002 guideline lifelong (Rankine‐Mullings et al., 2017). US experts (76% of 889 respondents) would stop prophylaxis at 5 years of age (McCavit et al., 2013). |
| Chlamydia trachomatis | Trachoma – Sub-Saharan Africa | Azithromycin – Annual or biannual single dose by mass administration | Reduction in trachoma & all-cause mortality in under 5 years (Keenan et al., 2018)  Transient increases in macrolide resistant *Streptococcus. pneumoniae.* Higher resistance when baseline resistance was high.  Ongoing trials analysing risk/ benefit. |

**Table 3:** Examples of prophylactic antibiotics to protect an infection prone site

|  |  |  |  |
| --- | --- | --- | --- |
| Infection prone site | Disease | Antibiotic | Comments |
| Heart | Endocarditis | Single dose amoxicillin pre procedure (invasive dental procedures) | 2008 NICE: changed to not recommending prophylaxis (NICE 2008) 2007 AHA: subset of patients at high risk should receive prophylaxis (Wilson et al., 2007)  2016 NICE: now not recommended “routinely”. |
| Urinary tract | Recurrent UTIs | Trimethoprim  nocte | 2018 Cochrane review: may reduce risk of repeat symptomatic UTI in children with 1 or more UTIs. Small benefit.  2019 Prophylaxis does not decrease the risk of renal scarring (Hoberman et al., 2014).  Used following individual case assessment |
| Lungs | Non cystic fibrosis bronchiectasis | Azithromycin  three days a week | BTS recommend starting long-term prophylaxis (Pasteur et al., 2010):  In adults with 3 or more pulmonary exacerbations a year.  In children with recurrent symptoms or severe disease. Frequently used.  Only 2 paediatric studies |

**Table 4:** Examples of prophylactic antibiotics to protect a vulnerable host

|  |  |  |  |
| --- | --- | --- | --- |
| Vulnerable host | Disease | Antibiotic | Comments |
| Primary immune deficiency | X-linked agammaglobulinemia  Other primary antibody deficiencies | Co-trimoxazole  Azithromycin | Used in approximately 50% of patients (Kuruvilla et al., 2013)  1 randomised controlled trial (RCT): AZM reduced pulmonary exacerbations  (Milito et al., 2019) |
| Primary immune deficiency | Chronic granulomatous disease – Prevention of Staphylococcal infections | Life-long co-trimoxazole | No RCTs.  Well defined used regimen based on:  1 retrospective series of 11 patients  Several observational studies (Kuruvilla et al., 2013) |
| Acquired immune deficiency | HIV | Co-trimoxazole | Duration varies according to country: Europe – may stop once CD4 >200 cells/mm3 or Sub-Saharan Africa – lifelong (Anywaine et al., 2018) |

Used appropriately, antibiotic prophylaxis can have significant benefits for the individual in terms of reducing the risk of acquiring infections. This is illustrated by those with chronic granulomatous disease. Without antimicrobial prophylaxis, patients suffered on average 1 life threatening infectious episode every 10 months, often with *Staphylococcus aureus*. With co-trimoxazole prophylaxis, this was found to be reduced to 1 life threatening episode every 40 months (Gallin et al., 1983). This may impact on short- and long-term morbidity, quality of life and mortality, as well as shortening hospitals stays in the example of perioperative surgical antibiotic prophylaxis. Despite these benefits, other side effects and consequences must be considered for both the individual and for the wider community. These include side effect profiles of the drugs (ranging in severity from minor gastrointestinal effects to life-threatening anaphylaxis or bone marrow suppression), promoting AMR and changes to the bacterial communities residing within us, the microbiota, potentially leading to specific (e.g. *Clostridium difficile* infection) or as yet uncertain consequences.

## Prophylactic macrolide use in paediatric respiratory disease

Macrolides are a class of broad-spectrum antibiotics with both gram-positive and some gram-negative cover. They exert their antimicrobial effects by inhibiting protein synthesis through binding to the large prokaryotic ribosomal subunit (Dinos, 2017). Newer generation macrolides such as azithromycin have a longer half-life and larger tissue volume distribution, allowing a favourable dosing regimen which is particularly important in paediatric populations. For acute paediatric respiratory infections, the commonly prescribed macrolides are erythromycin, clarithromycin and azithromycin, whilst azithromycin is the commonest agent for prophylaxis. In addition to the antimicrobial effects, over the past 2 decades, there has been increasing interest in the immunomodulatory effects of azithromycin, although many of the actions attributed to this agent are likely to be shared to a greater or lesser extent by other macrolides. The mechanisms of macrolide immunomodulatory action are still being characterised. They involve interactions with both innate and adaptive immune systems as well as with local host defences (Pollock et al., 2021). Furthermore, azithromycin also has other direct effects on bacteria through disruption of biofilm formation as well as some anti-viral properties by interfering with internalisation of viruses into host cells and disrupting the optimal environment for viral replication (Damle et al., 2020; Ichimiya et al., 1996). Prolonged courses of azithromycin are used in a variety of paediatric chronic diseases for its “anti-inflammatory” effect. This is particularly the case in conditions characterised by neutrophilic inflammation such as cystic fibrosis and bronchiectasis.

Adult prophylaxis with azithromycin is usually administered as 250mg -500mg thrice weekly. The paediatric dosing regimen used for long-term azithromycin varies. Metaxa et al report 3 different regimens being used in the East of England (Metaxa et al., 2019):

1. Thrice weekly (Monday, Wednesday, Friday).
2. Friday-Sunday alternate weekends.
3. Three doses over 48 hours every 2 weeks.

The standard dose in children used is 10mg/kg/dose. Other paediatric trials have used once weekly prescriptions at 30mg/kg/dose (Valery 2013). To date, only 2 studies in cystic fibrosis have compared dosing regimens (Kabra et al., 2010; McCormack et al., 2007). The first compared 5mg/kg daily vs 15mg/kg daily in children and the second 250mg daily vs 1250mg weekly in participants aged 6-58 years. No difference between the dosing regimens was found in either study with regards to pulmonary exacerbations.

Long-term macrolides are prescribed in a variety of paediatric respiratory diseases. The duration of therapy may range from a few weeks to several years. Their predominant use is in settings where their anti-inflammatory and immunomodulatory properties are felt to augment their antimicrobial actions. For over 20 years, long-term azithromycin has been used in cystic fibrosis (CF), a strategy supported by many randomised controlled trials (see section 1.7.1; these studies have mostly been undertaken in adults but with some paediatric cohorts). A more recent RCT has however questioned its efficacy in CF (Stick et al., 2022). Subsequently, studies have been conducted in patients with non-CF bronchiectasis (again with some studies enrolling children) with benefit demonstrated in reducing pulmonary exacerbations. As a result of the success of this treatment in the setting of CF and non-CF bronchiectasis, macrolides are now being prescribed in other paediatric respiratory conditions, both short- and long-term, but with little evidence to support this long-term use and concerns about the emergence and spread of antimicrobial resistance (AMR).

### Cystic fibrosis

Cystic fibrosis is an inherited condition in which mutations lead to loss-of-function of a transmembrane protein (cystic fibrosis transmembrane regulator – CFTR), which regulates chloride ion transport. In the airway this leads to thick secretions, predisposition to recurrent chest infections and airway damage/dilatation (bronchiectasis). *P. aeruginosa* is the major pathogen that forms biofilms leading to chronic inflammation and infection and eventually respiratory failure. Ciprofloxacin is the only effective oral antibiotic to treat established pseudomonas infections, but ciprofloxacin resistance is readily acquired by mutation in the quinolone target DNA gyrase, and by alteration of drug permeation through the outer membrane of the cell. Other treatment such as intravenous/inhaled antibiotics may be required in this setting. Of note, azithromycin and other macrolides do not kill *P. aeruginosa* in vitro but have anti-virulence activity (Imperi et al., 2014) that confers in vivo benefits demonstrated in a number of clinical trials as discussed below.

There are at least 12 RCTs (randomised controlled trials) investigating long-term macrolide use in CF. Of these, five were paediatric studies recruiting almost 600 children and 5 other trials recruited both children and adults. Azithromycin was compared to placebo in 9 of these. Meta-analysis of trials up to 2012 showed those taking azithromycin were almost twice as likely to be free of pulmonary exacerbations at 6 months; Odds Ratio 1.96 (95% CI 1.15 to 3.33) (Southern, Barker, Solis‐Moya, et al., 2012). The second finding was of a comparative change in FEV1 from baseline; mean difference 4% (95% CI 1.74% to 6.19%) in favour of azithromycin (Southern, Barker, Solis‐Moya, et al., 2012). This was significant up to 6 months, but the advantage was lost after 6 months in the one trial that continued for 12 months (Clement et al., 2006). It is debatable in any case whether a 4% change is clinically significant. A decline in pulmonary function has been associated with lower 2-year survival and therefore pulmonary function is frequently used as a surrogate end point in many CF trials (Konstan et al., 2009; VanDevanter et al., 2012). Over short periods there is variability in individual FEV1 and individuals may not be able to perceive a change in their pulmonary function hence quality of life is not usually impacted by small changes (Konstan et al., 2009). These studies enrolled patients with or without chronic *P. aeruginosa* infection or both. The impact of *P. aeruginosa* infection on outcomes required further examination of individual patient data, however it was not thought to be a significant confounding factor with regards to changes in FEV1 (Southern, Barker, Solis-Moya, et al., 2012). A more recent RCT did not find a reduction in the extent of structural lung disease at three years of age in children with CF exposed to long-term azithromycin from diagnosis. There was however a significant reduction in the number of day hospitalised for pulmonary exacerbations (mean difference=-6·3, 95%CI=-10·5, -3·1; p=0·004) and neutrophilic inflammation (Stick et al., 2022).

Only one RCT has looked at macrolides other than azithromycin. Long-term clarithromycin use in 63 adults and children over 12-months was not found to improve lung function, weight gain, quality of life or reduce sputum cytokine levels compared to placebo, despite its chemical similarities to azithromycin (Robinson et al., 2012).

Of note the role of prophylactic azithromycin in children with CF may change in the future as CFTR modulator therapy to improve CFTR function is adopted in the paediatric population. In adults homozygous for the delta F508 mutation, marked improvements in lung function were seen with triple modulator therapy, Elexacaftor/Tezacaftor/Ivacaftor (Heijerman et al., 2019), and ongoing studies have shown a reduction in exacerbation frequency (Patel et al., 2020). It is hoped that treating from an early age may reduce lung damage and hence all complications of CF including infection, reducing the need for prophylaxis, but this remains to be determined.

### Non-cystic fibrosis bronchiectasis

Bronchiectasis is a respiratory condition characterised by permanent dilation of the peripheral airways, often manifesting as a chronic cough with increased sputum production, recurrent exacerbations and shortness of breath. A cycle of lower airway inflammation and infection worsens airway damage and has been associated with long term disability, accelerated lung function loss and premature death in adults (Loebinger et al., 2009). CF is a significant cause of bronchiectasis however many other conditions are also associated with bronchiectasis including, primary immune deficiencies, cilial abnormalities, atypical mycobacterial infection, other severe respiratory infections (including recurrent aspiration), connective tissue diseases such as rheumatoid arthritis, and post-obstructive causes (Pasteur et al., 2000). Many cases are idiopathic, however. The prevalence of bronchiectasis in the UK 2013 was 566 per 100,000 in women and 485 per 100,000 in men (Quint et al., 2016).

In keeping with the role of long-term macrolides in CF, there is a growing evidence base for its use in non-CF bronchiectasis. The main evidence has come from five double-blind, randomised placebo-controlled trials (Altenburg et al., 2013; Masekela et al., 2013; Serisier et al., 2013; Valery et al., 2013; Wong et al., 2012). Only two of these were however conducted in paediatric populations (total of 120 children), demonstrating the paucity of evidence on which recommendations for children are based. The first study conducted in a paediatric population recruited 89 children aged 1-8 years and randomised to once weekly azithromycin or placebo over 12-24 months. They reported significantly lower pulmonary exacerbation rates in the azithromycin group compared to placebo: 104 vs 195 exacerbations, (incidence rate ratio 0.5; 95%CI 0.35-0.71, p<0.0001) (Valery et al., 2013). This study was carried out amongst indigenous populations in Australia and New Zealand and hence the results may not be fully applicable to a UK paediatric population. Additionally, no children over the age of 8 years were recruited as it was thought children in puberty have a different pattern of illness (Valery et al., 2013). Also noteworthy is that the inclusion diagnosis of bronchiectasis was not confirmed on high resolution computed tomography (HRCT) in all children, with varying percentages of HRCT-confirmed diagnoses in the 2 study groups. The second paediatric study was conducted in 31 HIV positive South African children aged 6-18 years (again not representative of a typical UK cohort) and was the only trial not to show a difference in pulmonary exacerbations with long term macrolide use; daily erythromycin (rather than azithromycin) over 52 weeks (Masekela et al., 2013). However, they had an extremely high attrition rate of almost half the participants making this result unreliable.

It is important to note when assessing these studies that the definition of “pulmonary exacerbation” varied between them, potentially changing the threshold for diagnosing an exacerbation. A related confounding factor is the experience of the clinician making this diagnosis. Altenburg suggests that specialist hospital clinicians may diagnose fewer pulmonary exacerbations when they have access to longitudinal sputum microbiology (Altenburg et al., 2013). The question of whether a GP would be more likely to diagnose a pulmonary exacerbation has however not been studied. This would be an important point to address in order to appropriately risk stratifying those who may benefit from prophylactic macrolide treatment as GPs are often those who see exacerbating patients.

A meta-analysis of 10 RCTs involving 602 patients found a significant benefit of macrolide therapy in reducing the exacerbations particularly in children (relative risk= 5.03, (95%CI 2.02-12.5, p=0.0005) as well as adults (relative risk= 1.66 (95%CI 1.37-2.02, p<0.00001) (D. Wang et al., 2019). In the adult studies they note significant heterogeneity in the type of macrolide, dosing regimens and severity of illness at recruitment. This suggests the result may not be universally applicable (D. Wang et al., 2019).

There are several gaps in this area of literature. No studies in this population have compared different macrolides and dosing regimens, the optimal duration of therapy, or which patient subgroups might benefit most. A further limitation of current trials is that of short follow-up times (usually 6-months to one year). Although the number of exacerbations may reduce with long-term macrolide therapy, prevention of disease progression has not been demonstrated, although as a surrogate marker of disease progression a beneficial effect of azithromycin on radiological appearances was noted after 1 year of treatment in 1 study (Terpstra et al., 2022). The impact of emerging antimicrobial resistance following long-term macrolide use must also be considered given the growing concerns in this field and has been little documented (see section 1.5.3).

On the basis of the current evidence, the British Thoracic Society (BTS) have recently published guidelines for the management of non-CF bronchiectasis in adults. They recommend long-term antibiotics be considered in those experiencing more than 3 pulmonary exacerbations a year (A. T. Hill et al., 2019). In children with non-CF bronchiectasis, the BTS suggest considering preventative azithromycin in those with frequent symptoms or severe disease.

More recently, a further three randomised, double blind, placebo-controlled trials have contributed evidence to the use of long-term azithromycin in primary ciliary dyskinesia, HIV-related lung disease and primary antibody deficiencies (Ferrand et al., 2020; Kobbernagel et al., 2020; Milito et al., 2019). All three report significant reductions in the number of pulmonary exacerbations in the groups exposed to azithromycin compared to placebo. Due to difficulties in recruitment, the primary antibody and primary ciliary dyskinesia trials were unable to meet their target recruitment. However overall, the evidence supports the use of azithromycin prophylaxis to reduce infective exacerbations in patients with structural lung disease and/or impaired immunity, but does not fully address longer term outcomes or complications such as AMR.

### Long term macrolides in chronic cough

Predominantly adult trials reporting benefits of long-term macrolide therapy in conditions characterised by neutrophilic airway inflammation, e.g. COPD and bronchiectasis, have prompted studies in those with chronic cough with no detectable underlying condition. There is some evidence to suggest neutrophilic airway inflammation is present in these patients, but only limited treatment options are available.

No studies were found investigating long-term macrolides in children with chronic cough despite using a variety of associated search terms. Extrapolating results from adult studies has limitations due to the presence of comorbidities, polypharmacy, smoking and anatomical airway differences in adults. Although good quality epidemiological data are not available to assess prevalence, there are undeniably significant numbers of adult patients whose chronic cough cannot be explained despite extensive investigations to exclude diseases such as bronchiectasis, COPD or asthma. Three studies have looked at long-term azithromycin in these adults who were classified as having “idiopathic cough”.

A recent observational study (Martin et al., 2019) looked at the response to 12 weeks of thrice weekly azithromycin. They report a significant improvement in cough as indicated by the Leicester Cough Questionnaire (LCQ) (median cough score -6.3 points p<0.001). However, this study was limited by lack of placebo group, small numbers recruited (30 participants) and the use of a post-hoc statistical analysis (Martin et al., 2019). Prior to this study, two double blind RCTs were conducted in similar cohorts of adults. The first did not find that 12 weeks of low-dose erythromycin reduced cough frequency compared to placebo as assessed by the Leicester cough monitor (Yousaf et al., 2010). They did not isolate typical respiratory pathogens in any of the sputum samples obtained. The second study investigating 8-weeks of thrice weekly low-dose azithromycin, did not find a significant improvement in cough compared to placebo as determined by the LCQ (Hodgson et al., 2016). However, neither trial reached their recruitment targets as assessed by power calculations. As such, high quality trials are lacking in this area and further studies are required. Recently published guidelines for the use of long term macrolides in adults with respiratory disease, do not recommend macrolides for chronic cough (Smith et al., 2020).

### Long-term azithromycin and AMR

Several key trials investigating long-term macrolides (6-12months) in CF, non-CF bronchiectasis, chronic obstructive pulmonary disease and asthma all report associated significant increases in macrolide-resistant respiratory pathogens (Albert et al., 2011; Saiman et al., 2010; Serisier et al., 2013; Uzun et al., 2014; Valery et al., 2013). The evidence is however low grade, as only one trial was powered to detect emergence of resistance, with the number of tested microbiological samples typically small, and often sampling the upper airways only. A meta-analysis of 3 trials in non-CF bronchiectasis found the rate of macrolide resistance was significantly higher in the long-term macrolide-exposed groups than control groups (RR = 3.59, 95% CI = 2.60–4.96, p<0.001) (D. Wang et al., 2019). Those studies that did not identify a significant increase reported trends to increase, based on very small numbers of samples. Although these studies generally report a reduction in pulmonary exacerbations associated with macrolide use, it would be important to document whether resistant organisms were cultured during any exacerbations, and whether these impacted on the severity of the episode. This information is not currently available from the published trials.

The single RCT investigating the emergence of macrolide resistance as a primary outcome with oropharyngeal swabs randomised 204 healthy adult volunteers to just 3 days of azithromycin, 7 days of clarithromycin or 2 placebo groups (Malhotra-Kumar et al., 2007). They reported a significant increase in resistant organisms, with the largest increased difference in the mean proportion of resistance from baseline amongst the groups at day 28. Importantly, a significant proportional increase from baseline was still demonstrated at day 180 post exposure (full results shown in Table 5). The proportion of macrolide-resistant streptococci (comprising both S. pneumoniae and S. pyogenes) was higher after azithromycin treatment than after clarithromycin use, with the largest difference between the two groups at day 28 (17.4% difference, p<0.0001). However, use of clarithromycin, but not azithromycin, selected for the erm(B) gene, which confers high-level macrolide resistance. The reason for this difference between the 2 macrolides is not yet clear.

**Table 5**: Proportional increase in mean macrolide-resistant streptococci from baseline following macrolide exposure (p<0.001) (Malhotra-Kumar et al., 2007)

|  |  |  |  |
| --- | --- | --- | --- |
| Day post exposure | Azithromycin: 3 days  (p value) | Clarithromycin: 7 days  (p value) | Placebo: 3 or 7 days  (p value) |
| Baseline | 25.9% (22-30) | 30.1% (24-36) | 27.5% (22-33) |
| Day 8 | 56.8% (54-67)  (<0.0001) | 51.9% (45-58)  (<0.0001) | 3.8% (2-10)  (0.2520) |
| Day 28 | 54% (47-60)  (<0.0001) | 33% (26-40)  (<0.0001) | 2.4% (-4.2 -9)  (0.4790) |
| Day 180 | 14.5% (7-22)  (<0.0003 | 16.3% (8-25)  (<0.0001) | -0.9% (-9-7)  (0.8240) |

The bacteriological profile of respiratory samples has been documented to alter following long-term macrolide use in a number of studies. Valery et al. reported a lower nasopharyngeal carriage of *H. influenzae* and *M. catarrhalis* in the azithromycin group (non-CF bronchiectasis patients) at the end of the study compared to the placebo group, but increased carriage of azithromycin-resistant *S. pneumoniae* and *S. aureus* (Valery et al., 2013). A secondary analysis of this trial, looking at post-intervention swabs collected more than 30 days and less than 12 months post-intervention, demonstrated recovery of the nasopharyngeal carriage of *H. influenzae* and *M. catarrhalis* and a significant reduction of macrolide resistant *S. pneumoniae* which were replaced with susceptible strains in the azithromycin group (Hare et al., 2015a). The median time of post-intervention swab collection for the azithromycin group was 192 days for the Australian cohort and 187 days for the New Zealand cohort (placebo group 170.5 and 187 for the 2 countries). Reduction of *S. aureus* was also seen in the azithromycin group during this time period, however all strains remained macrolide resistant. Similarly, further analysis of the patients enrolled in the AMAZES study investigating the use of 48 weeks of azithromycin in adults with persistent uncontrolled asthma, found that azithromycin reduced airway *H. influenzae* load (but not total bacterial load) compared to placebo but detected a significant increase in the detection of macrolide and tetracycline resistance genes (Taylor et al., 2019).

A further problem of employing standard culture methods alone (as in most of the reported studies) is that carriage of pathogens in the nasopharynx and oropharynx are reduced by long-term macrolide use. Thus, obtaining pathogen-rich samples for culture and susceptibility testing is challenging. Few studies have reported MIC results for specific pathogens. Reporting MICs and macrolide resistant gene expression would enable a better understanding of the extent of high and low resistance patterns. Using culture independent techniques would potentially have the benefit of increasing the understanding of the mechanisms of resistance and the effects on the microbiome and resistome (discussed below in section 1.6).

## Microbiome and microbiota

PBB study 2 in chapter 5 focusses on the nasopharyngeal microbiome and the general concepts of the microbiome are introduced here. The human microbiota refers to all the microbial communities that reside within or on our bodies. Over the last decade, the number of identified prokaryotic species isolated from the human body has almost tripled. By 2021, a total of 3253 bacterial species had been associated with human beings either as pathogens or commensals (Diakite et al., 2021). The microbiome refers to the collection of genomes of all the microorganisms present. This was initially in terms of describing the composition of various communities within different niches in healthy humans, with an increasing number of studies now beginning to investigate the *function* of the microbiome in both health and disease.

The respiratory microbiome is discussed below (sections 1.6.1 – 1.6.4) but the most studied niche is the gut microbiota. In health, the microbiota confers beneficial effects including metabolic activities, modulation of the host’s innate and adaptive immune system and colonisation resistance (preventing establishment of potential pathogenic micro-organisms such as *Clostridium difficile*).

Next-generation sequencing is an overarching term covering a number of high-throughput methodologies that enable rapid sequencing of the base pairs in DNA or RNA samples (Hu et al., 2021). Metagenomics is the study of genetic material recovered directly from environmental samples. Applied to microbiome research, it uses a variety of culture-independent techniques to analyse the DNA from mixed communities of microorganisms. Analysis of the community structure may use targeted regions of genes such as those on the 16s rRNA gene, or shotgun sequencing to document all of the genes present. The 16S rRNA gene is present in all bacteria and archaea and is integral to the process of protein synthesis. This gene comprises of highly conserved areas as well as 9 hypervariable regions. The hypervariable regions allow for taxonomical classification. 16s gene sequencing allows high throughput of samples and is relatively cheap. The limitations however are that only a small proportion of the 16s gene is sequenced (although more recent technologies are addressing this), and no single variable region allows for complete differentiation of all bacteria (Weinstock, 2012). The abundance of the detected bacteria is unable to be assessed using this method, and viruses and fungi that may be relevant cannot be detected. Results using 16S PCR should be considered with these limitations in mind.

Until recently, an OTU (operational taxonomic unit) clustering approach was often taken in 16s metagenomic analysis to categorise bacteria. Sequences were clustered together based on a threshold of at least 97% similarity, attempting to distinguish bacteria to a genus level. This method aimed to blur similar sequences into a final consensus sequence, but there is a risk of losing individual species identities. More recently ASV (amplicon sequence variant) analysis has been used. This method determines the exact sequences that are present and how many times they are read. Using an error model allows reads to be compared and determine the probability of whether they are due to sequence errors or not. This approach, using exact sequences and not clustering, allows comparison between studies and a more precise identification down to the species level when compared to a reference database.

Dysbiosis of the microbiota as defined by Levy (Levy et al., 2017) is typically characterised by an increase in pathobionts, i.e., commensal bacteria that have the potential to cause pathology, loss of commensals or loss of diversity. Such changes in the composition of microorganisms may result in a reduction of the microbiota’s ability to function optimally. The microbiota is highly dynamic, responding to various selective pressures including diet, cigarette smoke exposure, medication such as antibiotics and infections, as well as undergoing significant maturation throughout childhood (Man et al., 2017). These confounding factors need to be taken into account when studying the microbiota in disease states.

Dysbiosis of various microbiota have been associated with a range of diseases. In the nasopharynx, Teo et al. reported a correlation between virus-associated acute respiratory infections in the first 60 days of life and nasopharyngeal colonisation with *Streptococcus*, *Moraxella* or *Haemophilus* (Teo et al., 2015)*.* This was in comparison to healthy infants, whose nasopharyngeal microbiome demonstrated colonisation with *Staphylococcus* or *Corynebacterium* species up to 2-months of age (Teo et al., 2015). Dysbiosis of the gut microbiota has been reported to play a role in the pathogenesis of inflammatory bowel disease, with differences in the microbiota of those with ulcerative colitis and Crohn’s disease compared to healthy controls (Lloyd-Price et al., 2019). However, it is still to be determined whether such changes can predict future events. It is hoped that features within the microbiome may be able to improve diagnostics, indicate prognosis and even act as a therapeutic target. The most widely documented success in manipulating the microbiome is the case of recurrent *Clostridioides difficile* diarrhoeal infections. Restoration of the gut microbiota through faecal microbiota transplantation has been found to be an effective treatment to eliminate *C. Difficile* (Baunwall et al., 2020)*.* A small open label study also reported reduced hospitalisation and improved cognition followingfaecal microbiota transplantation for hepatic encephalopathy (Bajaj et al., 2017), attributed to fewer detrimental microbiome-associated metabolites acting via the ‘gut-brain axis’ (Mayer et al., 2022). These studies provide evidence that the microbiota can, in principle be an important therapeutic target.

### The upper respiratory microbiome

The nasopharynx is colonised by diverse bacterial species. These include *Streptococcus* spp, *Haemophilus* spp, *Dolosigranum* spp as well as *Moraxella* spp, *Staphylococcus* spp. and *Corynebacterium* spp. Some of these species including *Corynebacterium* are not generally associated with disease. Others are important human pathogens e.g. *S. pneumoniae,* yet the host may remain an asymptomatic carrier unless they become compromised in some way. This may for example occur during a viral upper respiratory tract infection, when the respiratory epithelium is damaged and the bacterial load in the nasopharynx increases. Viruses such as human adenovirus and rhinovirus have been detected in the majority of healthy asymptomatic children (Man et al., 2017).

*S. pneumoniae* frequently colonises the nasopharynx in children. Many remain asymptomatic yet colonisation is a prerequisite for invasive pneumococcal disease with the local host immune response playing an important regulatory role in controlling carriage (Bogaert et al., 2004a). The composition of the nasopharyngeal microbiome varied between healthy children and those with invasive pneumococcal disease (Camelo-Castillo et al., 2019). A potential antagonistic effect was found of *Dolosigranulum* against *Pneumococcus*.

The microbiome may influence or be influenced by a more diverse range of respiratory infections. An abundance of *Haemophilus spp.* and *S. pneumoniae* in the nasopharynx and fewer potentially beneficial bacteria (*Corynebacterium, Dolosigranulum, Moraxella* and *Helococcus)* were associated with children admitted to PICU with LRTIs compared to healthy controls (Man et al., 2019). This suggests these children had or developed a reduced resistance to pathogenic bacterial overgrowth.

Additionally, the airway microbiota may play a role in the development or progression of chronic conditions such as asthma. A persistent ‘*Moraxella* sparsity’ nasal microbiota profile, compared to a persistent ‘*Moraxella* dominance’ profile during the ages 2-13 months, has been associated with a significantly higher risk of developing asthma (adjusted odds ratio 2.74; 95%CI 1.20-6.27) (Toivonen et al., 2020). However, in school aged children with mild to moderate asthma, an upper respiratory tract microbiota dominated by *Corynebacterium* and *Dolosigranulum* was associated with less severe exacerbations than seen in those with microbiota dominated by more pathogenic bacteria: *Moraxella*, *Streptococcus* and *Staphylococcus* (Zhou et al., 2019). Longitudinal changes in the nasal microbiota with early *Moraxella* sparsity, secondary to exposure to two or more courses of antibiotics during the period 0-11 months of age, have also been associated with an increased risk of developing asthma (Toivonen et al., 2021). The presence of some species within the nasopharynx such as *Prevotella spp*. and *Leptotrichia spp*. have been strongly associated with subsequent development of upper respiratory tract infections (URTIs) in children (Bosch et al., 2017).

The composition of the airway microbiome may also influence the efficacy of antibiotic treatment for exacerbations, as well as the duration of symptoms. An RCT in 68 children with asthma-like-symptoms randomised participants to azithromycin or placebo during acute asthma like episodes. A greater richness and diversity of the nasopharyngeal microbiota was found to be associated with a longer duration of asthma-like symptom episodes (Thorsen et al., 2021). Importantly, as sample richness increased, the efficacy of azithromycin was also reported to increase, with a 10% greater azithromycin response for each 10-OTU increase (Thorsen et al., 2021).

The nasal, oral and nasopharyngeal microbiomes are readily accessible and hence easy to study. Microbes within these niches influence carriage and potential invasion. However there is emerging interest in the microbiome of the lower respiratory tract, which is more challenging to access especially in children

### The lower respiratory microbiome.

Until recently the lungs were thought to be a sterile environment. With culture- independent techniques, numerous microorganisms including bacteria, viruses and fungi have been identified in samples from below the vocal cords. It is thought that the healthy lung microbiota may consist of transient populations of microorganisms, rather than the resident communities found in chronic respiratory diseases (Man et al., 2017). Obtaining samples from the lower airways has specific challenges, particularly in children. Firstly, the requirement for a general anaesthetic and associated risks make sampling less readily available than the upper airway. Secondly as the lower airways are accessed, there is potential for contamination by going through the upper airways. Using protected sampling via a sterile catheter only ‘opened’ within the lung aims to mitigate this risk. Finally, the very low biomass of lower respiratory tract samples make them particularly susceptible to contamination during the laboratory analysis (contamination in DNA extraction kits, reagents and laboratory environment).

It has been suggested that the lung microbiota is determined by three factors (Dickson et al., 2015).

1. Microbial migration: Direct inhalation, micro aspiration, and dispersal through

the respiratory mucosa.

1. Microbial elimination: Cough, muco-ciliary clearance, innate and adaptive

Immunity.

1. Local growth conditions: For example pH, pO2, inflammatory cells.

In many chronic lung diseases including COPD, CF and bronchiectasis there are specific patterns of lower respiratory tract microbiota seen, with a loss of diversity and over-representation of *Proteobacteria* related to disease severity and exacerbations (Faner et al., 2017). It is unclear whether the detected dysbiosis is secondary to the chronic disease or is contributing to it (‘chicken or egg’). The establishment of causation versus association in this setting is challenging. This is an active area of research and our understanding of the role of microbiota in determining a number of key features of various diseases, whilst limited, is growing.

The lung microbiome of those with bronchiectasis shows lung bacterial communities often dominated by *Pseudomonas*, *Haemophilus* and *Streptococcus.* Dominance with *Pseudomonas*, *Enterobacteriaceae* and *Stenotrophomonas* has been linked to severe disease and greater lung inflammation with *Pseudomonas* and *Enterobacteriaceae* associated with more frequent exacerbations (Dicker et al., 2021). Those with very low diversity in sputum have been associated with more rapid lung function decline (Woo et al., 2019). It is difficult to establish the role of the microbiome in exacerbations, which may mostly be caused by bacteria, viruses and other environmental factors. Treatments such as antibiotics may also influence results. There are thus heterogenous changes reported in studies investigating the microbiome during exacerbations. Further research is required to understand these subtypes of exacerbations associated with different patterns seen within the lung microbiome and this has recently been reviewed in detail by Richardson et al (Richardson et al., 2019).

Lower airway microbiome analysis in those with cystic fibrosis (CF) has shown that a decline in specific species such as *Haemophilus* precedes colonization with *P. aeruginosa*. Organisms identified on standard clinical culture, such as *P. aeruginosa*, *S. aureus* and *Burkholderia spp*, may be core members of the microbiome (Huang et al., 2016). Culture-independent analyses have yielded similar findings but in addition, other anaerobic species such as *Porphyromonas*, *Prevotella* and *Veillonella* genera contribute to the core microbiota (van der Gast et al., 2011). Previously it was thought that, whilst microbial airway community compositions vary greatly among patients, they are often relatively stable in individuals with CF over time (Huang et al., 2016). More recently however, adolescents with CF displaying a dynamic and diverse lung microbiome were shown to have slower decline of lung function compared to those with a more stable and less diverse lung microbiome (Metzger et al., 2021). Untargeted metabolomics and quantitative metagenomics has allowed further characterisation of the cystic fibrosis lung microbiome. Silveira et al report that during rapid periods of lung function loss, accumulation of fermentation products from anaerobic bacteria opens a niche for *P. aeruginosa*. The use of clindamycin reduced the abundance of *P. aeruginosa* despite its resistance to this antibiotic, perhaps indicating dependence of *P. aeruginosa* on anaerobic bacteria metabolites (Silveira et al., 2021). The microbiome in cystic fibrosis pulmonary disease has been recently reviewed by Francoise et al. (Françoise et al., 2020). As the microbiome and complex interaction networks become better understood, the challenge will be to see if clinical outcomes can be influenced by manipulating the airway microbiome, or if it can be used to prognosticate disease progression and complications.

### The lung microbiome in protracted bacterial bronchitis

Few studies have been carried out looking at the microbiota of children with PBB. An early study demonstrated that children with CF (25 subjects), non-CF bronchiectasis (19 subjects) and PBB (12 subjects) had similar core microbiota, perhaps suggesting a link between these diseases (van der Gast et al., 2014). Although the numbers of patients were limited, the microbiota described were distinctly different from adults with CF and bronchiectasis. This temporal change has been studied specifically in CF, with progressive patterns of dysbiosis over time. A reduction in lung microbiota diversity and increasing dominance of respiratory pathogens in patients with CF has been correlated with disease progression and decline in lung function (Cox et al., 2010).

Cuthbertson studied bronchoscopic protected brushings of 24 children with PBB compared to a control group of 20 healthy children. They found lower alpha diversity in children with PBB than healthy controls and changes in the community composition (significant dominance of *Haemophilus* and *Neisseria* and a non-significant increase in *Moraxella* and *Streptococcus spp* in the children with PBB, (Cuthbertson et al., 2017).

A more recent study investigated inflammatory markers and the microbiota in paediatric PBB. They reported an increase in BAL bacterial biomass, associated with increased neutrophil percentage, IL-8 and IL-1β, in 28 children with PBB compared to 8 healthy age matched controls (Marsh et al., 2019). Contrary to previous studies, they found similar alpha diversity in the 2 groups, possibly due to the use of unprotected BAL sampling leading to upper respiratory tract microbiota contamination. Cluster analysis partitioned BAL microbiota into 4 main clusters. These were either dominated by the respiratory pathogens *H. influenzae* and *M. catarrhalis* when alpha diversity was low, or a more diverse microbiota including the prominence of *Prevotella spp*. Another important finding was of increased inflammation in both the *Prevotella spp*. associated profiles and pathogen associated profiles. They suggested that bacteria other than the previously identified respiratory pathogens may contribute to airway inflammation in paediatric PBB (Marsh et al., 2019). Of note, the impact of azithromycin on the respiratory microbiome of children with PBB is completely unknown.

### The impact of macrolides on the microbiome

Due to a paucity of studies, particularly in paediatric populations, the effects of azithromycin on the respiratory microbiome are not well characterised. The majority of studies that have addressed this issue do demonstrate some alterations, however the extent, duration and resulting effects are not well documented.

In one RCT, a 14 day course of azithromycin was shown to alter the nasal microbiota in healthy infants hospitalised with RSV bronchiolitis. The relative abundance of *Moraxella* and *Streptococcus* decreased significantly following azithromycin treatment compared to a placebo group whereas the relative abundances of *Dolosigranulum* and *Corynebacterium* significantly increased (Zhou et al., 2016). A small number of trials have investigated the impact of long term azithromycin on the microbiome. The majority of these however have not been specifically designed to investigate changes within the microbiome and hence the number of samples included in the microbiome analysis have been small. A double blind RCT in adult smokers with emphysema, randomised to 8 weeks of daily azithromycin or placebo, reported a reduction in alpha diversity on broncho-alveolar lavage (BAL) following azithromycin but no change to the bacterial burden. This resulted in 11 low abundance taxa being reduced, none of which were classical pulmonary pathogens (Segal et al., 2017). The significance of this finding was unclear.

In adult asthmatic patients, randomisation to 48 weeks of azithromycin treatment did not result in a significant change in sputum bacterial load compared to placebo (Taylor et al., 2019). It did however significantly reduce Faith’s phylogenetic diversity (a phylogenetic generalisation of species richness (Faith, 1992)) and *H. influenzae* load (Taylor et al., 2019). In adults with non-CF bronchiectasis exposed to 12-months of erythromycin, a significant change in sputum microbiota composition was reported compared to placebo. This was demonstrated in those with infection dominated by organisms other than *P. aeruginosa* and was again represented by a relative reduction in the abundance of *H. influenza*e. However, there was an associated significant increase in the relative abundance of *P. aeruginosa* (Rogers et al., 2014). This highlights the importance of characterising and understanding the effects of long-term azithromycin, as *P. aeruginosa* may be more difficult to treat than *H. influenzae* due to its intrinsic antimicrobial resistance. A recent study in 38 adults with cystic fibrosis investigated the effects of long-term (at least 2 years) azithromycin. They drew samples from a prospectively collected biobank and again did not find an obvious effect on sputum microbiota pre- and post-treatment (Acosta et al., 2021). Participants in these studies have been older adults, who may well have more established respiratory microbiomes than children.

Overall therefore, there is limited information on the impact of long-term azithromycin prophylaxis on the respiratory microbiome, particularly in the paediatric setting.

## Qualitative research to explore parental perceptions

Unlike adult patients with capacity, children may have decisions about their health-care needs and treatment made by their parents. Parental views on such treatments may thus shape the adoption of, and compliance with treatments, particularly in the longer term. It is thus of importance to explore parental understanding and concerns regarding the condition their child suffers from, the value and side-effects of the treatments available, as well as their attitudes on the impact of such treatments. Such perceptions will affect the treatment that the child may be started on (or not) and the compliance with treatment. To study these parameters requires qualitative methodologies. Qualitative research is concerned with the subjective world and offers insight into social, emotional and experimental phenomena by exploring the what, how and why (Giacomini et al., 2000). Three commonly used methods are summarised below (Mack N, 2005).

1. Participant observation: To gain intimate familiarity with a group of individuals in

their usual contexts.

1. In depth interviews: To collect data on personal histories, perspectives,

attitudes and experiences.

1. Focus groups: To gain knowledge about social issues and cultural norms,

in general relating to the subgroups represented.

These methods are generally flexible. Researchers are able to use their subjective judgment to explore anticipated and unexpected themes or issues that may arise. Qualitative results typically generate narrative accounts, explanations of phenomena or develop conceptual frameworks (Giacomini et al., 2000). Once a theory has been developed and potentially relevant variables identified, quantitative methods can then be used to test these.

In the field of paediatrics, qualitative research methods may require novel approaches depending on the research question being asked and the developmental stage and maturity of the child. Often, information about a child’s behaviour is obtained from others rather than themselves. With imaginative methodology and the use of play, qualitative research can be carried out in children younger than three years if the information is thought to be best gained from the child themselves (Platt, 2016). They should be regarded as active participants rather than those on whom research is being done. In some circumstances, particularly when younger children are involved, it may be more appropriate to gather information from parents or caregivers (Platt, 2016). Adults may have better recall of events and be more reliable informants.

### The role of the parent in paediatric medicine.

As noted above, a key difference between paediatric and adult medicine is parental responsibility. Parents must make clinical decisions, in particular for younger children, often without their child taking part in this process. Parental views therefore have a major influence on acceptance and adherence of treatments. Including parents and children in decisions relating to medicines is key to medication optimisation (Trivedi, 2017). In chronic paediatric illnesses, adherence to long-term medication is a significant problem with 50-88% reported to be non-adherent to their prescribed regimens (McGrady et al., 2013). Conn et al. found that parental concerns regarding medication was a major determinant of poor adherence (Conn et al., 2005). Similarly, parental knowledge, beliefs and attitudes have been identified as essential factors to consider when changes in antibiotic prescribing are required (Andrews, Thompson, Buckley, Heneghan, & al., 2012).

Health and behaviour are related through a variety of complex interactions. Chronic disease has been described as a long-term stressor for families, with 3 underlying determinants (Institute of Medicine, 2001). These include the change required by the family members, the capacity of the patient to make the required changes and the ability of medical service to mitigate or exacerbate the stresses of illness (Institute of Medicine, 2001). As such, exploring a parent’s experiences of having a child prescribed prophylaxis is important in order to help families and ensure they have the support they need to comply with medication regimes and seek assessment when needed.

### Parental perceptions of antibiotics in respiratory disease

There is an increasing body of research into parental views of antibiotics specifically for acute respiratory tract infections. Most parents acknowledge that antibiotics are not used for common colds, do not expect antibiotics from their doctor for such episodes and only want advice or reassurance (Havens et al., 2016). However, in a study surveying 170 parents only 62% strongly agreed that antibiotics are used to treat only bacterial infections (Havens et al., 2016). An Australian telephone interview study of 401 care givers asked about recent antibiotic use in their children for acute respiratory tract infections. Most responders believed antibiotics provide benefits for common acute respiratory tract infections, especially ear infections, even when the majority of such infections are likely to be viral in origin and to be self-limiting. Care-givers were reported to overestimate the benefits of antibiotics on symptom duration by 5 to 10 fold (Coxeter et al., 2017). Only 44% reported having some discussion during the medical consultation about why antibiotics were being used and 93% preferred to be involved in future decisions about antibiotics (Coxeter et al., 2017).

Knowledge of antibiotic resistance is increasing and parents are increasingly aware of the need for judicious antibiotic prescribing (Szymczak et al., 2018). However, when questioned about acute courses of antibiotics, many parents perceive their children to be at low risk from antibiotic resistance due to their low use of antibiotics (Van Hecke et al., 2019).

### Literature on parental views with regards to preventative antimicrobials

A survey into parental health beliefs and compliance with prophylactic penicillin administration in children with sickle cell disease found that in general, mothers believed that infections were a serious threat and antibiotic prophylaxis was beneficial. Despite this belief, pharmacy records showed that only 12% adhered to a schedule of collecting the fortnightly prescriptions. Those reporting they were never late in collecting prescriptions averaged 19.4 days (SD+/- 5.4) between collections whilst those who reported sometimes being late average 41.9 days (+/-28.5) (Elliott et al., 2001). The results of the 50 mothers surveyed found that only 54% reported not missing any doses whilst 26% reported missing 3-5 doses a week. Other demographic factors such as having private transportation, fewer children and more than one adult at home were associated with better compliance. The main perceived burdens were of obtaining prescriptions every 2 weeks and remembering to administer the medication twice a day (Elliott et al., 2001). The survey however only included 7 questions in total relating to the 4 areas of the health belief model; seriousness of condition, susceptibility of child, benefits and burdens.

Parental attitudes towards the use of infection prophylaxis in a Canadian paediatric oncology setting revealed that parents were in favour of prophylaxis. The main drivers of decision making were the chance of infection and death (Diorio et al., 2012). Although 33 parents were involved in the study, only 5 were interviewed initially to identify the main influencing factors. The duration of the interviews was not reported and may not have been in great depth. The second part of the study used a “think aloud” hypothetical decision-making task involving 26 parents. The major parental issues identified related to antimicrobial resistance, financial burden, route of medication administration and adverse effects of medication (Diorio et al., 2012). Interestingly, the concerns relating to antimicrobial resistance were thought to be an issue at a community level rather than an issue for their individual children. The researchers acknowledge that views may change when faced with a real situation as opposed to a theoretical one.

These studies show that although most parents perceive prophylaxis to be beneficial in these specific situations, this does not translate into good adherence with medication regimes. A number of barriers and perceptions have begun to be explored. Given the paucity of information relating to parental perceptions and attitudes with regards to long-term antibiotic prophylaxis, in depth interviews would help to understand the parental experience of having a child prescribed prophylactic antibiotics. Once more is known in this field, a behavioural model could be developed, and relevant issues then surveyed.

## Knowledge gap

Azithromycin prophylaxis is being used to prevent pulmonary exacerbations in children with PBB. The evidence for this intervention is mostly extrapolated from studies in cystic fibrosis and non-cystic fibrosis bronchiectasis, where higher rates of macrolide resistant organisms have been seen in those exposed to long term macrolide prophylaxis. The current literature describing PBB is very limited with regards to the effects of long-term azithromycin prophylaxis, in terms of efficacy, potential to promote antimicrobial resistance and possible changes to the respiratory microbiome. The pathophysiology and natural history of PBB are also still poorly understood, and factors that determine whether children have a single episode of PBB, multiple episodes or develop bronchiectasis are not known.

I have therefore investigated antimicrobial resistance patterns and changes to the nasopharyngeal microbiome in children with PBB exposed to long-term azithromycin. Including parents in decisions relating to macrolide prophylaxis in paediatric respiratory conditions is key, hence we first explored parental views with regards to prophylactic antibiotics through a qualitative interview study.

## Hypotheses and aims

The studies in this theses aimed to test the following three hypotheses:

### 1.11.1 Hypothesis 1

Psychosocial factors will influence parents’ decisions relating to the uptake, and the way in which families experience long-term prophylactic azithromycin.

Aim:

To improve the way in which antibiotic prophylaxis is discussed with parents during medical consultations.

Objectives:

1. To understand what it means for parents to have a child prescribed long-term prophylactic azithromycin to prevent chest infections.
2. To develop a behavioural model to explain the parental experience of having a child prescribed long-term prophylactic antibiotics.

### Hypothesis 2

Children with protracted bacterial bronchitis exposed to long-term prophylactic azithromycin will develop macrolide-resistant airway flora.

Aim:

To explore whether the use of long-term azithromycin in children with PBB is associated with increased nasopharyngeal macrolide resistant bacterial colonisation compared to an unexposed group of children with PBB

Objectives

1. To conduct a pilot study comparing macrolide resistance patterns in the nasal flora of children with PBB exposed or not to long-term prophylactic azithromycin (PBB study 1).
2. To validate the results of the pilot study through an extension of the pilot study with regards to patterns of azithromycin resistant nasopharyngeal bacteria and antibiotic exposure between children with PBB exposed or not to long-term prophylactic azithromycin (PBB study 2).
3. To determine the frequency of viruses associated with exacerbations of PBB (PBB study 2)
4. To investigate phenotypic and genotypic antibiotic resistance profiles of nasopharyngeal bacterial flora in children with PBB exposed or not to long-term prophylactic azithromycin.
5. To optimise recruitment strategy and sampling methodology for future studies.

### Hypothesis 3

Children with protracted bacterial bronchitis exposed to long-term prophylactic azithromycin will display a reduced diversity of bacterial communities in the nasopharynx.

Aim:

To describe the changes that occur within the nasopharyngeal microbiome of children with PBB exposed to long-term prophylactic azithromycin.

Objectives

1. To compare the nasopharyngeal microbiota composition of children with PBB taking long-term prophylactic azithromycin with an unexposed comparison group of children with PBB, through the use of 16s rRNA analysis.
2. To investigate differences between the nasopharyngeal composition at the beginning and end of exacerbations of PBB.

# **Chapter 2. Methodology**



## Methods: The parental experience of having a child prescribed prophylactic antibiotics.

### Study conduct

This study was conducted following favourable ethical approval from the Northwest - Haydock Research Ethics Committee (reference number 18/NW/0579) and approval from the Health Research Authority and Care Research Wales. Written consent was obtained from parents or legal guardians prior to the interviews. The study was carried out in accordance with the International Conference for Harmonisation of Good Clinical Practice, and the UK Policy Framework for Health and Social Care Research (Department of Health, 2022). It was funded and supported by the Bassetlaw Research Fellowship programme and the charity Antibiotic Research UK (Grant reference ANTSRG 03/2018).

### Study design

I carried out a single centre phenomenological study at Sheffield Children’s NHS Foundation Trust (SCH) of parents and guardians (subsequently referred to as parents) whose children had been prescribed prophylactic azithromycin. Oral azithromycin is used first line as prophylaxis in the respiratory clinic at SCH for conditions such as bronchiectasis and PBB (See appendix 2 for guideline). I used face-to-face interviews to allow parents to fully describe their experience of having a child prescribed long-term prophylactic antibiotics.

### Participant selection and recruitment

Parents whose children had been prescribed prophylactic azithromycin for at least 3 months to prevent respiratory tract infections were invited to participate. Recruitment took place at SCH over an 8-month period between 1st September 2018 and 31st April 2019. A purposive sampling method was used to try and capture maximum variation in views on the basis of age of the parent and child, parental education, ethnicity and the severity of the child’s condition. Purposive sampling using a maximum variation strategy is a recognised sampling method for the purpose of developing a deeper understanding of the phenomenon being researched (Palinkas et al., 2015).

#### Inclusion criteria

* Prophylactic oral azithromycin use for at least 3-months to prevent respiratory tract infections
* Age of child between 2 years and 10 years
* Child under the care of the paediatric respiratory or immunology teams and diagnosed with an underlying condition predisposing them to recurrent respiratory tract infections

#### Exclusion criteria

* Medication administration via a percutaneous gastrostomy or nasogastric tube
* Non English-speaking parents (insufficient resources for translation)

### Participant recruitment

Parents attending routine respiratory or immunology outpatient appointments for their children were approached by the specialist nurses and given written information regarding the study if the inclusion criteria were met (appendix 3.1). All families were contacted by text message 1 to 7 days later and asked if they would like to participate. An interview time was arranged for those willing to take part and any questions answered.

### Interviews

I conducted face-to-face interviews at SCH or the family home. The venue depended on what was most convenient for the parent being interviewed. The interviews were semi-structured using an interview questionnaire devised by myself, Dr Andrew Lee (Professor of Public Health, School of Health and Related Research) and Dr Fiona Shackley (Consultant Paediatrician, Sheffield Children’s Hospital). It was based on topics of interest to the research question and themes previously identified in the literature relating to adult and parents’ perceptions of acute antibiotics (appendix 4). I aimed to complete the interview within 45-60 minutes.

The interviews focused on the parents’ lived experience of having a child who had been prescribed prophylactic azithromycin. We intended to further our understanding of how parents made decisions, the driving forces behind these decisions as well the practical aspects of administration and adherence to medication. At the beginning of each interview I informed parents that I was not directly part of the respiratory team caring for their child, their views would be kept confidential and that the respiratory team would only see the final study results with anonymised quotations. They were encouraged to give a full description of their thoughts, feelings, attitudes and any views regarding prophylactic antibiotic use. A flexible open-question interview approach was used. The research questions initially put to the participants were:

1. What does it mean to you to have a child who has had to take regular preventative

antibiotics?

1. What is your experience of having your child prescribed long-term preventative

antibiotics?

Interviews were recorded using an encrypted digital voice recorder and field notes were taken of any interesting observations and summaries of conversations that took place pre- and post-recording.

### Data handling

Acquired data was stored and collated on Microsoft Excel password-controlled databases. Data containing patient information was stored only on a password protected NHS network computer drive. Anonymous information was stored and analysed on a password protected, university of Sheffield computer in a locked office at SCH.

Data were collected and retained in accordance with the Data Protection Act 1998. All source documents will be kept for a period of 5 years following the end of the study and then destroyed.

### Analysis

#### Sample size

A purposive sample size in qualitative research should be determined once no new patterns or information emerges - theoretical saturation. Guest et al. suggest that data saturation (no new data are being found) often occurs around 12 participants in homogeneous groups (Guest et al., 2006). Marconi et al report that “meaning saturation”, the point where no new insights of identified issues arose, required up to 24 interviews. Hence a sample size of 12- 20 parents was felt to be appropriate (Hennink et al., 2017). The final sample size was 15 interviews involving 18 participants.

#### Data analysis

Each interview was transcribed verbatim into an excel spreadsheet. Thematic analysis was conducted on all interviews, using the approach suggested by Smith with regards to phenomenological studies (Smith, 2009). Two researchers, myself and Prof Andrew Lee independently conducted the analysis beginning with familiarisation of the interview transcripts by repeated reading.

Analysis began after the first 3 interviews. All comments were coded line by line using standard coding techniques (Saldana, 2015). The coded transcripts were independently checked by myself and Prof Lee. A framework of subthemes was developed which was iteratively adapted following each interview. We focussed on finding recurrent and unusual, collective and opposing subthemes trying to understand how parents had experienced the events they described, why they had made the decisions they had and the factors influencing their behaviour.

A consensus of the final three themes and associated subthemes was agreed upon through a continuous process of refinement and discussion (Chapter 3). Background descriptive medical and social information was used to help interpret comments. This information was coded as descriptive background and did not fall into the three main categories. Anonymised quotations were used in the final report and parents specifically consented for this. The results of this study have been published in full (Hardman et al., 2021).

## Methods: PBB study 1 – Pilot study of azithromycin and nasopharyngeal flora AMR

### Study conduct and funding

This study was conducted following favourable ethical approval by the West of Scotland REC3 Ethics committee (Reference number 18/WS/0176) and approval from the Health Research Authority. A substantial amendment to obtain GP prescription records (to ensure all additional antibiotic courses were captured as parental documentation/recall was not always complete) was approved (Amendment number: REC Ref AM02). Written consent was obtained from all parents or legal guardians at the start of the study. The study was funded by Antibiotic Research UK (Grant reference ANTSRG 03/2018) and the Bassetlaw Research Fellowship programme. It was carried out in accordance with the International Conference for Harmonisation of Good Clinical Practice, and the UK Policy Framework for Health and Social Care Research (Department of Health, 2022).

### Study design

We carried out a single centre, prospective observational study at SCH. The study was a pilot study to explore feasibility of sample collection, to obtain preliminary results, and to establish important parameters in future study design. There is little published guidance on sample size for pilot studies. Hertzog (2008) found sample sizes ranging from 10-40 per group met a variety of aims (e.g. problems of data collection strategies, answering methodical queries, estimating variability) (MA, 2008). We aimed to recruit a total of 20 children with a diagnosis of PBB (see below), split between 10 children to be prescribed long term azithromycin (10mg/kg three times a week) to prevent pulmonary exacerbations of PBB over the winter months on clinical grounds. and 10 children with PBB who were not anticipated to require long term azithromycin over the winter. Our choice of sample size was pragmatic; since this is a previously unstudied field, a power calculation was not performed. The group of children who were not expected to require prophylaxis was not a ‘healthy control’ group and will be referred to as the “comparison group”. Children in the comparison group were to be age-matched if possible within 12-months of those in the azithromycin group. It was not anticipated that participants in the comparison group would be prescribed prophylactic azithromycin. However, if their condition deteriorated and they were prescribed prophylactic azithromycin during the study period, intention to treat analysis would be used. This however did not occur in PBB study 1.

### Participant selection

Between October 2018 and March 2019, parents whose children had had at least 1 episode of PBB over the previous 12-months were approached if they fulfilled study entry criteria.

#### Inclusion criteria for Azithromycin group:

* Age between 2 years and 10 years
* Diagnosis of PBB as previously defined (Section 1.2)
* Management plan to be prescribed long term azithromycin over the winter months 2018-2019 as part of their standard care
* Have not received azithromycin prophylaxis for at least 6-months

#### Inclusion criteria for the Comparison group

* Age between 2 years and 10 years – age-matched to azithromycin subject to within 12 months where possible
* Diagnosis of PBB as previously defined (Section 1.2)
* Less than 3 exacerbations of PBB over the preceding 12-months and not anticipated to require long term azithromycin as part of their standard care over the winter 2018-2019
* Not previously prescribed prophylactic antibiotics

#### Exclusion criteria

* Children prescribed permanent rather than seasonal prophylactic azithromycin
* Diagnosis of cystic fibrosis or ongoing investigation for cystic fibrosis
* Primary aspiration
* Primary ciliary dyskinesia
* Primary immune deficiency requiring immunoglobulin replacement
* Known diagnosis of bronchiectasis
* Children prescribed 3 monthly courses of intravenous antibiotics
* Children on anticoagulants – small risk of bleeding when taking DNPS
* Non English-speaking families – inadequate funding for translational services
* Children who have previously been prescribed non-azithromycin prophylaxis
* Hypersensitivity to or known intolerance of azithromycin

### Participant recruitment

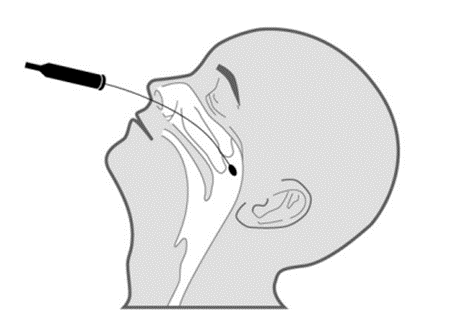
Parents were approached by specialist respiratory nurses whilst attending routine respiratory outpatient appointments for their children at SCH. Respiratory nurses see children prior to the respiratory consultant review, to check inhaler technique and discuss any issues of concern. The respiratory nurses gave families the written study information sheet. Following their consultant appointment, families had the opportunity to discuss the study with myself if they wished. The inclusion criteria were checked at this point. Families were then contacted by text message after 48 hours to see if they would like to discuss the study further or whether they would like to take part. No further contact was made with those who did not respond to the text message.

### Interventions and sample processing

Demographic data, medical history and previous antibiotic use were recorded for each participant. Deep nasopharyngeal swabs (DNPS – see below) were taken at baseline and every 3 months over a 12-month period (a total of 5 swabs for each participant). It was anticipated that 3-monthly sampling would be acceptable with regards to the swab burden for children and that this would enable sampling during and after the period of antibiotic prophylaxis in the azithromycin group. The baseline swab for those in the azithromycin group was taken prior to the first dose of azithromycin being administered. The clinical condition of the child was followed over the 12-months, including data on exacerbations, other courses of antibiotics, hospital admissions and any changes in medication. Parents were asked to note any courses of antibiotics their child was prescribed in a study booklet given to them on their first visit.

#### Deep Nasopharyngeal Swab (DNPS) Sampling

A cotton tipped wire swab (Sigma Transwab in liquid amies medium) was inserted to a depth of half the distance between the nostril and ear lobe as is the standard practice for taking such samples (Wouters et al., 2018) (Figure 2‑1). To avoid operator differences, all swabs were taken by myself either at the family home or SCH depending on what was easiest for the family. See Figure 2‑2 for the pathway of sample processing in PBB studies 1-3.



**Figure 2‑1**: Pictorial representation of collecting DNPS

*With the subject’s head tilted slightly back, a cotton tipped swab was inserted into the nasopharynx and rotated a few times to sample the bacterial flora. The insertion distance of the swab to sample this anatomical area was first estimated as half the distance between the participant’s nostril and ear lobe.*

**DNPS +/- viral throat swab taken from child**

**Study 1**

DNPS samples destroyed

Swabs delivered to the STH laboratory within 24 hours

**Viral swabs**

Processed for respiratory PCRs at STH **(Study 2 only;** at the beginning of exacerbations**)**

**Study 3**

Bacterial isolates frozen at -80°C and stored

**DNPS**

Bacterial culture

MIC testing

Carried out at STH (Studies 1 and 2)

Isolates sub-cultured at STH laboratory. Additional antibiotic susceptibility testing performed

**Study 2**

Remaining sample frozen at -80°C and stored

DNA extracted from isolates

(Professor Condliffe’s laboratory)

Samples transported on dry ice to the University of Edinburgh

(Professor Bogaert’s laboratory)

Subset of S. pneumoniae isolates sequenced (MicrobesNG)

All samples then destroyed

DNA extracted and 16s analysis performed

All samples destroyed

**Figure 2‑2:** Flow diagram of sample processing for PBB studies 1 and 2

#### Microbiology

Microbiology laboratory technicians at the Sheffield Teaching Hospitals NHS Foundation Trust laboratory cultured the swabs according to standard protocols. Aliquots of 10-μl were plated onto chocolate agar and blood agar. Plates were incubated at 35-37°C in 5-10% CO2 for 48 h before colony enumeration and identification. Any growth of *S. pneumoniae*, *H. influenzae*, *S. aureus* and *M. catarrhalis* was recorded and confirmed by standard techniques. Minimum Inhibitory Concentrations (MICs) were determined for azithromycin susceptibility in identified isolates using Etest strips (AB BioMérieux, France) and resistance determined according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2022). In study 1, once the DNPSs had been cultured, they were destroyed. Any bacterial isolates obtained were frozen at -80°C for later analysis (see sections 2.4.3 and 2.4.4). Specific consent was taken for this. This study did not involve any interventions with regard to the clinical care of these children, however results were uploaded onto the integrated computer system so that the clinical team including GPs could view any isolated organisms and their resistance profiles.

#### Adherence to medication

Participants prescribed prophylactic azithromycin over the winter months were asked to complete a self-reported prescription diary as a measure of compliance. This was based on honesty. Families were informed that it was not a test and they would not be reprimanded if they forget to take or record doses. Diaries were reviewed at 3 and 6 months when nasal swabs were taken.

#### Antibiotic exposure

To assess antibiotic exposure, parents were asked at recruitment about previous courses of antibiotics prescribed for their child. They were given a study booklet at recruitment to record any courses of antibiotics prescribed over the study period. At each visit to collect a DNPS, parents were asked if their child had had any courses of antibiotics since the last visit. The electronic data management system at SCH was used to look at clinic letters and discharge summaries with regards to antibiotics prescriptions. Since parental recall of previous antibiotic courses and the names of these was poor, an amendment to the study protocol was made and given ethical approval to obtain GP prescription records (Amendment number: REC Ref AM02) so that a more comprehensive analysis of antibiotic exposure could be made.

### Data handling

Data were anonymized, stored and collated on a password-controlled Excel database on a password-protected computer hard-drive, kept locked in the Research Fellow office at Sheffield Children’s Hospital. All data were backed-up to a personal, University of Sheffield password protected network drive. Patient details correlating to individual study numbers were only stored on a password protected, personal network drive on an NHS computer at SCH.

DNPS were taken and labelled with a study sticker, including the child’s unique SCH identification number, the study name and supervising clinician’s name (Dr Kelechi Ugonna, SCH Respiratory Consultant). This enabled standard laboratory administration procedures for the handling of samples to be used. Results of the samples were uploaded to the SCH results viewing system – ICE. Participants were allocated a unique study number. Research members recorded the results on an anonymised database using this study number (not the hospital number). Microbiology data were recorded on a password protected Excel spreadsheet. These were collated with baseline data and clinical information regarding exacerbations.

No patient identifiable data is or will be reported or published and the study adhered to the requirements of the Data Protection Act 1998. Trial documents (paper and electronic) will be retained in a secure location during and after the trial has finished. All source documents will be retained for a period of 5 years following the end of the trial prior to destruction.

### Statistical Analysis

The study was analysed and written up according to STROBE guidelines ([www.strobe-statement.org](http://www.strobe-statement.org)). Continuous data were summarized by the median (25th/75th centiles) and categorical data by n (%). Missing values were summarised. Statistical testing was kept to a minimum in line with recommendations for pilot studies (Lancaster GA, 2004). In accordance with formal statistical input from Professor Alan Rigby (Professor of Statistics, Hull York Medical School), some results from this study were combined with those from PBB study 2 (see 2.3.14) to enable statistical analysis.

## Methods: PBB Study 2 - Azithromycin and the nasopharyngeal microbiome

This study aimed to describe any changes that may occur within the nasopharyngeal microbiome of children with PBB exposed to long term azithromycin. The methodology of the first part of PBB study 2 is similar to that of PBB study 1. Refinements (see Sections 2.3.4 and 2.3.5) were made guided by issues of recruitment, and the feasibility and acceptability of taking DNPS that arose in the first pilot study. Study 2 involved sampling children longitudinally and analysing deep nasopharyngeal swabs for viral and bacterial isolates, antimicrobial resistance profiles and 16S rRNA gene sequencing to analyse the impact of antibiotics on microbial communities.

### Study conduct and ethical approvals

This study was conducted following favourable ethical approval by the Yorkshire and the Humber – Leeds East Research Ethics Committee (Reference number 19/YH/0207) and approval from the Health Research Authority. Substantial amendments were approved to obtain GP prescription records as above, to increase the duration of participant involvement from 12 to 20 months due to the COVID-19 pandemic, and finally to allow Professor Bogaert’s team at the University of Edinburgh to process the samples for the microbiome analysis (REC reference 19/YH/0207, Amendment numbers: Substantial Amendment 1, 09/09/19 and substantial Amendment 2 9/6/21). I had initially planned to extract DNA from the samples in Sheffield and outsource the 16S analysis to Genewiz, but due to the pandemic, the relevant resources for sample processing (specifically a dedicated sterile hood housing the Bead Beater for extracting DNA from low-abundance samples) had been moved into the Category 3 laboratory and were being extremely heavily used, leading to a high risk of potential sample contamination which would invalidate the study. We therefore collaborated with Professor Debby Bogaert (University of Edinburgh), who has extensive expertise in paediatric microbiome studies with facilities to extract DNA from low-abundance samples in a dedicated laboratory environment not used for other purposes.

Written informed consent was obtained from all parents or legal guardians at the start of the study and after each substantial amendment where required. The study was funded by the Sir Halley Stewart Trust (Grant reference R/160595, myself as principal applicant) and carried out in accordance with the International Conference for Harmonisation of Good Clinical Practice and the UK Policy Framework for Health and Social Care Research (Department of Health, 2022).

### Study design

After discussion with statistician Professor Rigby, we utilised a pragmatic sample size based on recruitment results from PBB study 1, anticipated patient numbers available, and the time constraints of the MD degree. However, the sample size is comparable to that of other studies exploring the microbiota in the setting of protracted bacterial bronchitis. We aimed to recruit a total of 30 children with a diagnosis of PBB to this single centre, prospective observational study at SCH. The same treatment and comparison cohorts were used as described in study 1 but 15 children were recruited to each group with the aim of pooling data where possible.

### Participant selection and recruitment

The same inclusion and exclusion criteria were used as described in study 1 apart from the following 2 amendments:

Firstly, the age range was changed from 2-10 years to 18 months-6 years. This was due to the fact that in PBB study 1, older children were more difficult to age match. In PBB study 1, a number of younger children starting azithromycin were not eligible, so the minimum age range was lowered for PBB study 2.

Secondly, to facilitate the recruitment of children to the comparison group, the time frame over which a child must have previously had an episode of PBB was extended from 12 to 18 months.

### Participant recruitment

The recruitment process was improved following PBB Study 1. To streamline recruitment and improve efficiency, additional ethical approval was requested and granted to pre-screen potential recruits (Reference number 19/YH/0207). I reviewed all relevant outpatient clinic lists on a weekly basis. The notes for each child who fulfilled the age inclusion criteria were reviewed (See section 2.2.3.1/2 for inclusion and exclusion criteria). A list of potential recruits was emailed to the Respiratory specialist nurses. The nurses once more approached families during the child’s outpatient clinic appointment and gave them the study information. Families were then contacted after at least 24 hours rather than 48 hours by text message to see if they would like to take part. This was because during study 1, we found some families had already started azithromycin by the time they received the text message.

### Interventions and sample processing

Demographic data, medical history and previous antibiotic use were recorded for each participant. Deep nasopharyngeal swabs (DNPS) were taken at baseline. We then aimed to take swabs every 4 months over a 12-month study period. The frequency of sampling was extended from 3 monthly (PBB study 1) to 4 monthly as further swabs were to be taken around exacerbations. In addition to a DNPS, a viral throat swab was planned to be taken at the beginning of any pulmonary exacerbations. Due to restrictions during the COVID-19 pandemic however, there were major changes in the timing of the swabs (see section 2.3.6).

Feedback from parents in PBB study 1 suggested that collecting swabs on 5-6 occasions over 12-months would be acceptable. Some families found collecting repeated nasal swabs distressing and hence the end of exacerbation swab was made optional. An exacerbation of PBB was defined as 2-6 weeks of antibiotic treatment commenced by hospital staff or the child’s general practitioner as advised by the respiratory team, for any of the following new symptoms that were present for more than 14 days:

* Increased cough.
* No new pointers to suggest other lung disease.
* Chest radiograph – minor changes such as bronchial wall thickening.

Details of the clinical course of the exacerbation were recorded. It was anticipated that children would have between 0-2 exacerbations a year. Families were asked to contact myself if their child was to be started on such courses of antibiotics and I would collect the beginning of exacerbation swabs. The clinical condition of the child was to be followed over the next 12 months as described in PBB study 1.

### Changes in methodology due to the COVID-19 pandemic

The COVID-19 pandemic began 5-months into the study. Due to two national lockdowns and government advice on self-isolation when unwell, the study methodology had to be significantly changed. During lockdowns, it was not possible to enter family homes to take swabs. The majority of participants thus missed having swabs taken at 4 months with some missing collection at 8 months. In order to collect the final swabs on participants, the time participants remained in the study had to be increased from 12 to 20 months, hence the time after azithromycin cessation was higher than anticipated for some patients. Collection of swabs around exacerbations was limited during the study due to self-isolation restrictions. Collectively, this limited the number of samples available for analysis and led to the timings of samples being suboptimal (for example, not always precisely timed with commencement or cessation of azithromycin).

### Compliance

The same self-reported prescription diary was used as in PBB study 1 for those taking prophylactic azithromycin. The importance of completing the diary was given greater emphasis in discussion with parents. They were offered suggestions such as attaching or placing the diary somewhere obvious as a medication reminder and to prevent them losing it. Parents were further reminded about the diary during any correspondence regarding exacerbations.

### Deep nasopharyngeal swab sampling

All DNPS were taken by myself exactly as described in PBB study 1. The pathway for sample processing is documented in Figure 2-2.

### Bacterial culture

All DNPS were processed at the STH laboratory using the same protocol as described in study 1 (Section 2.2.5.2). The DNPS transport medium and any bacterial isolates were frozen at -80⁰C pending further analysis.

### Virology sampling and studies

A viral swab (Sigma Virocult swab and transport medium, medical wire) was inserted into the child’s mouth and rubbed on the back of the throat. These swabs were processed at the STH virology laboratory by the laboratory staff according to standard protocols. Nucleic acids were extracted from the media in accordance with standard procedures. Real-time PCR assays were used to detect respiratory syncytial virus, adenovirus, influenza viruses (A and B), parainfluenza 1-4, human metapneumovirus, human coronavirus and rhinoviruses.

### DNA extraction and 16s rRNA gene sequencing

For each nasal swab taken for bacterial culture, we performed 16s rRNA gene sequencing to characterise the bacterial community composition. All the frozen transport medium samples were couriered on dry ice to Professor Deborah Bogaert’s laboratory in Edinburgh. Laboratory technicians with expertise in the processing of low bacterial biomass samples extracted DNA using both mechanical and chemical lysis. Following this a PCR amplicon library was generated by amplification of the V4 hypervariable region of the 16s rRNA gene. The pooled amplicon library was then sequenced at the Edinburgh genomics facility. I explored the possibility of visiting the laboratory to participate in sample processing, but ongoing COVID restrictions precluded this. Instead, the laboratory staff provided me with videos of all the steps and details of the protocol and techniques used. The following protocol has been optimised in Professor Boagaert’s laboratory and is identical to the protocol I had planned to use had I carried out the extractions in Sheffield. The extractions were carried out by Paula Lusarreta Parga, Research Assistant to Professor Bogaert, exactly as described below.

DNA was extracted using a phenol/ bead beating protocol in combination with an Agowa Mag DNA extraction kit (LGC genomics, Berlin, Germany). The nasopharyngeal swab transport medium was thawed. 200 µl of this medium was aliquoted into sterile screw-cap Eppendorf tubes containing 600µl of lysis buffer (Agowa Mag DNA extraction kit) with zirconium beads (diameter 0.1mm, Biospec Products, Bartlesville, OK, USA) and 550µl of phenol. Samples were mechanically disrupted using a bead beater (mini-Beadbeater-24, Biospec products) through 2 cycles of two minutes of beating at 3500 oscillations/minute. After each beating cycle, the homogenate was placed on ice for 2 minutes. After centrifugation at 12,000g for 2 minutes, the clear supernatant with aqueous DNA was transferred to new Eppendorf tubes containing 10 µl of magnetic beads in 1.3ml of binding buffer. To maximise recovery of DNA, samples were incubated for 30 minutes instead of 10 minutes as recommended by the kit’s manufacturer. The tubes were put in a magnetic separation rack and the supernatant discarded. The magnetic beads were washed with wash buffers 1 and 2 and then air-dried for 15 minutes at 55⁰C. Finally, elution buffer was added to the dried magnetic beads and the eluted DNA transferred into a new storage tube.

Following DNA extraction, the 16s rDNA gene was quantified using quantitative PCR. The samples were validated to check that sufficient 16S rDNA was present. Quality control standards for reliable analysis were set as samples having DNA levels of ≥0.2 pg/µl over negative controls. The concentration of DNA was normalised using high performance liquid chromatography grade water to a maximum of 20 pg/µl.

Generation of the PCR amplicon library was performed by amplification of the V4 hypervariable region of the 16S rRNA gene using barcoded universal primer pairs 515F (5’-GTG CCA GCM GCC GCG GTA A-3’) and 806R (5’-GGA CTA CHV GGG TWT CTA AT-3’). The PCR reactions were conducted using the following steps; 98 ⁰C for 30 seconds; 30 cycles at 98⁰C for 10 seconds, 55⁰C for 30 seconds and 72⁰C for 30 seconds with a final hold of 5 minutes at 72⁰C. Amplicons were quantified with PicoGreen (Thermofisher). Samples as well as positive and negative controls were pooled (150 ng/sample). Pool purification was carried out twice with AMPure beads added to the pooled sample (ratio 0.9x)to remove primer dimers and unwanted biproducts. . After leaving for 10 minutes at room temperature, the sample was placed in a magnetic rack for 5 minutes to separate the beads from the liquid. The supernatant was removed and the beads washed twice with 70% ethanol. A resuspension buffer was added and the eluate pipetted into a new tube for storage. The pooled amplicon library, containing samples and controls, was finally sequenced using paired-end, 250 base pair reads generated on an Illumina MiSeq.

### Steps taken to reduce contamination

Due to the low bacterial density of nasopharyngeal samples, it was imperative to eliminate contamination insofar as possible. The following steps were taken:

1. Dedicated linear workflow starting in a PCR cabinet in a designated and dedicated “clean room”. Here Eppendorf tubes were prepared with reagents and beads and PCR plate preparation conducted. No DNA extractions, amplicon generation or microbial work occur in this area. Thawing of samples and DNA extraction was undertaken in the main laboratory and amplicon generation was restricted to a separate room.
2. Single hand control of opening and closing Eppendorfs was used, with time open to the air minimalised. Disinfection of workspace, equipment, in between tubes and protocol steps was undertake as routine.
3. Blanks containing, transport medium or reagents without the addition of a sample and PCR blanks with no DNA present, were used to detect any contamination occurring despite the above precautions.

### Data security

Data was handled as described in study 1 with regards to the storage of data files.

Frozen transport medium samples sent to Edinburgh were labelled with study numbers only, without any patient identifiable details. A word document with the key to the study numbers and patient details was stored only on a password protected, personal network drive on an NHS computer at SCH. Anonymised data sets were used to carry out the analysis on non-NHS computers. No patient identifiable data was or will be reported or published and the study adhered to the requirements of the Data Protection Act 1998. Trial documents (paper and electronic) have and will be retained in a secure location during and after the trial has finished. All source documents will be retained for a period of 5 years following the end of the trial prior to destruction.

### Bioinformatics and statistical analysis

PBB study 2 was analysed according to STROBE guidelines ([www.strobe-statement.org](http://www.strobe-statement.org)) as described in study 1. Statistician, Professor Rigby (University of York and Hull) advised and reviewed the statistical methodology used throughout the study. Given the similar study design and participant inclusion criteria, results from PBB study 1 and 2 were combined for some analyses. The combined sample size allowed for statistical analysis. A paired T-test was used to compare the means between the two groups for continuous data and Fisher’s exact test for categorical data. Poisson regression analysis was used to report the incidence rate ratio when comparing incident rates between the 2 groups. A p value of < 0.05 was taken as statistically significant however, Martin Bland’s interpretation of significance was used to indicate the strength of the evidence as reported in Table 6**:** Martin Bland's interpretation of significance (Bland, 2020). An intention to treat analysis was used acknowledging the fact that 2 participants in the comparison group were prescribed long term antibiotics and remained in the study.

**Table 6:** Martin Bland's interpretation of significance

|  |  |
| --- | --- |
| P value | Evidence for a difference or relationship |
| P >0.1 | Little of no evidence |
| P = 0.05-0.1 | Weak evidence |
| P = 0.01 – 0.05 | Evidence |
| P < 0.01 | Strong evidence |
| P < 0.001 | Very strong evidence |

#### Quality control for 16sPCR

Raw sequences were processed using the packages ‘DADA2’, ‘decontam’, ‘phyloseq’ and ‘vegan’ in R. The bioinformatic department in Edinburgh firstly removed the barcodes and primers and plotted the quality scores. Reads were then trimmed and filtered as the quality dropped (length threshold of 200 base pairs for forward reads, 150 base pairs for reverse reads). Given that the V4 region is usually 253 base pairs, there was still adequate overlapping of forward and reverse reads. Standard filtering parameters were used: maxEE = 2 (maximum number of expected errors allowed in a read), truncQ = 2 (truncation of reads with a Q score of less than 2). The DADA2 core sample inference algorithm was run. This software models and corrects for Illumina-sequenced amplicon errors and infers sample sequences exactly and resolves differences of as little as 1 nucleotide (Callahan et al., 2016). Further filtering of sequences longer or shorter than the expected base length (acceptable 250-256 base pairs) occurred and removal of chimeras. Taxonomy was assigned in DADA2 using a native Bayesian classifier method (Wang et al., 2007) This takes the input set of unknown sequences and a training set of reference sequences (silva v138 training set) and outputs taxonomic assignments.

All subsequent analysis steps were undertaken by myself, with support from Dr Alicia Ruiz Rodriguez (a postdoctoral researchers in Professor Bogaert’s team) and Justyna Binkowska (a PhD student in Professor Bogaert’s team). Further quality control was undertaken with removal of contaminants and ultra-rare taxa using the package ‘decontam ‘in R.

### Analysis

I carried out the analysis in R using the packages ‘Phyloseq’, ‘Vegan’ and ‘ggplot2’. Firstly, an overview of the microbial composition was obtained using stacked bar charts. Differential abundance testing was carried out using the ‘metagenomeseq’ model to detect differences in the relative abundance of ASVs between the 2 groups. This programme is designed to account for sparsity due to under sampling (Paulson et al., 2013). The top 50 ASVs were selected and an adjusted p-value of <0.05 was considered to be significant. Alpha diversity (within-sample diversity) was computed using the Shannon diversity index on raw sequence data. This index takes into account the richness (number of species) and evenness (relative abundance) of species within a community. Fisher’s test was used to compare the 2 groups. Following this, beta diversity (between-sample diversity) was investigated using the Bray-Curtis dissimilarity measure. To assess this, a dissimilarity matrix was constructed whereby the composition of each samples is compared to all the other samples and assigned a numerical value. This measure ranges between 0 and 1 where 0 indicates the 2 sites have zero dissimilarity i.e. they share exactly the same number and abundance of microbes. A Bray-Curtis measure of 1 means that the 2 sites have complete dissimilarity. After creating a dissimilarity matrix from the ASV table, non-metric multidimensional scaling (NMDS) was undertaken. In order to test whether there was significant dissimilarity between groups, a permutational multivariant analysis of variance (PERMANOVA) was calculated. PERMANOVA is a non-parametric multivariant statistical model. It is used to compare groups of objects and test the null hypothesis that the dispersion of the groups, as defined by a measure in space, are equivalent for all groups (Ebner, 2018). Rejection of the null hypothesis means that the spread of objects differs between the groups.

## Methods: PBB Study 3 – Antibiotic resistance patterns of nasopharyngeal bacteria

The initial PBB studies 1 and 2 investigated similar cohorts of children with PBB. Half of these participants were exposed to long-term azithromycin and half were not exposed to long-term azithromycin (25 in each group). PBB studies 1 and 2 initially looked at just azithromycin susceptibility of bacterial isolates. An additional pump priming grant enabled further investigation the resistance patterns of stored bacterial isolates from PBB studies 1 and 2, initially with regard to susceptibility to other antibiotic classes and subsequently by genotypic resistance.

### Study conduct and funding

This study was conducted under the umbrella of the favourable ethical approval from PBB studies 1 and 2 (Ethics reference numbers 18/WS/0176 and 19/YH/0207, IRAS numbers 246148 and 255076). Specific written consent was taken during the first 2 studies to allow storage of the nasopharyngeal bacterial isolates and further research without additional consent. The study was funded by the Florey Institute for Host Pathogen Interactions (grant number R/159946-12-8).

### Study design

PBB studies 1 and 2 had initially looked at just azithromycin susceptibility of bacterial isolates from serial DNPS collected from 50 children with PBB (half were exposed to long-term azithromycin and half were not). During the study period, many children received additional acute course of antibiotics in particular amoxicillin and co-amoxiclav. All samples were analysed for phenotypic resistance and samples of interest were then sent for whole genome sequencing.

### Phenotypic resistance

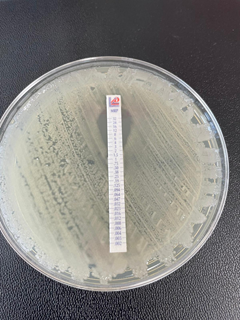
All 167 frozen nasopharyngeal bacterial isolates from participants in PBB studies 1 and 2 were used for this study (79 from PBB study 1 and 88 from PBB study 2). All isolates were sub-cultured at the Sheffield Teaching Hospitals NHS Foundation Trust microbiology laboratory by the laboratory staff. The antibiotics for susceptibility testing were in accordance with the standard antibiotic susceptibility panels run on NHS samples determined by clinically relevant antibiotics and local resistance patterns. Susceptibility to the antibiotics listed in Table 7 was determined using disk diffusion. Discs impregnated with specific concentrations of antibiotic (supplied by MAST) as noted in Table 7 were placed on pre-inoculated agar/blood agar plates (Agar from Oxoid ltd) and zones of inhibition (Figure 2-3 A) were measured in millimetres. Measurements were compared to the reference EUCAST zone diameter breakpoints for the bacterial species and antibiotic investigated. An effective antibiotic creates a large zone of inhibition, whilst an antibiotic that is not effective will not kill bacteria as the antibiotic concentration falls with diffusion and hence the zone of inhibition will be smaller. The diameter that determines resistance versus susceptibility is defined as the breakpoint (Table 7). E-test strips (Liofilchen) were used on a selection of samples to confirm erythromycin susceptibility (Figure 2‑3 B). The plastic strips coated with erythromycin (maximum antibiotic concentration 256mg/L) were placed on pre-inoculated agar/blood agar plates. Elliptical inhibition zones indicate the MIC at the intersection point between the inhibition zone and edge of the strip. Resistance was determined in accordance with the EUCAST breakpoint tables (EUCAST, 2022). Finally, an agar incorporation method (Table 8 and Figure 2-3 C) was used for *S. aureus* (due to the high numbers of *S. aureus* that require testing daily - the agar incorporation method is more efficient than testing with the disk diffusion method). A pre-defined concentration of antibiotic (MAST Adatabs®) was incorporated into agar plates (poured in house using Oxoid Muller-Hinton powder). The concentration of antibiotic used relates to the MIC breakpoint that determines resistance versus susceptibility. A standardised bacterial inoculum was prepared to give 104 colony-forming units per spot on the agar plate (EUCAST, 2000). If bacterial growth is inhibited then the bacteria is classified as susceptible to that antibiotic. If bacterial growth persists in the presence of the known antibiotic concentration that determines resistance, the bacteria is classified as resistant. An antibiotic free agar plate comprises the control. As part of the laboratory United Kingdom Accreditation Service (UKAS), all agar media is quality controlled. For all assays, antibiotic discs are tested weekly against quality control organisms as recommended by EUCAST. MIC strips are quality control checked against the same organism when a new batch comes into use to ensure they produce acceptable results.

**Table 7:** Antibiotics used for susceptibility testing using disc diffusion method for specified bacteria

|  |  |  |  |
| --- | --- | --- | --- |
| Bacteria | Antibiotic (µg on disk) | Zone diameter break points (mm) (EUCAST, 2022) | |
| Sensitive ≥ | Resistant < |
| *S. pneumoniae* | Erythromycin 15  Oxacillin 1  Tetracycline 30  Chloramphenicol 30  Levofloxacin 5 | 22  20  25  21  50 | 19  20  22  21  16 |
| *H. influenzae* | Chloramphenicol 30  Ampicillin 2  Cefuroxime 30  Ciprofloxacin 5  tetracycline 30  Co-amoxiclav 3 | 28  18  27  30  25  50 | 28  18  25  30  22  15 |
| *M. catarrhalis* | Chloramphenicol 30 Erythromycin 15  Cefuroxime 30  Ciprofloxacin 5  Tetracycline 30  Co-amoxiclav 3 | 31  23  21  31  28  19 | 30  20  18  31  25  19 |

**Table 8:** Antibiotics tested for susceptibility in *S. aureus* using the agar incorporation method

|  |  |  |
| --- | --- | --- |
| Bacteria | Antibiotic used | MIC breakpoints used  (mg/L) |
| *S. aureus* | Amoxicillin,  Penicillin, Erythromycin, Vancomycin,  Fusidic acid, Chloramphenicol,  Rifampicin,  Tetracycline,  Ciprofloxacin,  Gentamicin,  Trimethoprim, Nitrofurantoin,  Teicoplanin,  Oxacillin | 0.125  0.125  1  2  1  8  0.06  1  2  4  64  2  0.125 |

  A picture containing table, indoor, kitchenware, cup

Description automatically generated

1. B. C.

**Figure 2‑3:** Methods used to determine antibiotic susceptibility

1. *Disc diffusion method: Discs impregnated with specific concentrations of antibiotic as specified in Table 7 were placed on pre-inoculated agar/blood agar plates. Zones of inhibition were measured in millimetres. Measurements were compared to the reference EUCAST zone diameter breakpoints for the bacterial species and antibiotic investigated. (Table 7).*
2. *E-test strip method: Plastic strips coated with predefined antibiotics concentrations were placed on pre-inoculated agar/blood agar plates. Elliptical inhibition zones indicate the MIC at the intersection point between the inhibition zone and edge of the strip.*
3. *Agar incorporation method: A predefined concentration of antibiotic was incorporated into agar plates. A standardised density of bacterial inoculum was prepared to give 104 colony-forming units per spot on the agar plate. If bacterial growth is inhibited (middle plate) then the bacteria is classified as susceptible to that antibiotic. If bacterial growth persists (bottom plate) in the presence of the known antibiotic concentration that determines resistance, the bacteria is classified as resistant. An antibiotic free agar plate is used as a control (top plate in diagram).*

### Genotypic resistance

Once phenotypic antibiotic resistance data had been obtained, the frozen bacterial isolates were transferred to the University of Sheffield (Professor Condliffe’s laboratory). I subcultured the bacterial isolates by plating them onto chocolate agar (*H. influenzae*), blood agar (*S. pneumoniae* and *M. catarrhalis*), or Brain Heart Infusion (BHI) agar (*S. aureus*) and incubating them overnight at 37°C in 5% CO2 (No CO2 for *S. aureus*). Between 30-50 colonies were sampled using a 10µl inoculation loop. These were suspended in 1ml of PBS and then the Qiagen DNeasy blood tissue protocol for DNA extraction was followed using the reagents from the supplied kit (appendix 5). In summary, gram-positive bacteria were lysed in an enzymatic lysis buffer for 2 hours. Proteinase K was added to remove protein contamination. Buffering conditions were adjusted to provide optimal DNA-binding conditions and the lysates loaded onto the DNeasy Mini Spin Columns. DNA binds selectively to the DNeasy membrane and contaminants were removed by centrifugation and washing steps. DNA was then eluted using 50µl of buffer AE provided in the DNA extraction kit. DNA extraction from *S. pneumoniae* isolates during practice runs resulted in minimal DNA being obtained when using an incubation time of 30 minutes. A protocol optimisation was required for processing of *S. pneumoniae*. The yield of DNA was optimised when the bacteria were incubated for 2 hours in the enzymatic lysis buffer rather than 30 minutes (as advised by Lucy Unwin, PhD student, University of Sheffield). This was thought to be due to the properties of the polysaccharide bacterial capsule and the cell wall.

DNA concentration and quality was assessed using a Nanodrop spectrophotometer. The ratio of the absorbance at 260 and 280nm was used to assess DNA purity with a ratio of ~1.8 being accepted as “pure” for DNA (Koetsier, 2019). The presence of RNA (pure RNA: A260/A280 ratio = 2.1) may lead to higher ratios and the presence contaminants such as proteins may lead to ratios of <1.6 (Koetsier, 2019). The DNA was then frozen at -80⁰C until further analysis. A selection of 40 *Streptococcus pneumoniae* DNAsamples that had been designated as ‘azithromycin-resistant’ by the microbiology laboratory were sent to the external company MicrobesNG for whole genome sequencing in order to identify any resistance genes involved. MicrobesNG required a minimum DNA concentration of 10 ng/µl in 30-100 µl. The selection was based on prioritising highly resistant isolates and where multiple isolates were obtained from the same participant.

### Whole genome sequencing at MicrobesNG

Whole genome sequencing was performed on the Illumina sequencing platform and sequenced using 2x250bp paired end reads.

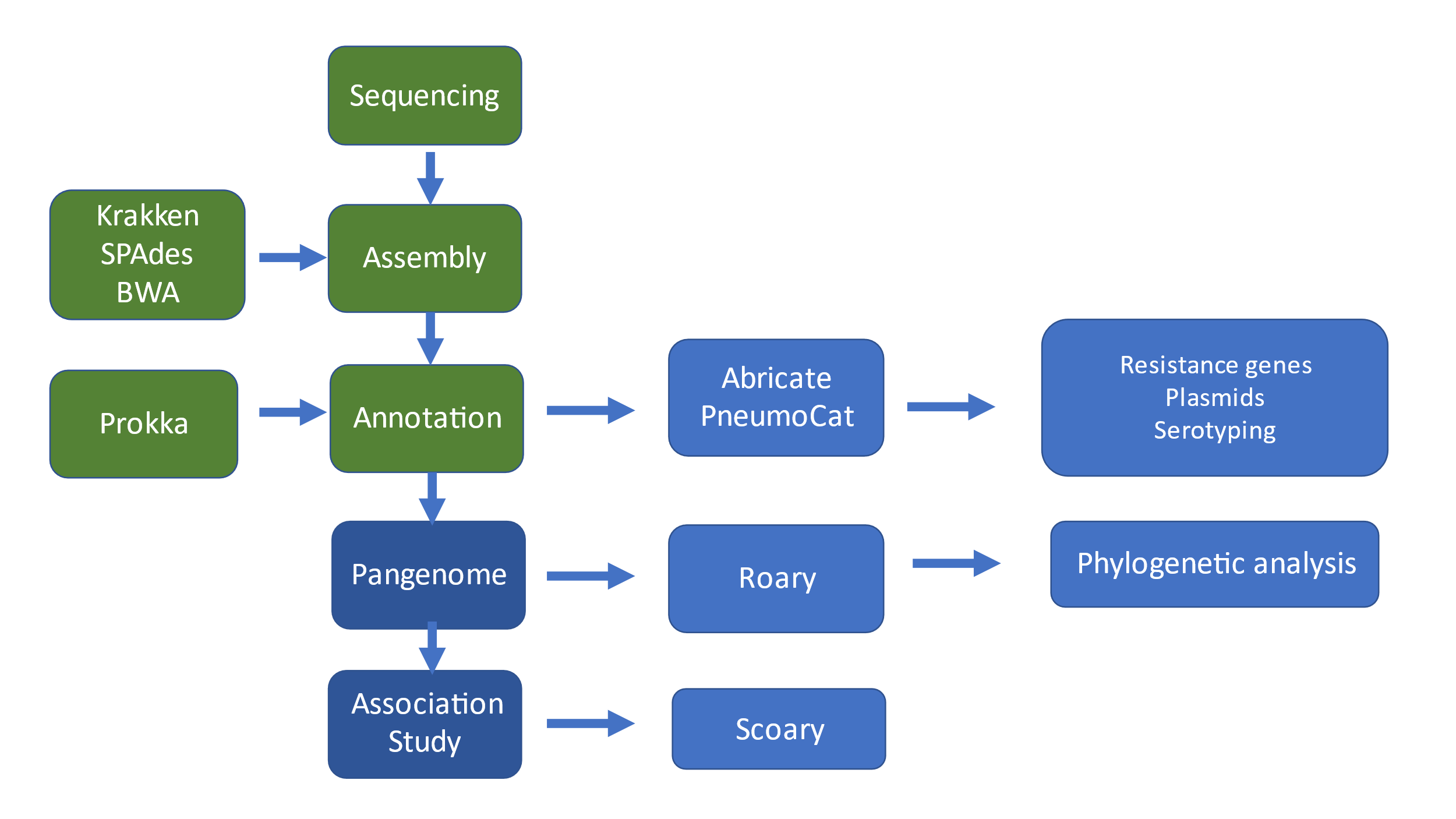
### Bioinformatics

An overview of the bioinformatics pipeline used is provided in Figure 2‑4. Assembly and annotation was performed by MicrobesNG using their standard pipeline. The closest identifiable reference genome was identified using the Kraken taxonomic sequence classification system. Reads were then mapped to the reference genome using Burrows-Wheeler Aligner (BWA) software and the quality of the data assessed. A de novo assembly of the reads was then performed using ‘SPAdes’ and the reads mapped back to the resultant contigs, again using BWA mem to get more quality metrics.

I then performed the subsequent bioinformatic analysis with guidance from postdoctoral researcher, Carlos Suligoy, department of Infection, Immunity and Cardiovascular Disease, University of Sheffield. Analysis was carried out using the Linux system Ubuntu and the following packages:

1. ‘abricate’ : To perform mass screening of contigs for resistance genes (NCBI and ResFinder databases) , plasmids (PlasmidFinder database) and SNPs mapping.
2. ‘pneumoCat’ : To identify the *pneumoncoccal* serotype.
3. ‘roary’ and ‘scoary’ : Utilised for pangenome studies.
4. ‘fastTree’: To create a tree file for use in the ‘interactive tree of life’ for phylogenetic tree construction.

In summary, coding instructions for the downloading and use of the packages were obtained through GitHub, Inc. This is an online collaborative platform that facilitates social coding and a web interface to open source, version-controlled projects such as the packages used in this analysis. The required sequence data files supplied by MicrobesNG, were inputted into each of the packages and the programme run. Interpretation of the output data obtained for the pangenome studies was assisted by Dr Carl Suligoy (PDRA in Dr Tom Darton’s laboratory).



**Figure 2‑4:** Pipeline used for the bioinformatic analysis of the S. pneumoniae sequence data.

The flow of the analysis starts with the sequencing and ends with the association study. A variety of programmes are used at each step. Green denotes steps carried out by MicrobesNG and blue shows the steps I performed.

# **Chapter 3. Results - The parental experience of having a child prescribed prophylactic antibiotics**

## Introduction

Parents must represent, advocate and make decisions regarding their child’s health until their child is old enough and competent to make such decisions for themselves. The degree of such responsibility may range from consenting to treatment and discussing management options through to developing ways of getting their child to take medication and remembering to do so. Parents will vary as to how much information and support they require from health care professionals when making decisions. Clinicians are in a unique position where, with good communication and insight, they can assist parents and ease the stress associated with having a sick child. There is a paucity of information about what parents think about antibiotic prophylaxis (see section 1.9.3). Once the prescription for antibiotics leaves the doctor’s hand, the story only just begins for the family. During outpatient appointments there is limited time to explore in depth the factors associated with long-term antibiotic use. This study therefore aimed to explore the parental experience of having a child prescribed prophylactic antibiotics. By doing so we hoped to facilitate clinicians in taking a holistic approach to prescribing and managing children on prophylactic antibiotics in general as well as specifically for respiratory conditions such as PBB.

Azithromycin is the antibiotic used first line at Sheffield Children’s hospital in the respiratory department for prevention of respiratory infections, and hence this antibiotic was chosen for investigation. Those prescribing antibiotics should consider antibiotic resistance and follow good antimicrobial stewardship practice. Factors within the family environment may contribute to resistance. These include adherence and availability of medication to comply with regimens which may influence the duration of the antibiotic course as well as appropriate antibiotic waste disposal. Given that parents take responsibility for their child’s health and ensuring they are given medication, this qualitative study was designed to complement and inform subsequent studies investigating the impact of prophylactic antibiotics on antimicrobial resistance and dysbiosis.

In order to develop a better understanding of how parents view the prescription of long-term antibiotics for their child, a qualitative interview-based study was undertaken. The results of this study have been published in full (Hardman, 2021). Due to a paucity of research investigating parental attitudes and perceptions in relation to having children prescribed long-term antibiotic prophylaxis, an interpretative phenomenological approach used. Phenomenology is a qualitative research method that takes a philosophical approach to explore peoples’ perceptions of a lived experience. The main philosophical principles underpinning this approach are (Turner, 2017):

1) Things happen (phenomenology).

2) We interpret this into something that makes sense to us (hermeneutics).

**I hypothesised** that psychosocial factors would influence parents’ decisions relating to the uptake and the way in which families experience long-term prophylactic azithromycin.

By conducting face-to-face interviews, I aimed to improve the way in which antibiotic prophylaxis is discussed with parents during medical consultations.

The **primary objectives** of this qualitative study were:

1) To understand what it means for parents to have a child prescribed long-term prophylactic azithromycin to prevent chest infections.

3) To develop a behavioural model to explain the parental experience of having a child prescribed long-term prophylactic antibiotics.

## Results

### Recruitment and participants

The recruitment process and methodology for this study are detailed in section 2.1. A total of 18 parents/guardians took part in the study through 15 conducted interviews. The consort diagram in Figure 3‑1 shows the recruitment process. All 56 parents meeting the inclusion criteria, who sequentially attended 4 specialist respiratory outpatient clinics at SCH, were approached by the respiratory nurses. Parents who did not speak English were not given the study information (appendix 3.1). The parent who cancelled their interview did not give a reason for the cancelation.

The mother of the child was interviewed on all occasions apart from one where the grandmother had parental responsibility. In 3 interviews the father or grandfather was present and contributed their views. The majority of parents were white British with a variety of different educational backgrounds which is representative of all the parents we approached. See Table 9 for participants’ characteristics.

56 parents approached during outpatient clinics

20 positive responses

15 interviews conducted involving 18 participants. 6 individuals were interviewed as couples

1 - Did not attend interview

1 - Cancelled interview

3 - Unable to commit to an interview time

**Figure 3‑1:** Consort diagram to show the recruitment process for the parental interview study

*Patients who fulfilled the inclusion criteria were given the study information. Of these, 20 responded to the text message sent 1-7 days after the study information had been given to families. A total of 15 interviews were finally conducted.*

**Table 9:** Participant demographics for those taking part in the parental interview study

|  |  |
| --- | --- |
| Demographics | N = Frequency  Total interviews = 15  Total participants = 18 |
| Participant interviewed | Mother = 14  Father = 2  Grandmother = 1  Grandfather = 1 |
| Age of interviewee (years) | 21-30 = 2  31-40 = 7  41-50 = 7  51-60 = 1  61-70 = 1 |
| Highest degree | None = 2  GCSE = 2  NVQ = 7  Higher degree = 7 |
| Employment status | Employed = 10 |
| Ethnicity | White British = 16  Arab (Libyan) = 2 |
| Mean Age of child (years) | 4 (Range: 2 – 6) |
| Mean duration on prophylaxis (months) | 11 (Range: 3-31)  12 = seasonal prophylaxis |
| Child’s diagnosis | Protracted bacterial bronchitis = 9  Bronchiectasis = 3  Hyper IgM = 1  Congenital tracheal stenosis – post operative = 1  Muscular dystrophy = 1 |

### Interviews

All interviews were face to face, semi-structured in nature and carried out by myself. Of these, six were carried out at Sheffield Children’s hospital and nine in the participant’s home. Each interview lasted approximately 30-70 minutes with the average time being 45 minutes. Parents seemed comfortable during the interviews and able to discuss their experiences freely.

### Findings

Through a process of thematic analysis (see Methods, section 2.1.7), the interview transcripts were coded and a framework of subthemes was iteratively developed. Three themes emerged that have given us a deeper understanding of factors influencing parental decision making, expectations and actions surrounding their use of prophylactic antibiotics to prevent respiratory tract infections. The main themes were decision making regarding prophylaxis, the context of prophylaxis within the family and their environment and response to acute illness whilst on prophylaxis. The themes, subthemes and codes are detailed in Table 10. Each theme is described in the following sections below. Pertinent quotes are used to illustrate the subthemes relating to the main themes during the analysis.

**Table 10:** Descriptive terms used for main themes, subthemes and codes from parental interviews

|  |  |  |
| --- | --- | --- |
| Main theme | Subtheme | code |
| **Decision making regarding prophylaxis** | Determinants of active involvement | Self-belief |
| Health literacy |
| Impact on parent |
| Passive Barriers | Social norms |
| Health beliefs |
| Environmental |
| Influencing factors | Perceptions of prophylaxis |
| Health beliefs |
| Impact on quality of life |
| Understanding of science |
| Balancing risk | Benefits |
| Present versus future |
| Concerns and side effects |
| **Context of prophylaxis within the family and their environment** | Normality | Establishing a routine |
| Barriers |
| Practicalities of medication | Preparedness |
| Administering issues |
| Waste and disposal |
| Adherence | Obstacles and difficulties |
| Initiatives |
| Impact on quality of life |
| **Response to acute illness whilst on prophylaxis** | Emotive reasoning | Immediate response |
| Previous experiences |
| Assessment | Threshold for consultation |
| Importance of specific symptoms |
| Driving pressures to take action |
| Management | Misconceptions of terminology and concepts |
| Expectations |
| Understanding of antibiotics |

#### Theme 1: Decision making regarding prophylaxis

Antibiotic prophylaxis with azithromycin was discussed with parents by paediatric respiratory or immunology specialists during outpatient clinics prior to commencement. Parents reported taking both active and passive roles when making decisions regarding the use of antibiotic prophylaxis. A variety of factors influenced how they balanced the perceived benefits and risks for their children.

***Determinants of active involvement:***

Those parents with strong feelings of being experts of their children felt they could fully engage, voice their concerns and question consultants. Subjectively this did not necessarily correlate with a high level of health literacy of the underlying condition or knowledge of potential risks of long term antibiotics. This was not however formally assessed. The limitations of my own medical gaze are discussed in more detail in section 3.3.4. Parents stated the importance of their parental responsibility when reflecting on why they felt they should actively take part in consultations. A desire to be more informed and increase their knowledge also drove active participation when considering management options.

*“We all help to decide. If we weren't doing our job as parents, keeping an eye on him, we wouldn't know he’s got that cold. So if they said let’s take him off (prophylaxis), and we said hold on a minute he’s had 4-5 (infections) in the past (month). If we thought that, they might refer their decision and say let’s keep it on for another couple of months.”* ***(Parent 10)***

*“I think people perceived doctors being that higher authority that sometimes can't be questioned but that is not how it is in society these days. Doctors are saying you are the expert on your illness and actually you're empowered to actually know what you are doing and look after your long term care.”* **(Parent 6)**

*“They (doctors) are qualified. They think they know best. But they don't. They really don't… They don't go through the nights of the child not sleeping because they can’t breathe, the child struggling, they do not know… they might know on a medical concept.”* ***(Parent 8)***

***Passive barriers:***

Parents who described taking a passive role in decision making, and trusting the professional to act in their child’s best interest, said that they did not feel fully informed. Environmental barriers identified by these parents included chaotic consultation rooms, time pressures and poor eye contact due to health care professionals looking at computer screens. Social norms of politeness, seeing the doctor as a higher authority who could not be questioned and not wanting to waste the doctors time led parents to take a more passive role. Not wanting to be seen or being made to feel like a “drama queen” or “paranoid overdramatic mother” also reinforced passivity. Some parents reported they were unable to understand jargon or key medical concepts. This in turn led to more passive behaviour in order to avoid feeling self-conscious and to cover their confusion. These barriers led to unvoiced concerns, excessive worry and feelings of a lack of control which in turn meant parents required increased support from health care professionals.

*“...the doctor tells me what the plan is and as a parent I just go along and think he's the doctor, he knows best... and you just take his word for it because he is the professional and I’m just a parent. There’s no guidebook for (parents)... And I think me being me and asking questions, I'm holding that doctor up. I don’t' want to bother him. But it isn’t till when I come home and I think, is it doing him any good (prophylaxis)? Is it harming him? Is it going to cause him any long term (side effects) ... and it’s not till after till I start think about things.”* ***(Parent 9)***

*“I don't want to seem like I’m a paranoid mum, but I feel she has taken a turn for the worst when she is coughing and being sick.”* ***(Parent 2)***

*” I go into clinic and they are sat at the computer and they are typing away or writing and it is very rare that they even give you eye contact. And you think they are just under so much pressure and you are just another body. That is how I feel. I just think I’m just another body, with another poorly child.”* ***(Parent 9)***

***Transition from passive to* active *involvement:***

Interestingly three parents described becoming more active with regards to decision making once prophylactic antibiotics were discussed in the consultation. Reasons for this transition included the perceived significant risks of long-term antibiotics, a desire to improve their knowledge given an increase in the severity of the situation and frustration at feeling uninformed. This process of transition to active involvement was also promoted when parents felt that their lives were being affected e.g. challenging pharmacists and doctors when there was “hassle” in obtaining prescriptions and medication. Some parents said they felt empowered with more knowledge.

*“We’re in such a position now that we just constantly ask questions. Not to be difficult but just because we want to know why. So we had big questions about long term antibiotic use. You know you hear a lot in the press about the use of antibiotics and so we did have a lot of questions regarding that but it was us asking rather than people proactively telling us anything.”* ***(Parent 1)***

***Influencing factors relating to the use of antibiotic prophylaxis:***

All parents either hoped or expected that starting antibiotic prophylaxis would reduce the number of respiratory tract infections, improve their child’s health and bring normality to their lives once more. The fear of a common cold progressing to a hospital admission and potential death or lung damage was a recurrent concern. The majority of parents felt that their child’s condition was detrimental not only to their child’s quality of life but that of the family. This was due to the distress of ill health and time spent in hospital as well as creating a hesitancy for spontaneity, moving further from the hospital or progression with their own careers.

Parents talked about how their own health beliefs of antibiotic use sometimes conflicted with a decision to start long term antibiotics. Many said they themselves had rarely required antibiotics and just got over illnesses whilst others had more alternative medical preferences.

*“We won’t’ go on holiday because we are fearful that it is going to be another hospital admission and we don’t want to be in a strange place in a strange hospital.* ***(Parent 8)***

*“I was brought up with homeopathy and it always worked… I hate antibiotics because I was brought up to hate them. But they have worked so I was grateful, but very uncomfortable with him having to be on them****.” (Parent 4)***

Parents’ understanding of science was another contributing factor to their decision making. They reported their children as high antibiotic users and had a skewed sense of normality with short courses of antibiotics lasting two to four weeks. The majority considered antibiotic resistance when deciding on antibiotic prophylaxis for their child. The problem of resistance was not seen as an immediate threat for their child rather as a problem for when they were years older.

*“You just don’t' want him to get something really bad when he is older and nothing works.”* ***(Parent 7)***

*“I mean I were brought up with you got antibiotics for pretty much anything. But I know nowadays they don't like it cos as you get older, not so much now, when you are older and you really do need them and you are quite vulnerable. If you have them too often you are eventually immune to it. They don't work anymore*.” **(Parent 12)**

All parents reported having seen or heard media campaigns challenging the use of inappropriate antibiotics and increasing awareness of the problem of antibiotic resistance. Many were influenced by these messages and said they took them into consideration when thinking about antibiotic prophylaxis. Some parents however reported feeling in a state of conflict when reading these public health messages. They felt that in their child’s circumstance, antibiotics were actually necessary.

*“It’s just a bit of scaremongering. It goes whichever way it wants to go, media in my opinion. I go with what the professionals say… They (the media) play it down a bit like antibiotics won't cure your cold and stuff. But for us it’s not a case of use. We're not abusing antibiotics. It’s quite clearly she’s dependent upon them at the moment so… We have to go with the specialists.”* ***(Parent 2)***

The final influencing factor was that of side effects related to azithromycin. The majority of parents were unable to recall knowing about side effects other than resistance in relation to antibiotic use. Some reported they wanted to avoid knowing about potential side effects stating it would make them worry more. The majority felt more knowledge would enable them to observe for potential complications and reduce their worries.

***Balancing risk:***

Most parents reported that they felt all other treatment options had been exhausted and only the option of antibiotic prophylaxis was left. They voiced concerns that their children were always unwell and this was distressing for the whole family. Pressures relating to poor school attendance and competing demands from siblings and employers, due to multiple hospital admissions, contributed to their decision-making process.

The parents’ perceived severity of their child’s condition did not always match that of the doctor at both ends of the spectrum. Some parents did not see prophylaxis as necessary despite their child having significant bronchiectasis (where evidence exists for prophylaxis) whereas parents of children without bronchiectasis, but recurrent episodes of wet cough, occasionally requested or demanded prophylaxis. This was sometimes reported to create a source of tension between parents and health care professionals.

The majority of parents’ said their main concern was the antibiotics no longer working in the future and uncertainty about treatment options at that point. Parents did not report concerns about other potential side effects influencing their decisions. The desire for their child to be well and have a better quality of life at that moment in time was overwhelmingly the most significant factor when deciding whether to trial prophylactic antibiotics. Concerns about reducing the risk of long term lung damage was mentioned by one parent.

*“I was a bit worried about how long is it going to be before they (prophylactic antibiotics) stop working. Because I know your body builds up a resistance towards them. But apart from that no… I just thought about him being well now. I mean in years to come there might be more antibiotics available or there might be something else out there. But here and now I just wanted him to be as well as he can be. Like he were only at school 50% of the time. So he were behind on school work and stuff like that. So I thought that it were the right thing to do to start him on the antibiotics now. Especially after last year. It were horrific how poorly he were.”* ***(Parent 13)***

#### Theme 2: The context of prophylaxis within the family and their environment

Parents described various ways in which they normalised their lives incorporating their child’s antibiotic prophylaxis. They reported fears of forgetting to administer antibiotics and anxiety about attending crowded places and exposure to possible infective sources resulting in isolation. Others said they didn’t want their children to feel as though they were different. Combined with the opinion of most parents, who thought prophylaxis was important and beneficial in reducing illnesses, these factors drove parents to strive for normality.

***Establishing a routine:***

Establishing a routine was the main way of creating a sense of normality. This practice allayed negative emotions about forgetting medication and inadvertently putting their child’s health at risk. Visual cues with the use of diaries, position of antibiotic bottles and star charts were reported to be helpful by most with some parents incorporating an educational element into colour matching and timetable reading. Electronic devices were used as alarms but also audio cues from virtual assistants to remind family members at home when the main care-giver was not present. A number of barriers were cited that had to be overcome including remembering during school holidays when the established routine was interrupted, busy morning routines, ensuring timely collection of prescriptions and avoiding confusion between different care-givers as to whether medication had been given.

*“I was given this bag of medicines and it was you need to give it to him Monday Wednesday Friday and it freaked me out to be honest... I were terrified of forgetting. What if I forget just one day? He’s going to get ill. If I don’t give it to him on time, what if I give them to him late, will that make any difference to him? Could he get an infection because I've given him his antibiotics in the afternoon and not the morning?”* ***(Parent 5)***

***Practicalities of medication, prescriptions and adherence:***

The vast majority of parents managed to administer the antibiotics without causing their child much distress. Ways in which they had established this routine of medicating were through non-negotiable administration, bribes, changing antibiotic brand to one with an acceptable taste and with time, and in almost all cases any initial behavioural issues resolved. A few parents admitted it was easy to forget to give the antibiotics due to the interrupted regimen of Monday, Wednesday and Friday. Adherence was reported to be extremely good in those who perceived the prophylaxis to be vital. If acute exacerbations became less frequent, complacency sometimes ensued. Hearing their child cough or wheeze and noticing lots of bottles of antibiotics in the cupboard prompted them to administer the prophylaxis more regularly. Most parents however reported only missing a few doses.

Azithromycin suspension is not stable beyond 7-10 days. For this reason, parents are asked to reconstitute the powdered antibiotic themselves at home. This negates the need to attend the pharmacy weekly for further bottles of suspension. Many parents were not expecting to reconstitute the antibiotics themselves at home and did not feel prepared for this. Some felt a demonstration would be better than written information to aid this process. This would avoid poor mixing resulting in unreliable dosing. Many parents had experienced difficulties with obtaining prescriptions in a timely fashion. They could not give the antibiotics if they were not available. During 2 interviews, guardians said that prescription difficulties influenced their decision to ask if the prophylaxis could be stopped. Many were concerned about the amount of antibiotic wasted as the bottles were never finished. They did not know how to dispose of unused antibiotics with many pouring them down the sink or toilet.

*“So sometimes it sounds horrible but it’s easy to forget it. I mean you go a week and actually no he’s not had it… Yeah we do find that he will start to be more unwell. Like we notice he just gets a bit more wheeze especially at night. Have we done it, have you done it? No, ahh that might be why. Whether It is the case of it being or not, but it has been we have forgotten to give it.”* ***(Parent 6)***

*“The consultant explained it very well, what it was for, what they hoped it would do, and he would stop it, and what the results were of other children who are on it…. That was very good. It was literally I had no idea it was a powder and I had to make it so that was a bit of a surprise.”* ***(Parent 14)***

*“The new antibiotics only last 5 days. You have to discard whatever is left. Cos it is made up into 15 (ml) and obviously she has 3.5 (ml) so that is like 10.5 (ml). I'm wasting antibiotics every week, which ... That is NHS money… (Interviewer: What do you do with the antibiotics?) Well I just have to flush it away. We don't know how to dispose of it really.”* ***(Parent 15)***

Parents were split with regards to how the prescription of prophylactic antibiotics impacted on their child’s quality of life. Most however felt that they were beneficial in reducing either infections, hospital admissions or improved school/ nursery attendance. Some parents reported that they previously isolated their child from situations of potential infection risk such as supermarkets, public transport and exposure to other children whether at nursery, school or other activities. They viewed antibiotic prophylaxis as a protective measure which enabled their child to take part in more activities with less restrictions and more freedom. A few parents described prophylaxis as a reminder that their child was susceptible and needed to be isolated or their activities restricted i.e., swimming and soft play. Others did not restrict their child whether they were taking prophylaxis or not.

*“On a personal side of things it were like, thank God we don't have to do that now (administer prophylaxis). It were that mental is it Monday, Wednesday when you are going through your daily check and things like that. It’s a case of that little bit of relief now.”****(Parent 10)***

*“Like I said with the ball pools and things like that. If he wasn't on those antibiotics, he wouldn't set foot in the place. I'd be too scared of him picking something up and ending up in hospital for a fortnight. It’s just, while ever he's on these antibiotics, I’ve got that little peace of mind that they are protecting him. But if he came off them I’d think that would stop and the only place he'd go would be the garden. Confined to the garden again.”* ***(Parent 5)***

*“She now does swimming lessons whereas I couldn't let her do that before because I'd be worried in case she picked more bugs up and that.”* ***Parent 3***

“*No not at all. I throw her in at everything. When we are outside, she can do mucky play. We've got horses... I don't treat her any differently (being on prophylaxis)”* **Parent 2**

#### Theme 3: Response to acute illness whilst on prophylaxis

Despite taking prophylactic azithromycin, almost all children had break through acute respiratory illnesses. Some of these were viral upper respiratory tract infections and required only supportive treatment. For other episodes, antibiotics were prescribed and occasionally children were admitted to hospital. Discussions with parents relating to ways in which these episodes are assessed, diagnosed and managed by both parents and health care professionals uncovered practical difficulties and parental anxieties leading to a cycle of expectations. This cycle potentially influences the perceived necessity for prophylaxis by hospital physicians.

***Immediate response***

There was often an initial emotive response when parents heard their child starting to cough. This evoked fears that the illness was going to progress to a hospital admission again causing stresses for everyone. Previous experiences of severe episodes were recalled and there was a nervousness that the prophylactic antibiotics weren’t working anymore. The intensity of these feelings often waned the longer children were on prophylaxis especially if the duration between episodes increased the severity of the acute respiratory illnesses reduced.

*“Back to chest infections and in and out of hospital. It’s a destruction of her life that she doesn't need. Erm.. so a common cold can quite easily, within a matter of days, turn into a severe chest infection... erm I don't know it is just stress. Stress for her, stress for me and progression from normal common colds to chest infections. It is just so drastic.”* ***(Parent 8)***

*“Even if he just does 2 coughs in his sleep, you go, here we go again. You know and I try and put it into perspective because I think well… We always get through it... So yeah when he starts with a cough, I’m just thinking.. right... sigh….* *I always expect the worse. We'll end up in hospital he’ll go onto oxygen and then have a week or so in hospital…I feel sorry for him.”* ***(Parent 1)***

***Assessment and symptoms***

Many parents described the following concerning symptoms that would lead them to get their child assessed: Fever, green sputum/ nasal discharge, uncontrolled crying with pain, signs of respiratory distress and pus on tonsils. Parents often described a feeling of helplessness and futility of self-help measures they tried such as over-the-counter analgesics and physio techniques. They felt that these factors and the fear of progression drove them to “take action” and that the symptoms were not just attributable to a common cold. Maternal instinct was also reported to be a key factor in deciding whether to consultant a health care professional.

A few parents had different recollection, recounting how their children coughed but seemed well with no fevers and continued to run around. Combined with their health beliefs of trying to minimise antibiotic exposure they often waited longer to present to medical services. The parental perception of their child’s susceptibility to infection and severity of the underlying condition did not always equate to that of the health care professional and underlying diagnosis.

Parents of 2 children with bronchiectasis had higher thresholds for seeking medical attention and isolated their children less, despite the more severe underlying diagnosis.

Deciding on the threshold for consulting a health care professional during an acute illness was found by many parents to be difficult and stressful. As well as looking for clinical cues they also reported social norms and logistical barriers to consulting. These included, “Not wanting to waste people’s time”, tackling the systems for making GP appointments as well changing their own schedules to make time for their child’s appointment. With experience and in 2 cases the respiratory team telling parents to reduce their threshold for seeking help, these parents felt they were improving their ability to assess their child.

*“(giving acute courses of antibiotics) you feel like you are doing something. I mean when kids get poorly you feel pretty helpless. Cough syrups don’t' work. You can't give them decongestants. You can't give them cold remedies because there isn't any for kids. Not that age. So you can pretty much just give them paracetamol and hope it works.”* ***(Parent 12)***

*“But a lot of the time it is viral and so there isn’t'.. And I know that antibiotics won't help but you feel like you are doing something. And I know that.. but as a parent you want to be doing something. And so sitting back and saying, oh yeah its bacterial (viral). But even when you're in hospital a lot of the time they start the course before they know.”* ***(Parent 1)***

*“Initially we were told, when she has a temperature that might be a sign that she has a worse infection so go and get her checked out. She gets temperatures less times than not. So that is not a very good gauge for us. And then we were told if her cough is lasting for a couple of weeks, go and get it checked out. And then we were told actually 2 weeks is too long to be waiting. So because of our not wanting her to be on antibiotics too much, coupled with her not getting high temperatures, we haven’t been going and getting extra antibiotics that much. So, I think that is why we have been told, we need to lower our threshold… So I guess the more it happens, the more I’m getting to know when she might need to have some extra help and when not.”* ***(Parent 11)***

***Management of acute episodes***

Parents described conflicting experiences in different health care settings which they thought were confusing and caused them significant distress and frustration. The cause of these conflicts often related to when it was felt the respiratory consultant plan was not followed and antibiotics not prescribed by other health care professionals.

Parental understanding of science relating to antibiotics and infections played a role in determining their expectations for antibiotics to be prescribed during acute respiratory illnesses. All parents stated that antibiotics should not be used for the common cold. Despite some knowing viruses caused a common cold, the term “infection” was often misinterpreted as being severe and requiring antibiotics.

*“You can get other bacterial infections like hand foot and mouth, that was going round school and I kept him at home. Not today, you can stop at home…They ask people not to take antibiotics for a cold and things like that because they don't work… there’s nothing you can do its gonna run its course unless it does change and you get an infection then you can have some antibiotics.”* ***(Parent 5)***

*“There’s quite a few bugs out there like viruses and stuff that can be beaten by the human body but obviously when it’s an infection it needs treating by antibiotics.”* ***(Parent 2)***

Confusion unsurprisingly arose when they were told by health care professionals their child had a virus but were then given antibiotics for the viral infection. Inconsistent messages and management led to future confusion and expectations related to antibiotic prescribing.

Some parents reported antibiotics were started despite diagnosing a viral infection due to the child’s background history. When antibiotics were prescribed “just in case”, this reinforced the severity of the situation and again started a cycle of expectations for further courses to be prescribed with future episodes.

In some cases, especially if antibiotics were not prescribed, parents felt they had either wasted the doctor’s time and been unable to assess their own child properly or became frustrated that the perceived consultant plan for antibiotics was not followed. These issues led to feelings of inadequacy, poor parenting or anger directed at the assessing doctor or at the seemingly poor communication between teams. Only 1 parent stated they would demand antibiotics and seek advice elsewhere if antibiotics were not prescribed.

## Discussion

### Main findings

The overriding factor influencing parental decisions about the uptake of antibiotic prophylaxis, is wanting their child to be well now. Factors such as quality of life, social circumstances and health literacy contribute to this decision-making process. The main concern voiced by most parents is that of antibiotic resistance. They accept that their children are at risk due to their high antibiotic usage but antibiotic resistance is usually seen to be a problem of the future not the present.

By reflecting on the main themes and subthemes that emerged from the interviews, I was able to further condense these into key concepts to describe the parental experience of having a child prescribed prophylactic antibiotics. I incorporated the concepts into a diagram through consideration of how they linked to each other. Through an iterative process and in discussion with Andrew Lee (Professor of Public Health, School of Health and Related Research, University of Sheffield), who has a particular interest in qualitative research, my initial linear model developed into a cycle of phases given the fact that many children are prescribed long-term prophylactic antibiotics for 6-months of every year. I then developed key words to represent the underlying concepts in discussion with Andrew Lee. We propose the following behavioural model to describe phases parents cycle through once their child is prescribed antibiotic prophylaxis (Figure 3‑2). The model illustrates how the key themes inter-relate. An awareness of this cycle may help clinicians prepare families for prophylaxis, pre-empt potential difficulties and focus on areas to improve the family’s experience (see appendix 6 for further explanation of the phases in the model).

* Assessment threshold reflection
* Consider parenting response
* Adherence: Reinforced
* Rationalise fears and concerns

**Antibiotic prophylaxis discussed**

* Balances risks and benefits
* Acceptance of outcome
* Preparation
* Emotive 🡪Objective
* Overcome barriers: Practical and social norms
* Drive to take action
* Normalisation through routine
* Security 🡪 Reassess life-style restrictions
* Adherence promotes importance or complacency
* Rationalisation – Increases expectations

**Figure 3‑2:** A proposed behavioural model to explain the cycle of phases parents experience when their child is prescribed prophylactic antibiotics. Adapted from (Hardman et al., 2021)

*Proposed behavioural model to explain the cycle of phases parents experience when their child is prescribed prophylactic antibiotics to prevent recurrent respiratory tract infections or progression of bronchiectasis.*

* *Phase 1: Parents go through a decision making process to commence prophylaxis and prepare for long-term antibiotics.*
* *Phase 2: A new normality is created incorporating prophylactic antibiotics into the family routine. The outcome of which varies with regards to restricting or promoting childhood activities and medication adherence.*
* *Phase 3: Most families cycle through a disruptive stage of acute illness. Parent must assess their child and decide when the threshold has been met to seek medical attention.*
* *Phase 4: After an acute illness, parents reflect on their parenting response and outcome of the healthcare assessments. These experiences may influence their assessment and expectation of future illnesses as well as reflecting on the efficacy of antibiotic prophylaxis. Parents re-evaluate their decisions.*

The proposed behavioural model was presented to 5 parents whose children have been prescribed prophylactic azithromycin to prevent respiratory exacerbations of PBB. Only one of these parents took part in the original study. Informal feedback from these parents was that the model represented their experiences accurately and they were interested and relieved to know that other parents were experiencing similar things. Their focus was predominantly on concerns around wanting their child to be well now, antibiotics not working in the future, anxieties about hearing their children cough, limited self-help measures and the difficulties in determining the threshold to get their child assessed by a medical professional during episodes of acute illness.

### Findings of the parental experience in relation to current literature

Parents have the responsibility of making medical decisions on behalf of their children, provided the treatment is in the interests of the child; competent children can input these decisions. Those parents actively participating in decisions are reported to have a feeling of security, be convinced that the treatment prescribed is correct and have a sense of control over the situation (Aarthun et al., 2018). In comparison, those passively taking part in decisions are noted to have post consultation decisional conflict and feel powerless and insecure (Boland et al., 2017). Such feelings were experienced by parents in our cohort when describing their involvement in consultations. Parents may not always want to be actively involved in decisions with regards to their child’s health. Their desire to be involved may change with time, their knowledge and understanding, the importance of the decision being made and their emotional response (Boland et al., 2017). Actively involved parents, supported by health care professionals who acknowledge importance of their contributions, have a positive experience and are more likely to become involved in future decisions (Aarthun et al., 2018).

Some parents did not feel fully prepared in practical terms for undertaking long-term antibiotic prophylaxis. Key problems identified related to reconstituting antibiotics, obtaining prescriptions and antibiotic disposal. Ensuring parents are prepared for prophylaxis is important. This requires that the practical aspects of medication are covered, and ways of medication normalisation are discussed. The aims are to facilitate adherence, acceptance and reduce anxiety. Unintentional effects of prophylaxis should be considered. Paradoxically, parental anxiety may increase with prophylaxis promoting a sense of susceptibility to infections resulting in childhood activities being further restricted. Preparatory counseling could be undertaken by a variety of health care professional including pharmacists.

Many parents poured the surplus antibiotics into the wastewater systems. This problem likely relates in part to the short shelf-life of the reconstituted liquid medication, and the fact that the formulation is tailored to adults who usually require a larger dose than young children. Safe medication disposal is important to reduce environmental contamination as well as reducing the risk of inappropriate ingestion. It is however reported that there is a lack of awareness amongst health care professionals and patients with regards to proper medication disposal (Kinrys et al., 2018). Urban wastewater plants have previously been found to be potential hotspots for the dissemination of antibiotic resistant bacteria and genes (Rizzo et al., 2013). Antibiotic resistance patterns in European wastewater treatment plants are reported to mirror that seen in clinics (Pärnänen et al., 2019). Although azithromycin is incompletely absorbed resulting in faecal excretion, safe disposal practices and return of unused antibiotics to pharmacies should be recommended.

In the setting of chronic illness, normalisation involves the efforts family members make to create a normal family life, incorporating how they perceive the consequences of these efforts and the meaning(s) they attribute to them. In a study of children with chronic genetic disease, Knafl et al. assigned parents to 2 separate groups: Normalisation Present (NP) or Normalisation Absent (NA). Parents in the NP group regarded normalisation as an important achievement, indicating that their family was doing a good job of adapting to the child's condition. In contrast, the absence of normalisation was associated with the perception that family life was disrupted or difficult and was associated with feelings of parental inadequacy (Knafl et al., 2010). In our study, establishing a routine of antibiotic administration within the family life was the main way of normalising prophylaxis. Families used a variety of different cues and reminders to facilitate this with most parents managing to administer the medication more easily once this normality had been achieved. In agreement with Knafi et al., normalising prophylaxis was important for parents, and they did not want their children to feel different. A sense of security and reassurance did follow this process with the majority of parents feeling that the prophylaxis was beneficial. Most parents said they no longer needed to isolate their child or restrict their activities due to the perceived protection of antibiotic prophylaxis.

Adherence to a medication regimen is generally defined as the degree to which the medications taken reflect the prescriber’s intention. Treatment adherence is challenging and a complex balancing act between competing concerns including parental beliefs, child resistance, preserving family relationships and promoting ”normal life” for the family (Santer et al., 2014). Poor adherence to medication regimens is known to be challenging for those with chronic diseases. Remembering to give medication has been reported to be one of the biggest challenges (Aston et al., 2019), something that was mentioned by our cohort of patients. The thrice weekly regimen was not thought by most parents, to contribute to difficulties in remembering to give medication once a routine had been established. Similar factors influenced adherence of older children including adolescents with preventative inhalers for asthma, including remembering to take the medication (establishing a routine), as well as difficulty obtaining prescriptions in a timely manner (De Simoni et al., 2017). Non-adherence with antibiotic prophylaxis in childhood respiratory disease may risk unnecessary treatment changes for presumed antibiotic failure, additional ‘rescue’ antibiotics, worsening of the underlying condition due to acute and ongoing chronic infections and emergence of resistant organisms, hence understanding the complex interplay of factors that determine adherence in this setting warrants further study.

As with other studies, we found the parental perception of threat from an acute respiratory illness to their child was influenced by perceived illness severity, child’s susceptibility and illness information as reported by Ingram et al. They found parents felt uncertain about identifying and interpreting their child’s symptoms and that it was safer to consult especially if sanctioned by friends or family (Ingram et al., 2013). Parental knowledge, understanding of their child’s condition and the healthcare system significantly impacts on decision-making (Boland et al., 2017). A recent household survey found that the majority (83% (1404/1691) of respondents) of the public recognised antibiotics kill or treat bacterial infections, however a significant minority (35% (592/1691) thought that antibiotics kill or treat viruses (McNulty et al., 2019). McNulty and colleagues also noted that more knowledge did not necessarily translate into better antibiotic practices. Clearer explanations about antibiotic indications in terms of signs and symptoms and clarification of the severity of bacterial and viral infections have been suggested would help reduce misconceptions about when antibiotics are required and not (Cabral et al., 2016). Our finding of a parental misconception of the word “infection” corroborates this literature.

Our study echoes findings previously reported of ongoing misconceptions relating to resistance, i.e. the body rather than the pathogen becoming resistant (Van Hecke et al., 2019) and confusion between viral and bacterial infections and hence the need for antibiotics (Cabral et al., 2016; Halls et al., 2017). Previous reports suggest parents perceiving their children as low antibiotic users do not see antibiotic resistance as an issue for their children (Van Hecke et al., 2019). However, parents in our cohort see their children as high users of antibiotics, who are at risk of resistance (albeit in the future) and weighed this factor when making decisions about prophylaxis. This was not reported in the only previous study looking at perceptions of parents on an oncology unit (Diorio et al., 2012) and is a novel observation.

It is hoped that the proposed behavioural model will give health care professionals a better understanding of the challenges parents experience and allow help them to support families through the cycle of different phases outlined. Of note it has been developed specifically in the context of prophylaxis for respiratory infection, and further studies would be needed to see if it could be adapted for other settings in which antibiotic prophylaxis may be required (examples in Tables 1-3).

### Implications for clinical practice

It is important to consider the potential practical applications of the above findings. When making decisions regarding prophylaxis, clinicians should be mindful of passive parents with potentially unvoiced concerns. Breaking down barriers that dissuade parents’ participation may help a transition to more active involvement. This includes acknowledging parents as experts in their own right, irrespective of heath literacy, focusing eye contact on parents rather than computer screens, providing adequate information including practical aspects such as antibiotic reconstitution and disposal, and addressing social norms. This preparation and counselling could be undertaken by a variety of health care professionals such as pharmacists when dispensing medication and facilitated through written material. Discussing normalisation and the importance of routine may mean stability is achieved sooner. Additional advantages of active involvement are feelings of control, security and confidence in the treatment. However, not all parents will want to actively take part in all decisions and this must also be respected.

During the stability phase, clinicians should be mindful that adherence may decline if the number of respiratory tract infections reduces. Addressing this when collecting repeat prescriptions might be possible as well as during consultations. The efficacy of prophylaxis may need to be considered not only in terms of the number of clinical exacerbations. If prophylaxis is seen as a protective barrier, the threshold for seeking medical attention for symptoms may be raised. This potentially influences the need for ongoing prophylaxis.

Discussing a threshold for seeking medical assessment during acute illnesses would be helpful for parents. By doing so, it will enable parents to feel they are seeking assessment at an appropriate time, alleviate anxieties and through good communication with GPs, raise awareness of suggested management plans. Discussing thresholds before the event may mean a cycle of expectations for further antibiotics, based on previous experiences, can be addressed.

The interviews showed parents often had a misunderstanding of the term infection implying the need for antibiotics. Clear and consistent messages from health care professionals may help reduce any confusion.

### Limitations and strengths

Using interpretive phenomenology has the strengths of providing detailed insight into participants’ experiences and allowing them to freely explain this. This methodology has however been criticised as the experiences explored are not witnessed by the researcher but rely on accounts from the participant being interviewed. It has been questioned whether the participant and researcher have the necessary communication and language skills to communicate the nuances of the experiences (Tuffour, 2017). All parents in the study had had a lot of contact with health care professionals having had children referred to hospital services and been prescribed prophylactic antibiotics. Knowing I was a doctor may have influenced how they responded. This could have been in terms of the aspects they thought I would deem important, or they themselves slipping into a medicalised role that they use when discussing issues with health care professionals. A non-medical interviewer may have focussed and guided the interviews into other areas of their lives. Additionally, the site of the interview could have influenced how comfortable parents felt talking to me. Those interviewed at home in their kitchen or front room with a cup of tea seemed more relaxed. The hospital locations were more formal. Although only rooms in non-clinical areas were used, this environment may have reinforced feelings of a doctor parent interview rather than an informal conversation.

My training to carry out the interviews was undertaken by Dr Andrew Lee, an expert in qualitative research. I did not have prior experience of interviewing apart from my clinical medical consultation skills. Through the use of open questions I allowed parents to tell me about important factors for them. These I followed up in further detail with more closed questions. It is likely that my competence increased during the study, allowing me to gather richer and more balanced views with regards to factors influencing parents’ behaviour. Despite this, the use of a single rather than multiple interviewers enhanced the consistency of the interviews and was helpful in harmonising the outcomes. The ability to run interviews flexibly in terms of location timing may have helped recruitment and the interview duration (average of 45 minutes) was flexible, giving parents enough time to consider and describe their experiences.

Finally, Interpretation of the interview could also have been influenced by my medical background, particularly as I aimed to disseminate my findings to the medical community. As such, the terminology I have used for coding and developing the themes is quite ‘medical’. Choosing words used by the parents may have better reflected their expressions, however it is harder to be concise and the terms to describe similar concepts differed widely between parents. Terms such as ‘health literacy’ and ‘understanding of science’ may indicate that I have made a judgement on the basis of my medial gaze. I have not used any objective criteria to assess the health literacy of a parent but instead have used my medical experience. This may have biased my interpretation of how they described events. Again I have interpreted a parents understanding of science from a position of specialist knowledge, without objective measures to determine how much understanding they have other than my opinion.

It is important to consider the sample size - how many qualitative interviews are enough to answer the research question? This is somewhat empirical, but in the literature, it is usually addressed using the concept of ‘data saturation’ - the point in data collection/analysis when little or no new information is added by additional interviews or other data collection methods (Guest et al., 2006). These researchers conducted a stepwise analysis of 60 interviews of female sex workers in West Africa and discovered that 70% of all 114 identified themes turned up in the first six interviews, and 92% were identified within the first 12 interviews. Other researchers reported similar findings (for example, (Francis et al., 2010; Namey et al., 2016). However, such generalisations have been challenged and a number of mathematical models developed and published for estimating required sample sizes for qualitative research (for example (Tran et al., 2017); however on pragmatic grounds I recruited to a sample size of 18 and this enabled 15 interviews to occur. In keeping with the above conceptual framework, no new themes emerged after the first 12-13 interviews.

The cohort of parents interviewed in this study does not fully represent the diversity of families that are seen in the children’s outpatient department (the majority were Caucasian). They do however represent the total group of parents approached to take part in the interviews. Only three male parents and one non-white British family were interviewed. There was inadequate funding to use interpretation services and hence only families who spoke English were approached. This will have reduced the ethnic diversity of the potential participants who could enrol in the study. Further research is needed to explore the ethnic and cultural dimensions that may influence the parent’s experience. Widening the cultural diversity might have had implications for the required sample size, but this would be important for future studies. The parental experience may also differ where antibiotics are prescribed with multiple other medications, for example, as occurs in patients with cystic fibrosis; again, widening participation might require a larger total sample size to ensure saturation.

The parents we interviewed may also be a self-selecting group who chose to take part for specific reasons such as voicing concerns or opinions. Parents who did not want to participate may potentially be different and have other diverse experiences. Other possible bias includes parental recall of non-recent consultations and under or over-reporting of experiences to either please the interviewer or for fear of being judged. A further factor is that purposive sampling has inherent risks of introducing bias and open to poor judgment from the researcher (Peat, 2018)**.** In order to mitigate this potential bias, parents of all children sequentially attending 4 respiratory outpatient clinics were invited by the respiratory nurses to participate. Thus my judgement did not eliminate any potential participants.

Throughout this chapter I have described parental perceptions towards prophylactic antibiotics, factors that influence the decisions they make and the potential impact it has on their child’s wellbeing. These attitudes may affect uptake and adherence of medication with inappropriate use potentially contributing to the development or antibiotic resistance. PBB was the most common reason for being prescribed long-term azithromycin and hence the subsequent studies have focussed on this condition. The results of this work have been published in full in the Archives of Disease in Children, BMJ group (Hardman, 2021), see appendix 7.

# **Chapter 4. Bacterial isolates and antibacterial resistance in protracted bacterial bronchitis.**

## Introduction

Protracted bacterial bronchitis (PBB), as described in section 1.2, is a common diagnose made in pre-school children being assessed for a chronic wet cough. There is increasing use of prophylactic azithromycin to prevent exacerbations of PBB with limited supporting evidence. The impact of frequent or continuous antibiotics on the paediatric airway microbial flora is largely unknown.

Engagement with parents of children with PBB (Section 3.3.1) and with the medical teams caring for them indicated that randomising patients to azithromycin (which would not be in accordance with our local guidelines recommending prophylaxis for children experiencing more than 3 episodes in 12 months, see appendix 2) would not be acceptable. Given this and the limited evidence base in children with PBB, I undertook observational studies to gather data that may support a future RCT to investigate the use of long-term azithromycin in PBB. In these studies, the decision to treat with prophylactic antibiotic or not was determined by clinical need in accordance with the local guideline. The overall study aimed to test the hypothesis that:

Children with protracted bacterial bronchitis exposed to long-term prophylactic azithromycin will develop macrolide-resistant airway flora.

The specific objectives were:

1. To conduct a pilot study comparing macrolide resistance patterns in the nasal flora of children with PBB exposed or not to long-term prophylactic azithromycin (PBB study 1).
2. To optimise the recruitment strategy and sampling methodology for future studies.
3. To validate the results of the pilot study through an extension of the pilot study with regards to patterns of azithromycin resistant nasopharyngeal bacteria and antibiotic exposure between children with PBB exposed or not to long-term prophylactic azithromycin (PBB study 2).
4. To investigate phenotypic and genotypic antibiotic resistance profiles of nasopharyngeal bacterial flora in children with PBB exposed or not to long-term prophylactic azithromycin (PBB studies 1 and 2)
5. To determine the frequency of viruses associated with exacerbations of PBB (PBB study 2)

We initially conducted a pilot study (PBB study 1) to optimise the participant recruitment process, scope the feasibility of using DNPS in this cohort of children, collect preliminary microbiological data on the numbers of potential respiratory pathogens residing in the nasopharynx, document the patterns of azithromycin susceptibility and explore whether the use of long-term azithromycin affected the need for rescue courses of antibiotics to treat breakthrough pulmonary exacerbations. The decision to utilise DNPS was based on the fact preschool children do not expectorate on demand and that obtaining sequential samples from the lower airways via bronchoalveolar lavage/ brushings or sputum would not be safe or feasible in a paediatric setting.

After completion of PBB study 1, we carried out a second observational study (PBB study 2), making minor refinement to the initial pilot study design. This enabled an increased participant sample size to validate the preliminary findings from the first study of higher than expected azithromycin resistance across both groups, and to gather further microbiological samples for more in-depth analysis, including subsequent 16s sequencing (see Chapter 5). PBB study 3 was undertaken to analyse the stored bacterial isolates from the first 2 PBB studies, to further investigate the antimicrobial resistance patterns found. This led to the sequencing of a selection of the S*. pneumoniae* isolates in order to characterise the genotypic resistance alongside the phenotypic data already obtained.

## Protracted Bacterial Bronchitis (PBB) Study 1 (Pilot/Feasibility Study)

This was a single centre, prospective, observational feasibility study carried out at Sheffield Children’s Hospital. All participants had been referred to the paediatric outpatient respiratory services and had a diagnosis of PBB made by a paediatric consultant physician. Four paediatric respiratory consultants and 2 general paediatric consultants with an interest in respiratory medicine hold outpatient clinics where children with PBB are seen.

The comparator groups were 10 children with PBB prescribed long term azithromycin over the winter months as part of standard clinical care, versus 10 children likewise with a diagnosis of PBB but who were not anticipated to require prophylactic azithromycin because they had not had 3 recurrences of PBB over the last 12 months (see section 2.2.3 for participant inclusion criteria).

In PBB study 1, **I hypothesised that children with PBB receiving prophylactic azithromycin would acquire respiratory flora with increased rates of azithromycin resistance.**

To investigate this, I carried out a single centre observational pilot study with the following research approach:

1. To collect serial nasopharyngeal swabs from children with PBB for bacterial culture and test bacterial isolates for azithromycin resistance.

2. To compare azithromycin resistance patterns in children with PBB exposed or not to long-term prophylactic azithromycin

3. To optimise recruitment strategy and sampling methodology for future studies.

### Recruitment and retention

As planned, a total of 20 children were recruited. Of these, 10 started prophylactic azithromycin (azithromycin group) over the winter and 10 did not (comparison group). The consort diagram in Figure 4‑1 shows the process of recruitment. Although the study information was given to 70 families who fulfilled the inclusion criteria, 44 out of 70 (63%) did not reply to the text message sent to ask if they would like to participate in the study (appendix 3.2). Two participants left the study, one due to parental health issues and 1 due to the burden of the sample collection, both in the azithromycin group. The median duration of long-term azithromycin in the azithromycin group was 5 months with a minimum of 3 days and a maximum of 8 months. One participant stopped taking azithromycin after 3 doses due to concerns that it was causing constipation. All analysis was by intention to treat.

Number of families that received study information:

N=70

Potential comparators = 53

Azithromycin = 17

* 3 declined the offer to take part
  + Too busy
  + Child too young and swabs unpleasant
  + Child too unwell
* 40 did not reply to the text message
* 3 wanted to participate but had already started azithromycin
* 4 did not reply to the text message

Azithromycin recruited

N=10

* 1 left the study – Issues relating to parental health
* 1 left the study – Burden of swabs too high
* 1 discontinued azithromycin after 3 days due to adverse side effects

Comparators recruited

N=10

**Figure 4‑1:** Consort diagram of the recruitment process for PBB study 1

*Consort diagram of the recruitment process. Patients who fulfilled the inclusion criteria were given the study information. Of these, we report how many responded to the text message sent 48 hours after the study information had been given to families, how many were recruited and how many left the study early or did not complete the appropriate treatment.*

### Demographics at recruitment

Children in the azithromycin group were on average older than controls (mean age 5 years and 1 month versus 3 years and 9 months) (Table 11). There were similar numbers of children with a microbiological (as opposed to a clinical) diagnosis of PBB in both groups. The reason for the 2 children requiring previous ventilatory support was prematurity, this was not felt to be related to PBB (Table 11). The mode of delivery and breast feeding were documented as these factors are reported to influence the development of the respiratory microbiome; it is not known whether they play a role in the development of PBB (Biesbroek et al., 2014; Reyman et al., 2019). Fewer children in the azithromycin group were born by vaginal delivery compared to those in the comparison group. Parental smoking was also documented as this affects respiratory health (Pattenden et al., 2006). The bacterial growth on BAL from those with a microbiological diagnosis can be found in appendix 8. The predominant species grown in the azithromycin group was *H. influenza*e. There was an even distribution of identified isolates in the comparison group between *H. influenzae*, *S. pneumoniae* and *M. catarrhalis*. Given the differing ages of children entering the study, the number of previous courses of acute antibiotics was calculated in relation to the child’s age in years at the point they entered the study (number of courses of antibiotics divided by the child’s age in years). Antibiotic prescribing data were obtained through GP prescriptions and electronic hospital records (Table 12). The median number of antibiotic courses prescribed for greater than 2 weeks for presumed PBB was 0.38 courses/ year (IQR, 0.32-0.55; mean 0.38) in the comparison and 0.41 courses/per year (IQR, 0.35-0.66, mean 0.59) azithromycin groups, suggesting disease severity was in fact comparable between the groups. It was not logistically possible nor was ethical approval sought to interrogate GP records to confirm whether all antibiotics of > 2 weeks duration were to treat exacerbations of PBB. The median number of acute courses of antibiotics prescribed per participant year was 2.58 courses (IQR1.78-3.51; mean 2.2) in the comparison group and 1.64 (IQR, 1.43-2.31; mean 2.8) in the azithromycin group. Previous macrolide exposure was slightly higher in the azithromycin group compared to the comparison group, 8 versus 6 participants had previously received macrolides respectively. Of the total number of antibiotic courses prescribed over the 12 months prior to starting the study, 8% (2 of 25) in the comparison group and 15% (3 of 20) in the azithromycin group were macrolides.

**Table 11:** Recruitment demographics of participants in PBB study 1

|  |  |  |
| --- | --- | --- |
|  | **Comparison Group**  **N = 10 (%)** | **Azithromycin Group**  **N = 10 (%)** |
| Age: Median  (25th-75th quartile) | 3.6 years  (2.9-4.8) | 4 years  (3-7.3) |
| Female | 4 (40) | 6 (60) |
| Vaginal delivery | 8 (80) | 4 (40) |
| Breast fed | 5 (50) | 5 (50) |
| Microbiological diagnosis | 5 (50) | 6 (60) |
| Parent smoker | 2 (20) | 2 (20) |
| Premature <37 | 1 = 31/40 (10) | 1 = 27/40 (10) |
| Ventilated | 1 (1) | 1 (1) |
| Penicillin allergy | 0 | 0 |
| Fully immunised in relation to age | 10 (100) | 10 (100) |

**Table 12:** Total antibiotic exposure of participants prior to commencing the study

|  |  |  |
| --- | --- | --- |
|  | **Comparison group**  N=10 | **Azithromycin group**  N=10 |
| Median total number of antibiotic courses per participant prior to study start/ participant age in years (25th-75th quartiles) | 2.58  (1.78-3.51) | 1.64  (1.34-2.31) |
| Median total number of > 2week courses of antibiotics / participant age in years  (25th-75th quartiles) | 0.38\*  (0.32-0.55) | 0.41\*\*  (0.35-0.66) |
| Number of participants prescribed acute courses of macrolides  (range per participant) | 6  (1-3) | 5\*\*\*  (1-3) |
| Median time from completing last course of antibiotics to study start (25th-75th quartiles) | 10 weeks (2.5-19.25) | 7.5 weeks (4-24) |
| Median time from completing last course of macrolides to study start (25th-75th quartiles) | 47 weeks (27.5-64) | 22 weeks (16-28.5) |

* *\* Breakdown of antibiotics courses lasting longer than 2 weeks: Co-amoxiclav (15), amoxicillin (1), cefixime (2), clarithromycin (2)*
* *\*\* Breakdown of antibiotics courses lasting longer than 2 weeks: Co-amoxiclav (19), erythromycin (2), clindamycin (1), amoxicillin (2), azithromycin (1).*
* *\*\*\* Not including the previous prophylactic azithromycin. When including prophylaxis 8 children had previous macrolide exposure as some children had both prophylaxis and acute courses*

### Nasopharyngeal swab results

DNPS were collected (see section 2.2.5.1) at baseline and at 3 monthly intervals over the 12 month study period (Figure 4‑2). The median duration of azithromycin prophylaxis prescribed in the azithromycin group during the study was 5 months (IQR 3.5 - 6 months, mean 4.5).

Baseline 3 months 6 months 9 months 12 months

DNPS collected

Azithromycin exposure over winter months

**Figure 4‑2:** Timeline of swab collection in PBB study 1

*Deep nasopharyngeal swabs were collected at baseline and then every 4 months for the 12 month study period. Swabs were cultured for S. pneumoniae, H. influenzae, M. catarrhalis and S aureus. Any isolates were susceptibility tested to azithromycin. The median duration of long term azithromycin administration was 5 months in the azithromycin group.*

Of the 20 children participating, 17 had all 5 swabs taken (3-monthly over a period of 12 months) and completed the study (See Figure 4‑3). Two children in the azithromycin group left the study, and one discontinued azithromycin prematurely after 3 doses but was included in the azithromycin group for analysis. One participant in the azithromycin group did not have the final swab taken due to the COVID-19 pandemic. At the time it was due to be taken, it was deemed to high risk to collect the swab. A total of 93 swabs were collected during the study period.

Of 93 swabs collected, 55 swabs grew one or more of the following bacteria: *S. pneumoniae (25 isolates)*, *H. influenzae (30 isolates)*, *M. catarrhalis (18 isolates)* or *S. aureus (6 isolates)*. At baseline, 4 out of 10 children had azithromycin resistant bacteria in the comparison group (1 MIC missing) and 2 out of 10 in the azithromycin group (Table 13). One child in the comparison group had 2 different azithromycin resistant bacteria grown from one swab hence 5 resistant isolates from 4 swabs.

**Table 13:** Baseline azithromycin resistance in PPB study 1

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Group  (Total number of swabs) | DNPS with positive growth | Total number of isolates | Number of azithromycin resistant isolates | Number of swabs with azithromycin resistant isolates  (Number of children) |
| Comparison  (10 swabs) | 6 | 9 | 5 | 4 (4) |
| Azithromycin  (10 swabs) | 6 | 8 | 2 | 2 (2) |

During the study, excluding the baseline swabs, 28 out of the 40 swabs collected in the comparison group had growth of at least one bacterial respiratory pathogen (Table 14). From these 28 swabs, 39 bacteria were isolated of which 23 were classed as azithromycin resistant by standard microbiology laboratory analysis (E-test strips, section 2.2.5.2). All 10 children in the comparison group had at least one swab with azithromycin resistant bacterial growth. In the azithromycin group, 33 swabs were collected. Of these swabs, 23 bacteria were isolated of which 14 were azithromycin resistant. During the study, 5 children in the azithromycin group had at least one swab with azithromycin resistant bacterial isolate of a potential respiratory pathogen (1 MIC missed). All the bacterial isolates from PBB study 1 were frozen for subsequent analysis after initial susceptibility testing.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Group  (total number of swabs) | DNPS with positive growth | Total number of isolates | Number of azithromycin- resistant isolates | Number of swabs with azithromycin- resistant isolates (number of children) |
| Comparison  (40 swabs) | 28 | 39 | 23 | 19 (10) |
| Azithromycin  (33 swabs) | 15 | 23 | 14 | 10 (5) |

**Table 14:** Azithromycin resistant isolates found during the course of PBB study 1

**Key:**

**Azithromycin susceptibility Duration from last macrolide exposure to start of study**

|  |  |  |  |
| --- | --- | --- | --- |
|  | Never had macrolide |  | >24 months |
|  | <6 months |  | Previous exposure. Uncertain duration |
|  | 6-12 months |  | Missing data |
|  | 12-24 months |  | |

|  |  |
| --- | --- |
|  | Resistant bacteria |
|  | Susceptible bacteria |
|  | No bacterial growth |
|  | Lab error (no MIC) |
|  | No swab (COVID-19) |

**Antibiotic courses during the study period**

|  |  |
| --- | --- |
| **1** | Number of acute courses of antibiotics |
|  | Prescribed acute course of macrolides |

**Figure 4‑3:** Representation of azithromycin susceptibility for DNPS cultures in PBB study 1

*Two observational cohorts each of 10 children with a diagnosis of PBB had sequential DNPS taken 5 times over a 12-month period. One group received prophylactic azithromycin (A) and the other did not (C). Each column on the X-axis represents an individual child. The Y-axis shows each of the 5 swab results, with different colours denoting azithromycin susceptibility of any isolates grown. Coloured circles under each participant represent the duration from the last course of macrolide antibiotic to the study start. Numbers in the upper boxes denote the number of acute courses of antibiotics each participant was prescribed during the study. Triangles in the boxes show when courses of macrolides were prescribed during the study. Resistance and susceptibility to azithromycin are attributed based on minimum inhibitory concentrations as per the EUCAST breakpoint tables: S. pneumoniae > 0.5 mg/L; M. catarrhalis > 0.5 mg/L; S. aureus > 2 mg/L; H. Influenzae > 4 mg/L*

The 44 azithromycin resistant organisms from both groups over the entire study period were predominantly *S. pneumoniae* (24 isolates) with smaller numbers of *H. influenzae* (9 isolates), *M. catarrhalis* (7 isolates) and *S. aureus* (4 isolates).

*Streptococcus pneumoniae*

Based on EUCAST breakpoints, isolates with an MIC > 0.5 mg/L were classed as azithromycin resistant. Over the course of the study, azithromycin-resistant *S. pneumoniae* was isolated from 8 out of 10 children in the comparison group and 5 out of 10 children in the azithromycin group (Figure 4‑4); several participants cultured more than one isolate. At baseline azithromycin-resistant *S. pneumoniae* was isolated from 3 participants in the comparison group compared to 2 in the azithromycin group. All 8 *S. pneumoniae* isolates in the azithromycin-exposed group were resistant, including 1 isolate with high level resistance (MIC >256mg/L). Only one out of the 17 *S. pneumoniae* isolates in the comparison group was fully susceptible to azithromycin. Although the number of resistant isolates was unexpectedly high, the majority of isolates exhibited only low-level resistance (0.75-4 mg/ml, i.e. 1.5x-8x the EUCAST breakpoint).

**Figure 4-4 A.**

**Key: Azithromycin susceptibility Duration from last macrolide exposure to start of study**

|  |  |
| --- | --- |
| Colour | MIC (mg/L) |
|  | ≤0.5 |
|  | 0.6-1 |
|  | 1.1 to 2 |
|  | 2.1 to 4 |
|  | >256 |

|  |  |  |  |
| --- | --- | --- | --- |
|  | Never had macrolide |  | >24 months |
|  | <6 months |  | Previous exposure. Uncertain duration |
|  | 6-12 months |  | Missing data |
|  | 12-24 months |  | |
|  | Macrolide courses prescribed during the study period | | |

**Figure 4-4 B.**

**Figure 4‑4:** Azithromycin susceptibility of Streptococcus pneumoniae isolates in PBB study

*Two observational cohorts, each of 10 children with a diagnosis of PBB had sequential DNPS taken 5 times over a 12-month period. One group received prophylactic azithromycin (A) and the other did not (C).* ***Figure 4-4 A****: Each participant is represented by a single column on the X-axis and each of the 5 swabs on the Y-Axis. The presented results show swabs with growth of S. pneumoniae and whether these were azithromycin susceptible (blue) or resistant (pink, red or brown depending on MIC, see key). Azithromycin susceptibility based on minimum inhibitory concentrations as per the standard EUCAST breakpoint tables: MIC > 0.5mg/L = resistant. Coloured circles under participants represent the duration from the last course of macrolide antibiotics to the study start.* ***Figure 4-4 B:*** *The MIC distribution of S. pneumoniae isolates in PBB study 1. Dashed red line indicates the standard EUCAST breakpoint.*

*Haemophilus influenzae*

The majority of nasopharyngeal isolates in both groups were classed susceptible to azithromycin based on the EUCAST breakpoint of > 4mg/ml (Figure 4‑5) although 10 isolates were at the breakpoint level (MIC = 4mg/ml). At baseline, 2 out of 4 participants in the comparison group had nasopharyngeal carriage of resistant *H. Influenzae*. In the azithromycin group, the 2 *H. Influenzae* isolates obtained at baseline were susceptible to azithromycin. All 4 final *H. influenzae* isolates were susceptible to azithromycin with these 4 participants having previously had azithromycin resistant *H. influenzae* isolates obtained during the study period.

*Moraxella catarrhalis* and *Staphylococcus aureus*

The majority (11 out of 18) of M. catarrhalis isolates were susceptible to azithromycin. Of the S. aureus isolates, 4 were azithromycin resistant out of 6 isolates. For S. aureus and M. catarrhalis MIC data, see appendix 9.

**Figure 4-5 A.**

**Key: Azithromycin susceptibility Duration from last macrolide exposure to start of study**

|  |  |
| --- | --- |
| Colour | MIC |
|  | ≤4 |
|  | 4.1 to 6 |
|  | 6.1 to 12 |
|  | 12.1 to 47 |
|  | >47.1 |
|  | No MIC |

|  |  |  |  |
| --- | --- | --- | --- |
|  | Never had macrolide |  | >24 months |
|  | <6 months |  | Previous exposure. Uncertain duration |
|  | 6-12 months |  | Missing data |
|  | 12-24 months |  | |
|  | Macrolide courses prescribed during the study | | |
|  |  | | |

**Figure 4-5 B.**

**Figure 4‑5:** Representation of participants with growth of *Haemophilus influenzae* on any DNPS in PBB study 1

*Two observational cohorts, each of 10 children with a diagnosis of PBB had sequential DNPS taken 5 times over a 12-month period. One group received prophylactic azithromycin (A) and the other did not (C).* ***Figure 4-5 A:*** *Each participant is represented by a single column on the X-axis and each of the 5 swabs on the Y-Axis. The graph shows swabs with growth of H. influenzae and whether these were azithromycin susceptible (blue) or resistant (see colour key for MICs). Azithromycin susceptibility based on minimum inhibitory concentrations as per the standard EUCAST breakpoint tables: MIC > 4mg/L = resistant. Coloured circles under each participant represent the duration from the last course of macrolide antibiotic to the start of the study.* ***Figure 4-5 B:*** *MIC distribution of H. influenzae isolates in PBB study 1. Dashed red line indicates the standard EUCAST breakpoint.*

### Antibiotic use during the study period

As noted, the median duration of azithromycin prophylaxis prescribed in the azithromycin group during the study was 5 months (IQR 3.5 - 6 months, mean 4.5). A single child had only 1 week of azithromycin and stopped treatment as his parents felt it was exacerbating his constipation (Participant A2).

Through the use of hospital records, GP prescription data and parental recall, antibiotic exposure for each participant was analysed. Of note, only 2 parents recorded courses of antibiotics in the study booklet provided at recruitment, prompting us to obtain an amendment to our ethics to access GP records, with additional consent obtained from participants. GP prescription records were reviewed for antibiotic prescriptions in 18 out of 20 cases. In 2 cases, the participants (in the azithromycin group) left the study before additional consent could be obtained to review their GP prescription records.

The total number of acute courses of antibiotics prescribed for presumed respiratory tract infections during the study period was lower in the azithromycin group than the comparison group (19 vs 27 courses respectively, see Table 15). Flucloxacillin was prescribed for 2 skin infections and not included in these totals. The number of courses of antibiotics between 2-4 weeks in duration, prescribed to treat presumed exacerbations of PBB, was lower in the azithromycin group compared to the comparison group, 4 versus 8 courses respectively. These were prescribed to 4 participants in the azithromycin group with a total of 8 weeks of antibiotics and to 5 participants in the comparison group with a total of 20 weeks of antibiotics. All 4 courses prescribed in the azithromycin group were administered during periods when the long-term azithromycin has been stopped. Whilst taking azithromycin a single participant received 3 separate, one week duration courses of antibiotics. The indications for antibiotics were reported by their parent (2=amoxicillin for an ear infection and 1= co-amoxiclav for a chest infection).

**Table 15:** Type of antibiotic prescribed for participants during the study period PBB 1

|  |  |  |
| --- | --- | --- |
| **Antibiotic prescribed** | **Comparison group**  Number of courses | **Azithromycin group\***  Number of courses |
| Amoxicillin | 14 | 10 |
| Azithromycin | 0 | 1 |
| Clarithromycin | 1\*\* | 0 |
| Co-amoxiclav | 12 | 8 |
| Flucloxacillin (skin infections) | 1 | 1 |
| Total antibiotic during study | 28 | 20 |
| Total antibiotics for presumed respiratory tract infections | 27 | 19 |
| Total courses of antibiotics > 2 weeks\*\*\* | 8 | 4 |
| Median number of antibiotic courses per child for respiratory illness (range) | 2.5  (1-6) | 1  (1-8) |

*\* Prophylactic courses not included in the total as all participants received prophylactic azithromycin*

*\*\* Participant C8 was prescribed 4 weeks between swabs 1 and 2.*

*\*\*\* Breakdown of courses of antibiotics > 2 weeks: Comparison group: Co-amoxiclav 2 weeks = 6 courses; clarithromycin 4 weeks = 1 course, co-amoxiclav 4 weeks = 1 course. Azithromycin group: co-amoxiclav 2 week = 4 courses*

### Adherence to azithromycin and diary use

All 10 participants in the azithromycin group were asked to complete the azithromycin adherence diary. Of these 4 out of 10 competed the diaries and in total 3 doses were marked as not given. The other participants’ parents verbally reported 100% compliance for those continuing in the study following the baseline swab.

### Adverse Events

Importantly, taking the nasopharyngeal swabs was acceptable to the majority of participants and parents, with only 1 child leaving the study early due to the discomfort of the swabs. Informally families said they felt that taking five swabs over the study period was not problematic and potentially 2-3 more could be taken over a 12-month period if the child did not become too distressed at the time of sampling. The only medical complications observed were two minor nose bleeds occurring immediately after taking two of the nasopharyngeal swabs. These stopped with direct pressure within a minute and neither participant withdrew from the study. No other complications were recorded. The only side effect of azithromycin reported was that of an exacerbation of pre-existing constipation.

### Feasibility issues and study limitations

Recruitment required 2 months longer than initially planned. Our initial ethical approval did not encompass pre-screening of participants who were attending clinic. This placed an increased workload on the clinical staff, who found it difficult to screen eligibility and distribute the study information to all families meeting the inclusion criteria as well as carrying out the medical consultation. This led to an amendment to the ethics to allow pre-screening and expedite recruitment to the subsequent PBB study 2 (see section 2.3.4 ). Recruiting families to participate in the comparison group was difficult, with poorer uptake than in potential recruits starting azithromycin. This in turn meant that accurately age matching participants within 12 months as initially planned was not possible. It was difficult to find age matched comparison participants for the older children in the azithromycin group, as few children with PBB are older than 6 years.

As noted, DNPS do not necessarily represent the lower airway flora but represent a potential source of lower airway pathogens. A total of four swabs were sub-optimally taken. The swab could not be inserted the full distance as the children were upset and moving (“wriggly children”). These swabs were processed but only 1 had a growth of bacteria. Due to laboratory errors, two cultured bacterial isolates were not tested for azithromycin susceptibility. The process of culturing and susceptibility testing of the nasopharyngeal swabs was undertaken by trained microbiology laboratory staff. Staff rotate around the various departments within the laboratory and therefore errors were made where staff were not aware of the specific protocol to be used on the research samples.

Professor Alan Rigby, statistician at York and Hull medical school, was consulted throughout the design and analysis phases with regards to statistical advice. As this is a small pilot study, I did not undertake statistical analysis on this data alone, in keeping with the standard practice of pilot study analysis.

### PBB study 1: Summary of study outcomes against study objectives

The two study objectives for PBB study 1 were achieved. The planned pilot study was conducted, 20 children with PBB were recruited, serial nasopharyngeal swabs were collected for bacterial culture and any bacterial isolates tested for azithromycin susceptibility. Azithromycin resistance patterns in children with PBB taking long term azithromycin were compared with a comparison group of children with PBB not prescribed long term azithromycin. Of all the bacterial isolates obtained from children in the comparison group, 58 % (28 of the 48 isolates) were resistant to azithromycin and in the group taking long term azithromycin, 51% (16 out of 31 isolates) of the bacterial isolates were resistant to azithromycin. Notably, the number of resistant isolates in the comparison group was higher than expected and in both groups resistance seemed to be largely driven by (mostly low level) azithromycin resistant *S. pneumoniae.* Given the small number of participants and bacterial isolates it was not possible to carry out any statistical analysis. These results however prompted PBB study 2 to be undertaken in order to build on the samples size, validate the findings from PBB study 1 and collect samples for microbiome analysis.

The second objective of optimising the study methodology was achieved and the refined study design is discussed in section 4.3 below. Compliance with taking long-term azithromycin was initially assessed through the use of self-reported prescription diaries. These were inadequately completed, and parents’ recall of recently prescribed antibiotics was poor when asked during visits to collect DNPS. As such, ethical approval was sought and granted (Amendment number: REC Ref AM02) to obtain GP prescription records in order to record antibiotic exposure during the study period.

## Protracted Bacterial Bronchitis (PBB) Study 2

This single centre, prospective observational study built upon the results of study 1 and was planned to allow us to both complement the data from study 1 and to obtain samples suitable for subsequent microbiome analysis (see Chapter 5). The PBB study 2 recruitment pathway was improved following scrutiny of the pathway used in PBB study 1. Firstly, outpatient clinic lists were pre-screened for potential participants allowing for a more organised and targeted approach to recruitment. Secondly, ethical approval was sought at the outset of the study to obtain GP prescription records, in order to record antibiotic exposure during the study period.

I recruited a further 30 children with PBB, of whom 15 were exposed to long-term azithromycin and 15 were not anticipated to require long-term azithromycin. Deep nasopharyngeal swabs were collected over the study period. This was initially planned to be 12 months but due to the COVID-19 pandemic, needed to be extended to 20 months in order to collect the final DNPS. Data on the bacterial isolates and antimicrobial resistance profiles complemented the results from study 1. This study additionally incorporated the intent to investigate exacerbations of PBB. At the beginning of exacerbations, we planned to take a viral throat swab and DNPS for bacterial culture and 16s analysis. On completion of the 2-4 week treatment course of antibiotics, there was the option for an end of exacerbation DNPS to be taken if this was acceptable for the child. We hoped this would give new insights into exacerbations of PBB. A microbiological diagnosis may be established by bronchoscopy on a single occasion, but a sequential study of the organisms driving exacerbations has not been undertaken to our knowledge. Unfortunately, this was again disrupted by COVID-19.

All the bacterial isolates from both PBB studies 1 and 2 were frozen so that more detailed resistance analysis could be undertaken at a later stage. The DNPS from PBB study 2 (but not study 1) were frozen in order to carry out 16s rRNA sequencing again at a later date. Following discussions with statistician Alan Rigby, the results of studies PBB 1 and 2 were combined in order to have a sufficient sample size for statistical analysis to be carried out.

The experimental approach for PBB study 2 was:

1. To validate and complement the findings of PBB study 1 with regards to patterns of azithromycin resistant nasopharyngeal bacteria and antibiotic exposure between two groups of children with PBB (one group exposed to long-term azithromycin and the other not so treated).
2. To collect and store deep nasopharyngeal swabs and any bacterial isolates for future detailed resistance profiling and microbiome analysis.
3. To collect deep nasopharyngeal swabs at the beginning and end of any PBB exacerbations and collect viral throat swabs at the beginning of any PBB exacerbations.

### Recruitment and retention

Ethical approval governing this study (REC reference number 19/YH/0207) allowed pre-screening of clinic attendees to identify those who might be eligible, enabling targeted discussions and distribution of study literature. This facilitated recruitment for study 2. As previously, many invitees did not respond, and there was a lower uptake by those not scheduled to start azithromycin prophylaxis. A total of 30 children with PBB were recruited as planned, 15 to the azithromycin group and 15 to the comparison group. The consort diagram in Figure 4‑6 shows the recruitment process. No child participating in PBB study 2 had taken part in PBB study 1, hence this was a true validation cohort. One child from each group left the study. C9 discontinued due to the fact additional swabs were being taken due to COVID and the swab burden became too high. A3 was not able to be contacted again despite telephone messages and written letters. Within the comparison group, 3 children started prophylactic azithromycin for recurrent PBB; Participant C7 received 4 months of azithromycin, C9 left the study as azithromycin was being commenced and no swabs were taken. And C10 received 1 dose of azithromycin which was not tolerated and changed to second line prophylaxis, co-trimoxazole. Analysis was by intention to treat.

### Demographics at recruitment

Children in the comparison group were aged matched within 12 months of the azithromycin group (Table 16). The groups were similar apart from two factors. The first difference was that those in the azithromycin group had a greater antibiotic exposure prior to the start of the study compared to the comparison group (Table 17). This may reflect the fact that these children were being commenced on long-term azithromycin due to an increased infection burden. Of the total prescriptions issued in the preceding 12-months prior to the study start, 15.9% (7 out of 44) were for macrolides in the comparison group and 17.3% (9 out of 52) in the azithromycin group. The second key difference was that unlike in study 1, the majority of participants in the azithromycin group had a microbiological diagnosis of PBB whereas those in the comparison group had a clinical diagnosis. Otherwise, the groups were similar to those in PBB study 1. The bacterial isolates grown from those with a microbiological diagnosis can be found in appendix 8.

Number of families given the study information

N=104

Potential comparators = 82

Azithromycin = 22

* 2 replied to text message
  + DNPS would be too unpleasant
* 5 no response to text message
* 1 replied to text message
  + too busy
* 68 no response to text message

Azithromycin recruited = 15

Comparators recruited = 15

1 child lost to follow up

1 child left the study due to the high swab burden.

2 children required long term antibiotics.

**Figure 4‑6:** Consort diagram showing the recruitment process for PBB study 2

*Consort diagram of the recruitment process. All patients who fulfilled the inclusion criteria were given the study information and invited to participate in the study.*

**Table 16:** Recruitment demographics for children in PBB study 2

|  |  |  |
| --- | --- | --- |
| **Demographic** | **Comparison group**  **N= 15 (%)** | **Azithromycin group**  **N =15 (%)** |
| Age: Median  (25th-75th quartile) | * 1. years   (2-3.5) | 3.4 years  (2.9-4.1) |
| Female | 8 (53) | 5 (33) |
| Vaginal delivery | 13 (87) | 11 (73) |
| Breast fed | 8 (53) | 7 (47) |
| Microbiological diagnosis | 8 (53) | 14 (93) |
| Premature <37 | 1 (7) | 3 (20) |
| Ventilated | 0 (0) | 1 (7) |
| Parent smoker | 4 (27) | 3 (20) |
| Fully immunised for age  (UK immunisation schedule) | 15 (100) | 15 (100) |
| Penicillin allergy | 1 (7) | 0 (0) |
| Family member on prophylactic antibiotics | 0 (0) | 1 (7) |

**Table 17:** Antibiotic exposure of participants prior to commencing the study

|  |  |  |
| --- | --- | --- |
|  | **Comparison group**  N= 15 | **Azithromycin group**  N= 15 |
| Median number of acute courses of antibiotics / participant age in years  (25th-75th quartile) | 1.89  (1.18-2.92) | 2.4  (1.83-3.71) |
| Median number of courses of antibiotics > 2 weeks / participant age in years  (25th-75th quartile) | 0.51  (0.42-0.84) | 0.8  (0.59-0.98) |
| Number of participants previously prescribed acute courses of macrolides | 8 | 6\*\*\* |
| Median time from completing last course of antibiotics to study start (25th-75th quartile) | 13 weeks  (5-25) | 1. weeks   (4-20) |
| Median time from completing last course of macrolides to study start, in those previously exposed to macrolides  (25th-75th quartile) | 30 weeks  (19-41.5)  6 previously had macrolides | 20 weeks  (12-25)  7 previously had macrolides |

*\* Breakdown of antibiotics courses lasting longer than 2 weeks: Co-amoxiclav (22 courses; 10x 2 weeks, 10x 4 week, 1x 5 week, 1x 6 week), Azithromycin (2 courses; 2x 2week)*

*\*\* Breakdown of > 2 week prescriptions:*

*\*\*\* Not including the previous prophylactic azithromycin courses received by 6 children. Total of 7 children previously exposed to macrolides.*

### Nasopharyngeal swab results

DNPS were collected at baseline from all participants. Following this a further three swabs were to be collected at 4 monthly intervals with additional swabs around exacerbations. As discussed previously (section 2.3.6) a number of swabs were missed due to the COVID pandemic and the swab schedule had to be amended (see Figure 4‑7). The median duration of long-term azithromycin over the study period was 12 months (IQR 7.25-12), mean 10.3 months). Of the 15 participants in the azithromycin group, 9 had a single episode of continuous azithromycin exposure ranging from 2 to 17 months. The study period covered 2 winter periods for some participants and hence 5 participants had 2 episodes of azithromycin with a break over the summer months. A single participant had 3 discrete episodes of azithromycin and 1 participant left the study. For further details see Table 34 in section 5.2.2.

Baseline 4 months 8 months 12 months 18 months

Regular background swabs – 4 monthly (initial study end planned at 12-months)

Test: Bacterial culture, MIC and 16s analysis

Beginning of pulmonary exacerbation – 2 swabs (1x DNPS and 1x viral throat swab)

Tests: Bacterial culture, MIC and 16s analysis + viral PCRs

End of exacerbation swab – taken on completion of antibiotic course

Tests: Bacterial culture, MIC and 16s analysis (optional swab)

Changes due to COVID: End of study swab collected at 18-months in some participants

Changes due to COVID: Some swabs at 4- and 8-months were missed

**Figure 4‑7:** Timeline for taking deep nasopharyngeal and throat swabs in study PBB 2

*Timings of sample acquisition for participants in study 2. Protocol is identical for Azithromycin and Comparison groups. All children were planned to undergo 4 swabs taken at 4-monthly intervals. Those children experiencing exacerbations of PBB over the 12-month period were to have additional swabs taken at the time of these exacerbations. Due to the COVID pandemic, swabs at 4- and 8-months were missed in a number of participants. The final swab had to be collected at 18-months in some participants rather than 12-months. The median duration of long term azithromycin administration was 12-months in the azithromycin group.*

From the 30 children participating in the study, 100 deep nasopharyngeal swabs were collected, 54 in the comparison group and 46 in the azithromycin group. Included in this total were 7 DNPS taken at the beginning of exacerbations, 5 from participants within the comparison group and 2 from participants within the azithromycin group. A single post exacerbation DNPS was collected in the comparison group.

Of the 100 swabs collected, 56 grew one or more of the following bacteria: *S. pneumoniae (33 isolates)*, *H. influenzae (25 isolates)*, *M. catarrhalis (13 isolates)* or *S. aureus (9 isolates)*. The distribution of azithromycin resistant isolates between the groups can be seen in Figure 4‑8. Due to laboratory protocol errors, 1 sample was lost and 6 isolates were not susceptibility tested to azithromycin.

At baseline, there was positive bacterial culture from 13/15 swabs (87%) in the comparison group compared to 10/15 in the azithromycin group (67%). Of the 24 isolates obtained in the comparison group, 13 were resistant to azithromycin (54%) compared to 12 out of the 15 isolates in the azithromycin group (80%). See Table 18. There was one laboratory error in the comparison group where one swab had a bacterial growth however was not susceptibility tested to azithromycin.

**Table 18**: Baseline azithromycin resistance in PBB study 2

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Group  (Total number of swabs) | DNPS with positive growth | Total number of isolates | Number of azithromycin-resistant isolates | Number of swabs with azithromycin-resistant isolates  (Number of children) |
| Comparison  (15 swabs) | 13 | 24 | 13 | 10 (10) |
| Azithromycin  (15 swabs) | 10 | 15 | 12 | 9 (9) |

**Key:**

**Azithromycin susceptibility Duration from last macrolide exposure to start of study**

|  |  |  |  |
| --- | --- | --- | --- |
|  | Never had macrolide |  | >24 months |
|  | <6 months |  | Previous exposure. Uncertain duration |
|  | 6-12 months |  | Missing data |
|  | 12-24 months |  | |

|  |  |
| --- | --- |
|  | Resistant bacteria |
|  | Susceptible bacteria |
|  | No bacterial growth |
|  | Lab error (no MIC) |
|  | No swab (COVID-19) |
|  | Lab error (smaple lost) |

|  |  |
| --- | --- |
| **1** | Number of acute courses of antibiotics |
|  | Prescribed acute course of macrolides |

**Antibiotic courses during the study period**

**Figure 4‑8:** Representation of azithromycin susceptibility for DNPS cultures in PBB study 2

*Two observational cohorts each of 15 children with a diagnosis of PBB had four sequential DNPS taken over the study period (Y-axis rows 1, 4, 5 and 7). Additional swabs were collected at the beginning and end of some pulmonary exacerbations (Y-axis rows 2,3 and 6). One group received prophylactic azithromycin (A) and the other did not (C). Each column on the X-axis represents an individual child. The Y-axis shows each of the swab results with different colours denoting azithromycin susceptibility of any isolates grown. Coloured circles under each participant represent the duration from the last course of macrolide antibiotic to the study start. Numbers in the upper boxes denote the number of acute courses of antibiotics each participant was prescribed during the study. Triangles in the boxes show when courses of macrolides were prescribed during the study. To note C7 commenced long term azithromycin after the acute course of macrolides and C10 long term co-trimoxazole. Resistance was based on minimum inhibitory concentrations as per the EUCAST breakpoint tables:*

*S. pneumoniae > 0.5 mg/L; M. catarrhalis >0.5 mg/L; S. aureus > 2 mg/L; H. Influenzae > 4 mg/L*

During the study period, excluding the baseline swabs, there was positive bacterial growth on 20/40 swabs (50%) in the comparison group compared to 11/33 (33%) in the azithromycin group. Of the 27 isolates in the comparison group, 15 were resistant to azithromycin (56%) compared to 11 of the 14 isolates in the azithromycin group (79%), see Table 19. As in PBB study 1, the resistance in both groups was predominantly driven by *S. pneumoniae* with only a single isolate being fully susceptible to azithromycin (see Figure 4‑9). Five *S. pneumoniae* isolates had high level resistance (MIC >256 mg/ml) with the remaining isolates having MICs of 0.75-3 mg/ml. There was less azithromycin resistance amongst the *H. influenzae isolates* with 9 out of 18 isolates tested in the comparison group being resistant to azithromycin and 5 out of 9 in the azithromycin group (Figure 4‑10). In the comparison group 3 swabs with bacterial growth did not get susceptibility tested to azithromycin and in the azithromycin group 1 swab due to laboratory errors.

**Table 19**: Azithromycin resistance of bacterial isolates during PBB study 2

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Group  (total number of swabs) | DNPS with positive growth | Total number of isolates | Number of azithromycin- resistant isolates | Number of swabs with azithromycin- resistant isolates (number of children) |
| Comparison  (40 swabs) | 20 | 27 | 15 | 14 (11) |
| Azithromycin  (33 swabs) | 11 | 14 | 11 | 9 (7) |

Participant Number C= Comparison group A= Azithromycin group

**Key:**

**Azithromycin susceptibility Duration from last macrolide exposure to start of study**

|  |  |
| --- | --- |
| Colour | MIC |
|  | **≤0.5** |
|  | 0.6-1 |
|  | 1.1 to 2 |
|  | 2.1 to 4 |
|  | >256 |
|  | No MIC |

|  |  |  |  |
| --- | --- | --- | --- |
|  | Never had macrolide |  | >24 months |
|  | <6 months |  | Previous exposure. Uncertain duration |
|  | 6-12 months |  | Missing data |
|  | 12-24 months |  | |
|  | Macrolide courses prescribed during the study period | | |

**Figure 4‑9:** Representation of azithromycin susceptibility of Streptococcus pneumoniae isolates: PBB study 2

*Two observational cohorts each of 15 children with a diagnosis of PBB had sequential DNPS taken every 4 months over the study period. One group received prophylactic azithromycin (A) and the other did not (C). Each participant is represented by a single column on the X-axis and each of the swabs on the Y-Axis. The presented results show swabs with growth of S. pneumoniae and whether these were azithromycin susceptible or resistant. Azithromycin susceptibility is based on minimum inhibitory concentrations as per the standard EUCAST breakpoint tables: MIC > 0.5mg/L = resistant. Coloured circles under participants represent the duration from the last course of macrolide antibiotics to the study start.*

**Key:**

**Azithromycin susceptibility Duration from last macrolide exposure to start of study**

|  |  |
| --- | --- |
| Colour | MIC |
|  | ≤4 |
|  | 4.1 to 6 |
|  | 6.1 to 12 |
|  | 12.1 to 47 |
|  | >47.1 |
|  | No MIC |

|  |  |  |  |
| --- | --- | --- | --- |
|  | Never had macrolide |  | >24 months |
|  | <6 months |  | Previous exposure. Uncertain duration |
|  | 6-12 months |  | Missing data |
|  | 12-24 months |  | |
|  | Macrolide courses prescribed during the study | | |

**Figure 4‑10:** Representation of azithromycin susceptibility of Haemophilus influenzae isolates: PBB study 2

*Two observational cohorts each of 15 children with a diagnosis of PBB had sequential DNPS taken every 4 months over a 12-20 month study period. One group received prophylactic azithromycin (A) and the other did not (C). Each participant is represented by a single column on the X-axis and each of the swabs on the Y-Axis. The presented results show swabs with growth of H. influenzae and whether these were azithromycin susceptible or resistant. Azithromycin susceptibility based on minimum inhibitory concentrations as per the standard EUCAST breakpoint tables: MIC > 4mg/L = resistant. Coloured circles under each participant represent the duration from the last course of macrolide antibiotic to the start of the study. (C15, first swab had 2 separate isolates: MIC 4 and MIC 6)*

The above results broadly support the findings reported in PBB study 1. In PBB study 1, 68% of the swabs in the comparison group grew bacteria with 58% of the isolates being resistant to azithromycin. This is very similar to the comparison group in PBB study 2 where 60% of the swabs grew bacteria and 55% of the isolates were resistant to azithromycin. In PBB study 1, 49% of the swabs grew bacteria in the azithromycin group compared to 44% in the azithromycin group of PBB study 2. There was however a higher percentage of azithromycin resistant bacteria in the azithromycin group of study PBB2 compared to that of PBB study 1, 79% vs 52% respectively. Azithromycin resistance was again predominantly driven by resistant *S. pneumoniae* in PBB study 2 although most resistant isolates had MICs of ≤ 4mg/ml. Within the azithromycin group of PBB study 2 there was, as in PBB study 1, a trend towards a reduction of *H. influenzae* following exposure to azithromycin yet in PBB study 2 a greater (though still small) number of azithromycin resistant *S. aureus* were isolated than in PBB study 1.

### Azithromycin MIC data

The azithromycin MIC data for each isolate from both the comparison and azithromycin groups in PBB study 2 are presented in Figure 4‑11. S. pneumoniae isolates from subjects in study PBB2 showed frequent but low level azithromycin resistance with the median level of 1.5 mg/L (17 isolates). A smaller number of highly resistant S. pneumoniae isolates were identified (5 isolates with MIC >256 mg/L). The majority of H. influenzae isolates are azithromycin susceptible with some low level resistance. The results are consistent with the findings from PBB study 1.



**Figure 4‑11**: MIC distributions of bacterial isolates from PBB study 2.

*Azithromycin MIC results for bacterial isolates obtained during PBB study 2. Azithromycin susceptibility was tested using E-test strips. Susceptibility was determined using the EUCAST break points for each isolate. The dotted red line indicates the breakpoint. Zero denotes isolates where an MIC was not tested due to laboratory errors. A: S. pneumoniae, B: H. influenzae. Dashed red lines indicate EUCAST breakpoints.*

### Antibiotic exposure during the study period PBB study 2

Through the use of hospital records, GP prescription data and parental recall, antibiotic exposure for each participant was analysed. To note, only 1 parent recorded courses of antibiotics in the study booklet provided at recruitment. GP prescription records were reviewed for antibiotic prescriptions in 27 out of 30 cases. In 2 cases (1 from each of the 2 groups), the participants left the study before additional consent could be obtained to review their GP prescription records and in one case in the azithromycin group, consent was not given to review the GP prescription records.

The total number of acute courses of antibiotics prescribed for presumed respiratory tract infections during study period in the azithromycin group and the comparison group were 14 vs 27 courses respectively, see Table 20. During this period, courses of flucloxacillin, trimethoprim and penicillin were prescribed for other indications. These were excluded from the total of acute antibiotics for presumed respiratory tract infections.

Long term antibiotics had to be prescribed to two participants in the comparison group during the study period. Participant C7 was unexpectedly prescribed AZM prophylaxis shortly after the first swab was collected. Participant C10 was prescribed azithromycin prophylaxis between the first and second swabs. The child did not tolerate the taste of AZM prophylaxis and was therefore prescribed co-amoxiclav prophylaxis instead.

### 4.3.5.1 Antibiotic prescriptions for presumed exacerbations of PBB

The number of courses of antibiotics prescribed for a duration of 2 weeks or more to treat presumed exacerbations of PBB, was slightly lower in the azithromycin group compared to the comparison group, 7 vs 11 courses respectively. This presumption was made on the duration and choice of antibiotic, usually co-amoxiclav for ≥2 weeks, which is recommended to treat exacerbations of PBB as per the SCH guideline (appendix 2). These were prescribed for 3 participants in the azithromycin group and for 7 participants in the comparison group.

Participants were anticipated to be prescribed long term azithromycin for 6 months over the winter months. Due to COVID-19, the median duration of long-term azithromycin over the study period was in fact 12 months (IQR 7.25-12), mean 10.3 months), see Table 34 for azithromycin exposure details. Participant A15 only received azithromycin for 2 months and was switched to co-trimoxazole due to ongoing symptoms attributed to PBB. Of the 7 courses prescribed to the 3 participants on long term azithromycin, 3 courses were prescribed whilst the participants were not taking long-term azithromycin. The other 4 courses were all prescribed to participant A15 and prescribed whilst on long-term antibiotics.

These results are consistent with the results from PBB study 1 where fewer courses of antibiotics of >2 weeks were prescribed for presumed exacerbations of PBB in the azithromycin compared to the comparison group, 4 vs 8 courses respectively. In PBB study 1 however no such courses were prescribed whilst participants were taking long-term azithromycin.

**Table 20**: Antibiotics courses prescribed during PBB study 2

|  |  |  |
| --- | --- | --- |
| **Antibiotic prescribed** | **Comparison group**  N = 15 | **Azithromycin group\***  N = 15 |
| Amoxicillin | 7 | 4 |
| Azithromycin | 3 | 1 |
| Clarithromycin | 1 | 0 |
| Co-amoxiclav | 16 | 9 |
| Flucloxacillin (skin infection) | 1 | 0 |
| Penicillin (tonsilitis) | 1 | 2 |
| Trimethoprim (UTI) | 1 | 0 |
| Total acute antibiotic during study\* | 30 | 16 |
| Total antibiotics for presumed respiratory tract infections | 27 | 14 |
| Total courses of antibiotics > 2 weeks | 11\*\* | 7\*\*\* |
| Median number of antibiotic courses per child for respiratory illness (range) | 2  (0-6) | 1  (0-4) |

*\* Not included 2 courses of prophylactic antibiotics: C7 prescribed azithromycin prophylaxis, C10 didn’t tolerate azithromycin prophylaxis therefore prescribed co-amoxiclav prophylaxis*

*\*\* Breakdown of antibiotic courses >2 weeks for comparison group: Co-amoxiclav: 2 weeks = 4 courses, 4 weeks = 3 courses, 6 weeks = 1 course; azithromycin: 2 weeks = 1 course, 4 weeks = 1 course; clarithromycin 4 weeks = 1 course*

*\*\*\* Not including courses of prophylaxis. Breakdown of course > 2 week for Azithromycin group: Co-amoxiclav: 2 weeks = 7 courses*

### 4.3.6 Adherence to azithromycin and diary use

All 15 participants in the azithromycin group were asked to complete the azithromycin adherence diary. Of these just 4 out of 15 competed the diaries and 1 parent marked the doses on her phone calendar instead of the diary. In total 12 doses were marked as not given. Of these 8 were missed over the Christmas holidays. The other participants’ parents verbally reported missing 2-3 doses.

### 4.3.7 Viral PCR results

At the beginning of any PBB exacerbations that required treatment, it was planned that a throat swab for respiratory viruses and DNPS would be taken prior to starting antibiotics. There was an option to have a further DNPS taken at the end of the course of antibiotics, acknowledging that these might not be taken due to the high swab burden. Unfortunately due to the restrictions during COVID-19 pandemic, and also to parents forgetting to inform the research team in a timely fashion that their child was unwell, only 7 viral swabs were taken at the beginning of PBB exacerbations prior to commencing at least 2 weeks of oral antibiotics; 3 of these swabs identified a virus that might have triggered the exacerbation (Table 21).

**Table 21**: Viral swab results taken during exacerbations of PBB in PBB study 2

|  |  |  |  |
| --- | --- | --- | --- |
| Participant group | Number of viral swabs taken | Swabs with no viruses | Viruses present on positive swabs |
| Comparison group | 5 | 3 | Parainfluenza 2  Rhinovirus |
| Azithromycin group | 2 | 1 | Seasonal coronavirus & parainfluenza |

### 4.3.8 Adverse events and protocol deviations

The only medical complications observed were 5 minor nose bleeds that occurred after DNPs were taken. The bleeding stopped within a few minutes once direct pressure had been applied to the nose. No other complications were noted.

Due to the COVID-19 pandemic, children were exposed to more nasopharyngeal swabs than anticipated at the outset of the study. This was due to the fact that national guidance during the pandemic was to have a nasopharyngeal/ throat swab taken due acute episodes of fever or cough. Participant C9 decided not to continue with the study due to the increased swab burden. The planned serial swabs could not be taken at the desired time points due to the national lockdown. As such a number of swabs could not be collected whilst children were actively taking long-term azithromycin and the duration of the study had to be extended from 12 to 20 months in order to eb ale to collect final swabs from the participants.

### 4.3.9 PBB study 2: Summary of the study outcomes against the study objectives

The first PBB study 2 objective was achieved. The results of higher than expected azithromycin resistance across both study groups from PBB study 1 were validated in PBB study 2. A further 30 children with PBB were recruited to PBB study 2, serial nasopharyngeal swabs were collected for bacterial culture and bacterial isolates were tested for azithromycin susceptibility.

The secondary objective of obtaining swabs around the times of PBB exacerbations was not achieved. Due to the lock down restrictions during the COVID pandemic, it was not possible to collect swabs from children at these times. There were also fewer respiratory tract infections during this period as will be discussed in section 4.6. All swabs were however frozen and stored for further analysis in order to investigate the phenotypic and genotypic resistance in more detail at a later date.

## Results for PBB studies 1 and 2 (combined data)

During PBB studies 1 and 2, a total of 50 participants were recruited, 25 in the azithromycin group and 25 in the comparison group. Although there was slight variation in the age inclusion criteria between the 2 studies and the timing of swabs differed, the other inclusion criteria and the overall study design were the same and hence this has meant that certain data from the 2 studies could be combined and analysed together. This has enabled some statistical analysis (see section 2.3.14 for details), all of which has been done in consultation with Professor of Statistician Alan Rigby, York and Hull Medical School.

### Demographics at recruitment

Children in the combined azithromycin groups were somewhat older compared to the combined comparison groups and had more often had a microbiological diagnosis made of PBB. These differences were not statistically significant (Table 22). The groups were otherwise highly comparable at baseline. A paired T-test was used to compare the means between the two groups for continuous data and Fisher’s exact test for categorical data.

**Table 22**: Combined demographic data from PBB studies 1 & 2

|  |  |  |  |
| --- | --- | --- | --- |
| **Demographic** | **Combined Comparison Group**  **N=25 (%)** | **Combined Azithromycin Group**  **N=25 (%)** | **Statistical test and results** |
| Mean age in months | 39.24 | 50.24 | T-test, p=0.063 |
| Female | 12 (48) | 11 (44) | Fisher  p = 1 |
| Vaginal delivery | 21 (84) | 15 (60) | Fisher, p=0.113 |
| Breast fed | 13 (52) | 12 (48) | Fisher p= 1 |
| Microbiological diagnosis | 13 (52) | 20 (80) | Fisher p= 0.071 |
| Premature <37 weeks | 2 (8) | 4 (16) | Fisher p= 0.667 |
| Ventilated | 1 (4) | 2 (8) | Fisher p=1 |
| Parent smoker | 6 (24) | 5 (20) | Fisher p=0.71 |
| Fully immunised | 25 (100) | 25 (100) | Fisher p=1 |
| Penicillin allergy | 1 (4) | 0 (0) | Fisher p=1 |

Antibiotic exposure at baseline

The median number of acute courses of antibiotics before the study in the combined comparison groups was 7 (IQR 4-10) compared to a median of 10 courses (IQR 6-13) in the combined azithromycin group. Poisson regression analysis determined the mean number of courses of antibiotics prescribed over both groups to be 8.6 with a variance of 26.93, indicating over-dispersion (the presence of higher variation than expected when using Poisson regression). Given that the mean and variance were not equal, negative binomial regression (NBR) was used instead. NBR is a generalisation of Poisson regression that corrects for over dispersion. See Table 23 for the antibiotic exposure at baseline for the combined studies.

**Table 23:** Antibiotic exposure at baseline for PBB studies 1 and 2

|  |  |  |  |
| --- | --- | --- | --- |
| **Exposure** | **Comparison group**  **Median (25th-75th)** | **Azithromycin group**  **Median (25th-75th)** | **Statistics** |
| Acute courses of antibiotics before study enrolment | 7 (4 – 10) | 10 (6 – 13) | IRR, p = 0.037 |
| Antibiotics >2 weeks duration before study enrolment | 1 (1 – 2) | 2 (2 – 3) | IRR, p = 0.070 |
| Time from last course of antibiotics | 11 (2 – 25) | 5 (4 – 21) | Fisher’s  p = 0.737 |
| Previous macrolide use before study enrolment | 15 out of 25 (56%) | 13 out of 25 (52%) | Fisher’s p = 0.776 |

Using NBR, participants in the combined azithromycin groups at baseline were found to have been prescribed more courses of antibiotics than those in the combined comparison groups with an incident rate ratio of 1.38 (95%CI 1.02-1.89), p= 0.037. However participants in the merged azithromycin groups were older and when correcting for age, the difference was not significant, p=0.12.

When reviewing the number of prescriptions of antibiotics for a duration of > 2 weeks (presumed treatment for exacerbations of PBB), the combined azithromycin groups trended towards a higher exposure, IRR 1.42 (95%CI 0.97-2.08), but this was not statistically significant, p=0.07. When adjusting for age at the start of the study, the IRR did not change 0.073.

The median time from the last course of antibiotics in the merged comparison groups was 11 weeks (IQR 2-25) and 5 weeks in the merged azithromycin groups (IQR 4-21). The difference in means was not significant using a T test, p=0.737. Of the participants in the comparison groups, 14 out of 25 (56%) has previously been prescribed a macrolide. In the azithromycin group, 13 out of 25 (52%) had previously been prescribed either an acute course or long-term macrolide therapy.

### 4.4.2 Swab results

At baseline, 25 swabs were collected in each merged group (Table 24). There was a non-significant trend towards fewer bacterial isolates being cultured in the combined azithromycin group, IRR 0.69 (95%CI 0.40-118), p=0.18. Of these isolates, slightly fewer were resistant to azithromycin in the azithromycin group although again this was not significant, IRR 0.71 (95%CI 0.35-1.43), p=0.34.

Participants in the merged azithromycin groups were prescribed long-term azithromycin for a median of 7 months (IQR 4.5-12 months) over the study period. During the study a total of 77 swabs were collected in the merged comparison groups and 64 in the merged azithromycin groups (Table 25). Significantly fewer bacteria were isolated from swabs collected in the azithromycin groups, IRR 0.58 (95%CI 0.34-0.98) p= 0.044. Of these bacteria, there was a non-significant trend towards fewer azithromycin resistant bacteria being present in the azithromycin group, IRR 0.63 (95%CI 0.37-1.06) p= 0.083. Adjusting for the number of bacteria isolated did not change this lack of significance (p=0.68) nor did adjustment for duration in the study (p=0.67).

**Table 24**: Combined baseline swab results from PBB studies 1 & 2

|  |  |  |  |
| --- | --- | --- | --- |
| **Baseline investigations** | **Comparison Groups**  N=25 | **Azithromycin Groups**  N=25 | **Statistics**  IRR |
| Total number of swabs collected | 25 | 25 |  |
| Number of swabs with positive bacterial growth | 19 | 16 |  |
| Number of swabs with azithromycin resistant bacteria  (% of baseline swabs with positive bacterial growth) | 14  (73.7%) | 11  (68.8%) |  |
| Number of bacterial isolates | 34 | 24 | p = 0.18 |
| Number of azithromycin resistant bacterial isolates  (% of azithromycin resistant bacterial isolates) | 18  (52.9%) | 14  (58.3%) | p = 0.34 |

**Table 25**: Combined swab result data during PBB studies 1 & 2

|  |  |  |  |
| --- | --- | --- | --- |
| **During study: Investigations** | **Comparison groups** | **Azithromycin groups** | **Statistics** |
| Total number of swabs collected | 77 | 64 |  |
| Number of swabs with positive bacterial growth | 49 | 26 |  |
| Number of swabs with azithromycin resistant bacteria  (% of swabs during study with positive bacterial growth) | 34 (69.4%) | 19 (73.1%) |  |
| Number of bacterial isolates | 72 | 38 | IRR, p = 0.044 |
| Number of azithromycin resistant bacterial isolates  (% of azithromycin resistant bacterial isolates) | 44  (61.1%) | 26  (68.4%) | IRR, p = 0.083 |

### 4.4.2 Combined MIC data

The MIC data from studies PBB1 and PBB2 build upon each other making the unimodal distribution clearer in the isolates *H. influenzae* and *M. catarrhalis* (Figure 4‑12). For these isolates, the median value equates to the MIC cut off for denoting resistant bacteria. All but 2 of the *S. pneumoniae* isolates were classed as azithromycin resistant by the EUCAST criteria (EUCAST, 2022). The median MIC for these isolates was 1.5mg/L (3-fold higher than the breakpoint) with the majority of isolates having an MIC in the range of 1-2mg/L. In these *S. pneumoniae* isolates, a second smaller peak of highly resistant isolates (MIC >256mg/L) was found. Few *S. aureus* isolates were collected with a broad range of azithromycin MICs.

1. MIC distribution of *Streptococcus pneumoniae* isolates (PBB Studies 1 and 2).
2. MIC distribution of *Haemophilus influenzae* isolates (PBB Studies 1 and 2).
3. MIC distribution of *Moraxella catarrhalis* isolates (PBB Studies 1 and 2).
4. MIC distribution of S. aureus isolates (PBB Studies 1 and 2).

**Figure 4‑12:** Distribution of azithromycin MICs for all bacterial isolates (PBB studies 1 and 2)

*Azithromycin MIC results for all the bacterial isolates obtained over PBB study 1 and 2. Azithromycin susceptibility was tested using E-test strips. Susceptibility was determined using the EUCAST break points for each isolate. The dotted red line indicates the breakpoint. Zero denotes isolates where an MIC was not tested due to laboratory errors. A : S. pneumoniae,*

*B : H. influenzae, C : M. catarrhalis, D : S. aureus.*

### 4.4.3 Antibiotic exposure during the study

The duration of long-term azithromycin for participants in the combined azithromycin groups was for a mean of 8.2 months (median 7 months, IQR 4.5 – 12).

During the study, there was a non-significant trend to fewer acute courses of antibiotics prescribed for presumed respiratory infection to those in the azithromycin group versus the comparison group, 54 versus 33 respectively (Table 26), IRR 0.69 (95%CI 0.41-1.14), p=0.15. There were also somewhat fewer prescriptions for courses of antibiotics >2 weeks in duration to treat presumed PBB exacerbations in the azithromycin group compared to the comparison group (Table 26), but again this was not significant, IRR 0.65 (0.27-1.55), p=0.33.

**Table 26**: Combined antibiotics prescribing data for PBB studies 1 & 2

|  |  |  |  |
| --- | --- | --- | --- |
| **Antibiotic prescribed** | **Comparison group**  N = 25 | **Azithromycin group**  N= 25 | **Statistics**  IRR |
| Amoxicillin | 21 | 14 |  |
| Azithromycin | 3 | 2 |  |
| Clarithromycin | 2 | 0 |  |
| Co-amoxiclav | 28 | 17 |  |
| Flucloxacillin (skin infection) | 2 | 1 |  |
| Penicillin (tonsilitis) | 1 | 2 |  |
| Trimethoprim (UTI) | 0 | 0 |  |
| Total acute antibiotic during study\* | 57 | 36 |  |
| Total antibiotics for presumed respiratory tract infections | 54 | 33 | p = 0.15 |
| Total courses of antibiotics > 2 weeks | 19 | 11\* | p = 0.33 |

*\* 7 courses prescribed whilst not on azithromycin prophylaxis. 4 courses prescribed to A15 whilst taking long-term antibiotics, initially azithromycin but changed to co-trimoxazole.*

Overall, these data showed our cohorts were well-matched, with those in the combined azithromycin groups at baseline having been prescribed more courses of antibiotics prior to study commencement than those in the combined comparison groups. There were significantly fewer bacterial isolates in total from swabs obtained from PBB patients in the combined azithromycin than the combined comparison groups, other comparisons did not reach significance (perhaps due to the limited sample size). The most notable finding from PBB studies 1 and 2 was the high level of apparent azithromycin resistance in *S. pneumoniae* isolates in all cohorts, and a decision was made to interrogate this further.

## PBB study 3: Extended antibiotic resistance testing of bacterial isolates

In PBB studies 1 and 2, baseline azithromycin resistance of bacteria isolated from DNPS was markedly higher than expected, a surprising and potentially concerning observation. Similar rates of resistant bacteria were seen throughout the study period in both groups and predominantly attributable to azithromycin resistant *S. pneumoniae*. Given the unexpectedly high resistance rate, the frozen isolates were re-cultured and tested against an extended antibiotic panel for antibiotic susceptibility.

### Phenotypic susceptibility testing

Of the 167 bacterial isolates from PBB studies 1 and 2, 159 were previously tested for azithromycin susceptibility using E-test strips (8 isolates were not tested due to laboratory errors). Of these 159 isolates, 100 (63%) were classed resistant to azithromycin. 95% of the *S. pneumoniae* isolates (55 of 58), 38% of the *H. influenzae* isolates (23 of 61), 32% of the *M. catarrhalis* isolates (11 of 34) and 79% of the *S. aureus* isolates (11 of 14) were categorised as resistant to azithromycin Figure 4‑12.

Of the 167 frozen bacterial isolates, 6 could not subsequently be located and were presumed to have been inadvertently discarded. Technicians at the STH laboratory were able to re-culture 150 of the remaining 161 isolates, see Tables 27-30. Although 55 of the 58 *S. pneumoniae* isolates were resistant to azithromycin, on testing for erythromycin susceptibility, only 5 were found to be resistant to erythromycin on disk diffusion susceptibility testing (zone diameter breakpoint of <19mm). Of the 54 *H. influenzae* isolates, 5 were resistant to erythromycin and 12 to ampicillin. Of the *S. aureus* isolates, 2 were erythromycin resistant and none were resistant to tetracycline.

**Table 27**: Antibiotic susceptibility of S. pneumoniae isolates

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Bacterial isolate | Antibiotic resistance | | | | | |
| AZM  \* | Tetracycline | Oxacillin | Chloramphenicol | Erythromycin | Levofloxacin |
| *S. pneumoniae*  N=55 | 54 | 3 | 5 | 0 | 5 | 0\*\* |

**\*** Of the 55 S. pneumoniae isolates, 30 were not susceptibility tested to levofloxacin due to laboratory error.

*\*\* AZM – Azithromycin*

Of the 5 erythromycin resistant isolates, 4 of these were susceptible to levofloxacin and 1 was not tested. All 5 were resistant to azithromycin. Beta-lactam resistance screening was performed using oxacillin with only 5 isolates being resistant to beta-lactams.

**Table 28**: Antibiotics susceptibility of H. influenzae isolates

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Bacterial isolate | Antibiotic resistance | | | | | | |
| AZM\* | Tetracycline and ciprofloxacin | Ampicillin | Chloram  phenicol | Erythro  \*\* | Cefuroxime | Co-amoxiclav |
| *H. influenzae*  N=54 | 19 | 0 | 12 | 2 | 5 | 1 | 7 |

*\*AZM – Azithromycin \*\*Erythro – Erythromycin*

**Table 29**: Antibiotic susceptibility of M. catarrhalis isolates

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Bacterial isolate | Antibiotic resistance | | | |
| Azithromycin | Tetracycline, Ciprofloxacin, Chloramphenicol, Cefuroxime | Erythromycin | Co-amoxiclav |
| *M. Catarrhalis*  N=30 | 9 | 0 | 2 | 2 |

**Table 30**: Antibiotic susceptibility of S. aureus isolates

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Bacterial isolate | Antibiotic resistance | | | | | | |
| AZM | Tetracycline, Ciprofloxacin  Vancomycin,  Chloramphenicol, Trimethoprim, Teicoplanin, Gentamicin, Rifampicin, Nitrofurantoin | Amoxicillin | Penicillin | Erythromycin | Fusidic acid | Oxacillin |
| *S. aureus*  N=11 | 6 | 0 | 4 | 9 | 2 | 1 | 8 |

*\*AZM – Azithromycin*

The number of *S. pneumoniae* isolates resistant to erythromycin was much lower than the azithromycin resistance previously found, 9% vs 95%. The 5 erythromycin resistant isolates were those previously found to have MICs to azithromycin of >256. A subset of 10 azithromycin resistant, *S. pneumoniae* isolates were therefore re-cultured again to see if the freezing process had selected for non-resistant strains. E-test for azithromycin susceptibility and disk diffusion and E-test for erythromycin susceptibility were carried out in parallel to further investigate this unexpected finding (see Table 31). Only 5 of the 10 isolates were able to be re-cultured on this occasion, the others did not grow. The repeat azithromycin MICs corresponded precisely with those previously measured for all 5 re-tested isolates (Table 31) with all classed as azithromycin resistant but erythromycin sensitive.

**Table 31**: Repeat parallel susceptibility testing of a subset of S. pneumoniae isolates

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Sample | \*AZM MIC mg/L  (first E-test strip) | Erythromycin Disk diffusion in mm (first) | \*AZM MIC mg/L  (2nd E-test strip) | Erythromycin Disk diffusion in mm  (2nd) | Erythromycin MIC  Resistant > 0.5mg/L  (E-test strip) |
| 1 | 1 | 31 | 1 | 31 | 0.25 |
| 2 | 1.5 | 31 | 1.5 | 31 | 0.19 |
| 3 | 2 | 29 | 2 | 29 | 0.125 |
| 4 | 0.75 | 30 | 0.75 | 30 | 0.25 |
| 5 | 2 | 29 | 2 | 29 | 0.25 |

*\*AZM – Azithromycin*

### 4.5.2 Genotypic profiles of *Streptococcus pneumoniae* sequences

The phenotypic resistance profiles demonstrated a major discrepancy between erythromycin and azithromycin resistance amongst the *S. pneumoniae* isolates, 5% vs 95% resistance respectively. In order to investigate this further, DNA was extracted from the *S. pneumoniae* isolates and sequenced to provide genomic data.

Of the 58 *S. pneumoniae isolates* obtained from PBB studies 1 and 2, 55 were able to be re-cultured at the microbiology laboratory in STH. Following this, 51 isolates (4 re-cultured samples could not be located by the STH microbiology laboratory) were transferred to the University of Sheffield for DNA extraction, with an extended lysis step to optimise DNA yield as described in section 2.4.4. Of these 51 samples, I was not able to re-culture 5 of the isolates. I extracted DNA from the 46 remaining *S. pneumoniae* isolates. The quality and concentration of DNA was measured using a nanodrop, Figure 4‑13. The median DNA concentration was 85.5 ng/µL with a median 260/280 ratio of 2.02 and median 260/230 ratio of 2.19. The slightly higher than optimal 260/280 ratio might have indicated minor RNA contamination of the sample although the 260/230 ration was reassuring.

Three samples had DNA concentrations of <10 ng/µl which was not sufficient for further analysis. From the total of 43 samples with adequate concentrations of DNA, 40 were selected to be sequenced by MicrobesNG. Participants with more than 1 sample were prioritised in order to investigate longitudinal changes. Of these, 37 were used in the final analysis. The three samples not included in the final analysis were due to the following reasons:

* Two samples had total contig lengths double that of *S. pneumoniae* suggesting possible contamination
* One sample was identified as *H. Influenzae* not *S. pneumoniae* (labelling error).

|  |
| --- |
|  |
|  |
|  |

**Figure 4‑13:** Quality of DNA extracted from S. pneumoniae isolates

*Box and whisker charts showing the concentration of DNA of the 40 samples sent to MicrobesNG for further analysis.*

Other samples passed the quality assessment of the genome assemblies as provided by MicrobesNG (Table 32). The genome of pneumococcus consists of ~2 – 2.2 million base pairs depending on the strain, with a GC coverage of around 39% (Nzoyikorera et al., 2021; Tettelin et al., 2001).

**Table 32**: Quality assessment of genome assemblies provided by MicrobesNG

|  |  |
| --- | --- |
|  | Median (25th-75th quartiles) |
| Number of contigs | 29 (24 – 44) |
| Largest contig | 374452 (322588 – 434645) |
| Total length | 2087469 (2051440 – 2119495) |
| GC(%) | 39.57 (39.54 – 39.62) |
| Mean Coverage (Standard deviation) | 80.34 (20.4) |

*A summary of the quality assurance of the 37 sequenced S. pneumoniae genomes. Descriptive analysis of sequencing showed the mean total length and GC(%) coverage to be consistent with that found in S. pneumoniae genomes. The number of contigs is the number of small fragments of DNA sequences that when overlapped, provide a continuous representation of the genomic region.*

### 4.5.3 Resistance gene screening

Mass screening of contigs for antimicrobial resistance genes using the databases NCBI and ResFinder found four isolates where known macrolide resistance genes were present (Table 33). All four of these isolates were highly resistant to both azithromycin (MIC >256) and erythromycin (inhibition zone <19mm). In three isolates the gene Erm(B) and tet(M) were present and the plasmid rep43US identified. In one isolate, the genes mef(A) and msr(D) were present with no identifiable plasmid. In 2 isolates with lower level azithromycin, the plasmid rep36 was identified.

### 4.5.4 Pangenomic studies

Pangenomic studies allowed the core genome of isolates to be identified using the application software ‘roary’. Following this, differences and similarities in the non-core genome between isolates could be analysed with ‘scoary’. Clinical data was then correlated to these data.

When selecting samples with high level azithromycin resistance (MIC ≥ 256 mg/L) compared to all other samples, one hypothetical protein sequence was positively identified in the 4 highly resistant samples and not identified in any of the other 33 samples; sensitivity 100%, specificity 100%. The Bonferoni p value was used as a conservative approach to control for multiple comparison corrections; p <0.00001. The sequence of this hypothetical protein was identified as (letters represent amino acids):

‘MTKELQSSRYIVISFLVREMGIDIVEAISLMAELEKSGLVRLESSGDLILKELGGAL’

This represents the nucleotide sequence of:

ATGACAAAAGAATTACAATCATCACGCTATATTGTCATTTCATTTTTAGTACGTGAAATGGGAATTGACA

TTGTTGAAGCCATCTCTCTTATGGCTGAATTAGAAAAAAGTGGCTTGGTTCGATTGGAATCAAGTGGAGA

TTTAATACTCAAAGAACTTGGAGGAGCGCTATGA

No other sequences were found that were specific to a group of samples when looking at the clinical indices of sex, azithromycin exposure or not, nor when groups of different azithromycin MICs was examined. The *Streptococcus Pneumoniae* Comparative System and PaperBLAST databases were searched and the protein identified as SPN23F13170 polypeptide, a putative uncharacterised protein consisting of 57 amino acids, present in the core *S. pneumoniae* genome.

The pneumococcal serotypes were identified for all 37 isolates. All but one of the isolates were non-vaccine serotypes. The single isolate identified as a vaccine serotype was 19A. Using the aligned sequence data, a phylogenetic tree was constructed to examine the relationship of samples with regards to serotype, year the sample was obtained and highlighting the 4 highly resistant isolates, Figure 4‑14. There is not a relationship between year of sample collected nor a closely related clade. The highly resistant samples group together, with 2 of these being from the same participant.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Study Number** | **Erythromycin**  **Resistant <19mm** | **Tetracycline resistant**  **<22mm** | **AZM MIC**  **Resistant >0.5mg/L** | **Res**  **Genes\*** | **Serotype\*\*** | **Plasmid**  **Finder \*\*\*** |
| A1 PBB1⁰ | 31 | 33 | 1 | 0 | 31 | 0 |
| A4 PBB1 | 6 | 24 | >256 | Erm(B) tet(M) | 33F | repUS43 |
| A5 PBB1 | 30 | 33 | 1.5 | 0 | 10A | 0 |
| A5 PBB1 | 31 | 33 | 1 | 0 | 8 | 0 |
| A6 PBB1 | 29 | 22 | 1 | 0 | 7C | 0 |
| A6 PBB1 | 30 | 34 | 1 | 0 | 19A | 0 |
| C1 PBB1 | 31 | 35 | 4 | 0 | 35B | 0 |
| C1 PBB1 | 29 | 33 | 0.75 | 0 | 11A | 0 |
| C5 PBB1 | 29 | 33 | 2 | 0 | 7C | 0 |
| C5 PBB1 | 31 | 37 | 1.5 | 0 | 23A | 0 |
| C6 PBB1 | 29 | 35 | 1.5 | 0 | 11A | 0 |
| C7 PBB1 | 31 | 34 | 1 | 0 | 23A | 0 |
| C7 PBB1 | 29 | 36 | 2 | 0 | 35F | 0 |
| C9 PBB1 | 31 | 35 | 4 | 0 | 31 | 0 |
| C9 PBB1 | 29 | 34 | 1 | 0 | 17F | 0 |
| C9 PBB1 | 30 | 35 | 1.5 | 0 | 6C | 0 |
| C10 PBB1 | 33 | 34 | 2 | 0 | 17F | 0 |
| C10 PBB1 | 30 | 36 | 1.5 | 0 | 35B | 0 |
| A1 PBB2 | 30 | 34 | 1.5 | 0 | 35F | 0 |
| A4 PBB2 | 28 | 31 | 1.5 | 0 | 21 | Rep36 |
| A5 PBB2 | 35 | 40 | 1.5 | 0 | 8 | 0 |
| A9 PBB2 | 6 | 11 | >256 | Tet(M)  erm(B) | 15A | repUS43 |
| A9 PBB2 | 6 | 12 | 256 | Erm(B) tet(M) | 15A | repUS43 |
| A13 PBB2 | 30 | 34 | 1 | 0 | 17F | 0 |
| A15 PBB2 | 27 | 31 | 1.5 | 0 | 23B | 0 |
| A15 PBB2 | 30 | 35 | 3 | 0 | 23A | 0 |
| C1 PBB2⁰⁰ | 29 | 32 | 1.5 | 0 | 15A | 0 |
| C1 PBB2 | 29 | 32 | 1.5 | 0 | 24 | 0 |
| C4 PBB2 | 29 | 33 | 1.5 | 0 | 6C | 0 |
| C4 PBB2 | 31 | 36 | 1.5 | 0 | 6C | 0 |
| C4 PBB2 | 29 | 33 | 1.5 | 0 | 6C | 0 |
| C9 PBB2 | 11 | 35 | >256 | Msr(D) mef(A) | 33F | 0 |
| C11 PBB2 | 28 | 32 | 2 | 0 | 35B | 0 |
| C6 PBB2 | 30 | 35 | 1.5 | 0 | 10A | 0 |
| C13 PBB2 | 30 | 35 | 1.5 | 0 | 7C | 0 |
| C5 PBB2 | 30 | 35 | 1.5 | 0 | 23A | 0 |
| C5 PBB2 | 29 | 32 | 1 | 0 | 21 | Rep36 |

**Table 33**:Results of mass screening of contigs for antimicrobial resistance genes

*\*Antimicrobial resistance genes identified using the databases NCBI and ResFinder*

*\*\* S. pneumoniae serotype identified using PneumoCAT software*

*\*\*Plasmids identified using the database PlasmidFinder*

*⁰A1 PBB1; Signifies that the participant was in the azithromycin group of PBB study 1*

*⁰⁰C1 PBB2; Signifies that the participant was in the comparison group of PBB study 2*

Chart

Description automatically generated

**Figure 4‑14:** Phylogenetic tree of the 37 S. pneumoniae isolates.

Key: Participant number\_PBBstudy\_year sample taken\_pneumococcal serotype\_resistance gene

*The 4 highly resistant isolates cluster together (highlighted in red). A single non-vaccine serotype was present (highlighted in blue). All other isolates were non vaccine serotypes.*

## Discussion of PBB studies

The 2 prospective observational studies we carried out have given further insight into the impact of long-term azithromycin use in children with PBB. We have been able to show trends into the nasopharyngeal carriage of antibiotic resistant bacteria, the prescribing of antibiotics and a high prevalence of azithromycin-resistant but erythromycin-sensitive isolates. Although the numbers recruited have been small and timing of some samples disrupted by COVID-19 restrictions, hurdles together limiting statistical analysis, the information gathered has been incorporated into further research protocols so that we can continue to investigate this common yet understudied condition and the impact of its treatment on bacterial flora.

### Feasibility and acceptability

These 2 studies have demonstrated that collecting serial nasopharyngeal swabs to monitor children with PBB is feasible and acceptable to the majority of parents and children. Out of the 50 children recruited, only 2 withdrew due to the distress of having swabs taken and the high swab burden. The recruitment aspect of the study protocol was refined, following the pilot study. Pre-screening potential recruits enabled a more targeted and efficient approach by the specialist nursing staff. This enabled successful age matching in the second study and will facilitate recruitment in future studies investigating children with PBB at SCH and potentially elsewhere.

The diaries documenting antibiotic treatment and compliance with prophylaxis were poorly completed by most families. Given that most families were unable to recall the number of acute antibiotics prescribed during the study period, it is unlikely that those who did not complete the diary could reliably remember only missing 2-3 doses of azithromycin. In those who did fill the diaries in, high adherence rates were noted. However, it is not known how accurately the families were when completing them. The main reason given for not filling the diary in was losing it, something that would be difficult to mitigate except by perhaps using an online or electronic reporting mechanism, which was beyond the scope/resourcing of our study. A review of assessing methods of measuring medication adherence in chronically ill children concluded that the perfect method to measure adherence does not exist (Al-Hassany et al., 2019). Questionnaires documenting medication compliance have been validated for specific chronic conditions such as asthma, epilepsy, diabetes mellitus and HIV. A general questionnaire can be applied to different chronic diseases, however all questionnaires have limitations related to recall and response bias. Electronic adherence measurement devices have been regarded as the “gold standard” of adherence measurement (Al-Hassany et al., 2019). In the case of oral medication Ingerski et al. suggest the use of an electronic vial opening device, however this still does not confirm that the medication was ingested (Ingerski et al., 2011). Azithromycin is mainly eliminated unchanged in the faeces via biliary excretion (Singlas, 1995). This makes therapeutic drug monitoring of stool samples a further possibility, although obtaining such samples presents its own challenges.

### DNPS culture

During the study period, significantly fewer swabs collected in the azithromycin group had a positive bacterial growth compared to swabs in the comparison group. This likely reflects long-term azithromycin supressing bacterial colonisation of the nasopharynx, but might also reflect inhibition of growth in culture conditions by antibiotic ‘carried over’ in the sample. The use of culture independent techniques to investigate bacterial communities may overcome some difficulties relating to bacterial culture and will be discussed in section 5.3. The clearance of bacterial species, including *S. pneumoniae*, *H. influenzae* and *M. catarrhalis*, from sputum and carriage within the nasopharynx, following exposure to long-term azithromycin is consistently reported in the literature (Abotsi et al., 2022; Hare et al., 2015b; Li et al., 2014). Our results of a reduction in bacterial isolates in azithromycin-treated patients are consistent with those reported in previous studies, albeit in other conditions. Investigators conducting the BREATHE trial (in children with HIV-associated lung disease) report that there was a significant reduction in bacterial carriage (sputum and nasopharyngeal swabs) at 48 weeks of treatment with azithromycin. This however was no longer seen 24 weeks following azithromycin cessation (Abotsi et al., 2022). Hare et al. also reported a partial recovery on stopping long-term azithromycin at a median of 170-190 days. Although the children in the azithromycin treatment groups did not take azithromycin continuously throughout the study, due to the low numbers of cultures and the fact that most exacerbations occur during the winter when prophylaxis is given, I did not analyse the numbers following azithromycin cessation separately; however there did not seem to be an obvious increase in positive isolates when azithromycin was discontinued (see Figures 4.3 and 4.8).

### Antibiotic prescribing

Fewer courses of antibiotics were prescribed over the study period in the azithromycin group compared to the comparison group, although this was not statistically significant. The IRR (incidence rate ratio) did however change from a value greater than 1 at baseline, to a value of less than 1 during the study. This indicates a change from an increased risk of antibiotic prescribing in the azithromycin group to a reduced risk of antibiotic prescribing, despite the p value not being significant. This change was seen both for the prescribing of any antibiotics as well as for antibiotics of 2 or more weeks duration for presumed PBB. Of the 11 courses of antibiotics prescribed for more than a 2 week duration in the azithromycin group, 7 were prescribed during periods ‘off’ long-term azithromycin, and the other 4 were all prescribed to a single participant who changed long-term antibiotics from azithromycin to co-trimoxazole. As discussed in section 1.3.2, in conditions such as non-cystic fibrosis bronchiectasis, current BTS guidelines recommend the use of long-term azithromycin to reduce pulmonary exacerbation frequency. The efficacy of long-term azithromycin use in PBB is not known and this finding is encouraging for clinical benefit but further research in this field is required. The trend towards fewer courses being prescribed in the azithromycin group may not have reached significance due to the small sample size and low number of prescriptions for 2 or more weeks during the study period. Our findings will help power future studies to enable more definitive results to be obtained.

It may be difficult to distinguish between an uncomplicated LRTI and an exacerbation of PBB in a child with previous PBB. Differentiating between viral and bacterial LRTIs may also be difficult. It is thought that many exacerbations of PBB develop following an acute viral lower respiratory tract infection associated with damage to the ciliated epithelium (Ishak et al., 2017). In keeping with this, 3 of 7 viral swabs we obtained at the beginning of a suspected exacerbation identified a plausible viral cause. Trial definitions of an acute LRTI (not associated with PBB) often include an acute onset of cough lasting less than 21 days with other symptoms or signs localising to the lower airways such as shortness of breath, sputum or pain with the majority of uncomplicated cases spontaneously resolving without the need for antibiotics (Little et al., 2021). The diagnosis of PBB requires the presence of a wet cough for >4 weeks and response to 2-4 weeks of an oral antibiotic (Kantar et al., 2017). The antibiotic of choice for PBB is usually co-amoxiclav as is recommended in the local SCH guidelines (appendix 2). The most frequently prescribed antibiotic in both azithromycin and comparison groups was co-amoxiclav, with 28 prescriptions in the comparison group versus 17 in the azithromycin group; however many of these were for just 1 week. Amoxicillin was the second most frequently prescribed antibiotic. Amoxicillin is recommended by NICE for the majority of common childhood infections including acute cough, otitis media and community acquired pneumonia. Our results may suggest that co-amoxiclav is frequently used as first line for standard respiratory tract infections in children with PBB, which maybe outside the recommended guidelines. It seems likely that either an inappropriate choice of antibiotic was being used for an uncomplicated infection, or the correct antibiotic was being used but for too short a time to treat an exacerbation of PBB. It would be helpful to discuss with GPs to investigate their understanding of the PBB guidelines and how/if they would distinguish between a simple LRTI and an exacerbation of PBB.

During the COVID-19 pandemic there are indications that increased empirical prescribing of antibiotics has been significant in some (Castro-Lopes et al., 2021; Ul Mustafa et al., 2021) but not all settings (Duffy et al., 2021). It is reported that the total consumption (community and hospital sector) of anti-bacterials for systemic use across the European Union and European Economic Areas decreased compared to 2019, a reduction from 19.9 to 16.4 defined daily doses per 1,000 inhabitants per day (ECDC, 2021). This was mainly due to reduced prescribing in the community sector (18.3% decrease) whereas in the hospital sector there was just a 4.5% decrease (ECDC, 2021). Similarly in the UK, the absolute number of community antibiotic prescriptions fell by 15.48% over the period April-August 2020 compared to the previous year. Given the absolute number of appointments over this time period was reduced the number of prescriptions was actually 6.71% higher than expected (Armitage et al., 2021). Qualitative interviews in UK GPs report that the threshold for prescribing antibiotics early in the pandemic reduced. This finding was influenced by an increase in remote consultations. Post-lockdown participants perceived their antibiotics prescribing returning to “normal” (pre-pandemic). A further contributing factor to reduced paediatric antibiotic prescribing over the pandemic was the significant reduction in paediatric respiratory tract illnesses over the national lockdowns due to markedly decreased exposure to infections. Paediatric hospital admissions reduced for all respiratory tract infections and all severe infections apart from pyelonephritis over the 12-months from March 2020 (Kadambari et al., 2022). In the Yorkshire Humber region there was a ~50% reduction in the number of cases admitted to hospital with pneumonia seen in all demographic subgroups including those with pre-existing conditions more likely to be admitted to hospital (Kadambari et al., 2022). Families whose children were deemed severe enough to require long-term azithromycin may have taken more precautions during the study to avoid getting respiratory tract infections compared to those not prescribed azithromycin, although this is not proven. At the beginning of the pandemic the use of azithromycin as treatment for COVID-19 was questioned. For a number of these reasons, the respiratory team continued the prescriptions of long-term azithromycin for PBB longer than they normally would. The mean duration of azithromycin in PBB study 1 (pre-pandemic) was 4.5 months compared to 10.3 months in PBB study 2. These factors may also have impacted on the number of PBB exacerbations seen during the study period particularly if the groups perceived the risk of COVID differently.

### Bacterial isolates and azithromycin resistance

The results from our studies do not support our hypothesis that those children exposed to long-term azithromycin would develop azithromycin resistant bacteria within their nasopharynxes. They are also not in keeping with current literature, where long-term azithromycin has been associated with increased likelihood of azithromycin resistance (Abotsi et al., 2022; Saiman et al., 2010; Samson et al., 2016; Valery et al., 2013). One of the factors underpinning our results is that baseline azithromycin resistance in both groups was much higher than expected. Bacteria classed as azithromycin-resistant were found in 16 of the 18 participants who had apparently never previously been exposed to macrolides (having reviewed their GP and hospital outpatient prescription records). This higher than expected azithromycin resistance may be particular to children with PBB (who are exposed to multiple courses of non-macrolide antibiotics), may represent high community carriage of azithromycin resistant bacteria, or could reflect a technical issue with the assay measuring azithromycin MIC.

Background rates of macrolide prescriptions in the 12-months prior to starting either of the studies was 8-15% of acute antibiotic prescriptions in the comparison group and 15-17% in the azithromycin group. This prescribing rate is just above the local primary care prescribing rate of 12.8% as a total of all antibiotic prescriptions (personal communication from Ian Hutchinson, prescribing data from Sheffield CCG 2015-2019). Only 4 children in the comparison group were prescribed a macrolide during the study period. Together this data suggests that macrolide exposure was not the driving force behind the azithromycin resistance we have reported. Other factors are potentially involved such as community transmission of resistant bacteria or development of resistance due to high exposure to other classes of antibiotics.

As expected, the two most prevalent species cultured were *H. influenzae* and *S. pneumoniae*.

UK carriage prevalence of non-typable *H. Influenzae* in children under 5yearswas reported to be 22.65% over the winter 2012/13 (Cleary et al., 2017). Many strains of *H. influenzae* exhibit low level intrinsic non-susceptibility to macrolides primarily due to the presence of an efflux pump homologous to the acrAB efflux pump in *E. coli* (Tristram et al., 2007). Occasional strains lack this efflux pump and are more susceptible to macrolides whereas others with ribosomal protein mutations have higher MICs (Tristram et al., 2007). Newer macrolides such as azithromycin have long half-lives and high tissue penetration. By achieving high intracellular concentrations, it is thought new generation macrolides may be useful antibiotics for community acquired infections caused by *H. influenzae* even in less susceptible strains (Euba et al., 2015). *H. influenzae* is generally thought to be predominantly an extracellular organism, suggesting this would not be a relevant fact, however newer data have suggested *H. influenzae* may in fact have the ability to persist specifically within macrophages (Ackland et al., 2021).

In 2005, the TARGETed surveillance programme monitoring antimicrobial resistance, reported that 97-100% of all strains of *H. influenzae* were susceptible to azithromycin (1,530 *H. influenzae* isolates analysed from six countries including France, Germany and Spain) (Rennie et al., 2005). More recently, emerging azithromycin resistant strains of *H. influenzae* have been reported. Data from the survey of antibiotic resistance (SOAR) 2014-2016 in Greece reported 5.8% of 52 *H. influenza*e isolates from community acquired respiratory infections were resistant to azithromycin (Torumkuney et al., 2018). Intermediate resistance was reported in 90.4%. This study used older EUCAST breakpoints of ≥8mg/L for azithromycin resistance and 0.25-4mg/L for intermediate susceptibility. Clarithromycin susceptibility was intermediate for 100% of these isolates. In comparison, the results from our study showed 19 % of *H. influenzae* isolates as being resistant (EUCAST cut off >4mg/L). This finding may be higher than the previous surveillance studies due to newer (and lower) breakpoint definitions being used. Those in the azithromycin prophylaxis groups showed a trend towards having a higher proportion of *H. influenzae* resistant isolates following exposure, but this did not reach significance, perhaps owing to the small sample size.

It should be noted that there is conflicting evidence for the efficacy of macrolides in *H. influenzae* respiratory infections due to spontaneous cure rates. Epidemiological cut-offs are therefore advised by EUCAST rather than clinical breakpoints. A clinical breakpoint predicts clinical success whereas an epidemiological cut off is the MIC that separates a population into isolates with and without acquired or mutational resistance (Lockhart et al., 2017). Where there is limited data on the correlation between MIC and clinical response, as is the case in *H. influenzae* due to spontaneous cure rates, epidemiological cut offs are provided rather than breakpoints (EUCAST, 2022).

The cohorts treated with azithromycin prophylaxis showed a reduction in the total number of isolates of *H. influenzae* detected during the study period compared to the comparison groups, 11 (28% of the isolates obtained during the study period) vs 25 respectively (35% of the isolates obtained during the study period). There is precedent for this finding. In a cohort of adults with severe asthma, the AMAZEs study found a significant reduction in *H. influenzae* load on sputum samples following 48 weeks of thrice-weekly azithromycin compared to placebo (Gibson et al., 2017). Valery et al also reported a reduction in *H. influenzae* isolates in children exposed to azithromycin for 12- 24 months (Valery et al., 2013). In this study, of the 15 children in the azithromycin group who isolated *H. Influenzae* at baseline, only 1 still carried it at their last visit; this compared to the placebo group where 9 isolated with *H. Influenzae* at baseline and 5 remained colonised at the last visit. This may suggest azithromycin prophylaxis can in some cases eliminate *H. influenaze* colonisation of the upper airways, although there is also the potential for spontaneous clearance; alternatively the initial isolates may represent transient rather than long-term colonisation with the azithromycin reducing the likelihood of re-colonisation.

*S. pneumoniae* is another common cause of childhood respiratory tract infections. Following the introduction of the 7-valent pneumococcal vaccine (PCV) in 2006 to the UK paediatric immunisation schedule, invasive pneumococcal disease caused by PCV7 serotypes reduced by 98% in children under 2 years ((Miller et al., 2011). In April 2010 the 7-valent vaccine was changed to a 13-valent vaccine. By 2016/17 PCV-7 type invasive pneumococcal disease had decreased by 97% in all ages compared to pre PCV-7 era and PCV-13 type invasive pneumococcal disease had decreased by 64% since following the introduction of PCV-13 (Ladhani et al., 2018). Invasive pneumococcal disease due to nonvaccine serotypes has increased although this is predominantly seen in adults (Ladhani et al., 2018). The dominant serotypes causing community acquired pneumonia in adults requiring hospitalisation have been reported to be serotypes 3 and 8 (Pick et al., 2020). Carriage of 2 serotypes, 3 and 19A have continued to still be detected in fully vaccinated children (Southern et al., 2018). The pneumococcal serotypes we report are consistent with the literature in that all but one of the serotypes were non PCV-13 serotypes apart from a single isolate that was 19A. All children in the study had been fully vaccinated as per the recommended UK immunisation schedule. Those noted to have a poor response to PCV13 were boosted and an adequate response subsequently documented. Pneumococcal vaccination is known to reduce nasopharyngeal colonisation density as well as colonisation with vaccine strains (O'Brien et al., 2007). Carriage rates following the introduction of PCV 13 in 2010 in the UK have been reported to be 30% in children under 4-years (Gladstone et al., 2015). This reflects our findings of carriage at baseline of 36% in the comparison groups and 32% in the azithromycin groups.

Azithromycin-resistant *S. pneumoniae* was much more common than anticipated in both groups; 94% and 95% in the comparison group and azithromycin group respectively. The baseline proportion of azithromycin resistant *S. pneumoniae* isolates was similar across groups with the subsequent spread of S*. pneumoniae* isolates relatively even throughout the study in the comparison group, suggesting that the use of long-term azithromycin was not driving this resistance. Data from the European centre of Disease Prevention and Control on macrolide (erythromycin/ clarithromycin/ azithromycin) resistant invasive strains of *S. pneumoniae* report UK levels of just 5.5% (ECDC, 2021). Only results using the EUCAST breakpoints and methodology are accepted. At SCH, erythromycin rather than azithromycin resistance is standardly reported – we used azithromycin resistance as we were specifically using azithromycin prophylaxis. Local data from sputum samples in children sent from Sheffield Children’s hospital between 2012 and 2015 report erythromycin resistant *S. pneumoniae* to have been cultured in 51 of 341 (14.9%) sputum samples (personal communication, Trevor Winstanley *Clinical Scientist and I.T. lead* *Department of Microbiology STH*). Given that sputum samples are generally not obtainable in those under 5 years this may not be representative of background resistance in younger children however it may be indicative of higher than expected rates of resistance locally. Data from sputum samples sent from children under 16-years in general practice over the same period showed no macrolide-resistant isolates of *S. pneumoniae* from 37 samples. Adult sputum samples over the same time period found growth of *S. pneumoniae* on 2385 samples. Of these 518 were erythromycin resistant, 21.7%. (personal communication, Trevor Winstanley *Clinical Scientist and I.T. lead* *Department of Microbiology STH).* In a UK adult prevalence and antibiotic resistance study, *S. pneumoniae* carriage was 1.8% with clarithromycin resistance of these isolates being 13.9% (95%CI 2.6-25.2) (Yahiaoui et al 2016). The apparently high levels of azithromycin resistance prompted us to undertake additional antibiotic sensitivity testing, supported by a pump priming grant from the Florey Institute.

### Extended antibiotic resistance

The extended phenotypic antibiotic resistance data and genotypic data enabled a more detailed investigation into the surprisingly high level of azithromycin resistant *S. pneumoniae* carriage within the nasopharynxes of children with PBB both exposed and unexposed to long-term azithromycin. Given that only 5 of the 55 azithromycin resistant *S. pneumoniae* isolates were resistant to erythromycin there were initial concerns that during the freezing and storage process, antibiotic resistance had been lost. By re-culturing a subset of the original azithromycin resistant *S. pneumoniae* isolates and carrying out in parallel susceptibility testing with azithromycin and erythromycin E test strips and erythromycin disk diffusion, we were able to confirm that the subset of the isolates re-tested were susceptible to erythromycin yet remained resistant to azithromycin. This suggested that there was not simple error in the original azithromycin E-test susceptibility testing. The use of E-test strips is a validated and widely used method for determining antibiotic resistance. In *S. pneumoniae* it has been reported that there is 98% agreement between E-test strips and a standard microbroth dilution method in determining erythromycin resistance (Jorgensen et al., 1991). Susceptibility testing can be affected by many factors including inoculum size, enrichment broth concentration and carbon dioxide. The use of ‘AB biodisk’ E-test strips were found to falsely overestimate *S. pneumoniae* MICs in the presence of carbon dioxide which also lowered the pH of the agar plates (Johnson et al., 1999). When retested in the absence of carbon dioxide, susceptibility testing by E-test and microbroth dilution were comparable. The diagnostic accuracy of E-test strips for susceptibility testing in *S. pneumoniae* has not been more recently updated. To note, the instruction manual for the BioMerieux E-test strips used to test for azithromycin resistance in the PBB studies, specifies that *S. pneumoniae* should be incubated in 4-6% CO2 when using BioMerieux E-test strips (BioMerieux, 2016). *S. pneumoniae* was incubated in 5% CO2 for the PBB studies hence it is unlikely that this a source of error. The margins of the zone diameters to determine erythromycin susceptibility of *S. pneumoniae* using disk diffusion in our study were not small and therefore not likely to have led to subjective measuring errors. A zone diameter of <19mm is used to determine resistance and a zone ≥ 22mm to determine susceptibility (EUCAST, 2022). All susceptible samples had a zone diameter ≥ 27mm which is well above the 22mm breakpoint. These erythromycin susceptible isolates include isolates with an azithromycin MIC of 4 mg/L, representing an 8-fold increase in MIC over that which determines azithromycin resistance. Subjective measuring errors are therefore again not likely.

Literature reporting macrolide resistance in *S. pneumoniae* rarely document comparisons between individual macrolides given that erythromycin resistance has been said to be a valid surrogate marker for other macrolide resistance (EUCAST, 2022). Mosleh et al. investigated local *S. pneumoniae* macrolide resistance in Hamadan (Iran). They report that of the 55 *S. pneumoniae* isolates obtained from clinical samples, 25.5% were resistant to erythromycin, 18.2% to clarithromycin and 16.4% resistant to azithromycin (susceptibility testing using E-tests) (Mosleh et al., 2014). Genotypic data from this study showed ermB to be present in 10.9% (6 samples) and mefA in 18.2% (10 samples). The Survey of Antibiotic Resistance (SOAR) is an international antimicrobial surveillance study focussing on respiratory pathogens across a variety of countries in the Middle East, Latin America and Asia-Pacific. Pakistani macrolide resistance data in *S. pneumoniae* isolates obtained from non-hospitalised patients with community acquired respiratory tract infections, was reported in 2004-2006 for 3 macrolides with 12.8% erythromycin resistance, 9.8% azithromycin resistance and 9.2% clarithromycin resistance (180 samples analysed using E-test strips) (Zafar et al., 2016). SOAR data from the Kuwait and Saudi Arabia 2015-2017 reported *S. pneumoniae* resistance to 3 macrolides; Kuwait erythromycin 58.%, azithromycin 57.3% and clarithromycin 57.3% resistance (82 samples using broth dilution); Saudi Arabia, all 3 macrolides 47.2% resistance (36 samples) (Torumkuney et al., 2020). Thus across different countries and different time points, only slight discrepancies between the percentage of resistant *S. pneumoniae* isolates are reported for the 3 different macrolides with isolates generally being more resistant to erythromycin than later generation macrolides such as azithromycin. The finding from PBB study 3 of apparent azithromycin resistance together with erythromycin sensitivity is thus very unexpected.

As per the EUCAST guidelines, erythromycin susceptibility in *S. pneumoniae* can be used to infer susceptibility to other macrolides *(EUCAST, 2021)*. A 2 fold increase in MIC from 0.5 to 1mg/L may not be clinically relevant given that there is intracellular accumulation of azithromycin in cells such alveolar macrophages and the fact that MICs are set conservatively. Knowing that there is an 8-fold increase in the azithromycin MIC might however change the choice of antibiotic used (personal communication, Professor David Partridge, STH microbiology, who is responsible for clinical advise regarding antibiotic choices based on MIC data). From an antimicrobial stewardship perspective, the use of a potentially subtherapeutic antibiotic could further drive azithromycin resistance. This is particularly pertinent given the increased use of azithromycin during the COVID-19 pandemic (Abdelmalek et al., 2022) (Taylor, 2021), although current evidence now suggests that azithromycin should not be used in the treatment of COVID-19 (Hinks et al., 2021; Oldenburg et al., 2021). Due to the potential clinical relevance, the results of this discrepancy between erythromycin and azithromycin resistance in the *S. pneumoniae* isolates has been extensively discussed with the local microbiology team at STH. Given erythromycin is used as a surrogate marker for azithromycin resistance, and azithromycin is increasingly used in many settings, these data were felt to have potential direct clinical relevance. As such, the microbiology department have contacted the UK Health Security Agency in order to take this further.

The mechanism of the low level azithromycin resistance in the erythromycin sensitive strains of *S. pneumoniae* is currently unexplained – no mutations were identified that might underpin this finding. Although I was unable to find a precedent in *S. pneumoniae*, in a study of *Legionella pneumophila* isolates from China, all 149 strains examined were fully sensitive to erythromycin but 25 of the strains exhibited azithromycin resistance (Jia et al., 2019). Increased expression of efflux pump component lpeAB was responsible for the reduced azithromycin susceptibility in all 25 of these strains, with mutations identified in some but not all of these strains (Jia et al., 2019). In a previous study of this efflux determinant (Massip et al., 2017) mutations in the upstream sequence of lpeAB operon were associated with an increased protein expression, however increased expression was also observed under sub-inhibitory macrolide concentrations in strains with both non-mutated promoting regions. Thus it is possible that increased expression of (as yet unidentified) resistance determinants in *S. pneumoniae* might be promoted by exposure to azithromycin in the absence of mutations. Bacterial genes that change transcriptionally upon stress exposure have been investigated as potential diagnostics to predict antibiotic sensitivity, but are context dependent (i.e. dependent on the bacterial species and the precise stressor). It would have been of interest to explore the transcriptome of the azithromycin-resistant isolates, but unfortunately I did not have time or funding to undertake this.

The four *S. pneumoniae*  isolates that demonstrated high levels of azithromycin resistance were also fully resistant to erythromycin. These exhibited known genes responsible for macrolide resistance, erm(B), mef(A) and msr(D) as described in section 1.3. The plasmid repUS43 was found in the 3 isolates with erm(B) genes. No further analysis was conducted on the plasmid to define the exact structure. The presence of plasmid rep36 in 2 isolates without any resistance genes identified is of unknown significance and was not investigated further. A hypothetical protein was also identified in these 4 highly resistant isolates but not seen in any of the other isolates tested. This hypothetical protein, SPN23F13170 is largely unstudied. A single study investigating the regulation of the *S. pneumoniae* virulence factor, cytolytic toxin pneumolysin (Ply) describe the location of SPN23F13170 on the mobile genetic element ICESp23FST81. Its position is at the end of the linear chromosomal form of ICESp23FST81 (Stevens et al., 2020) however a functional effect cannot be interpreted from this. Integrated and conjugate elements (ICE) are thought to be important to the success of *S. pneumoniae* due to the antibiotic resistance capabilities they bring to the bacteria and are likely to contribute a significant proportion of the *S. pneumoniae* pan-genome (Croucher et al., 2009). The role and function of this protein are as yet unclear and further studies are likely to be needed to determine whether this finding is reproduced when other resistant isolates are examined.

### Limitations of PBB studies

Due to the paucity of literature surrounding the use of long-term azithromycin in PBB, a power calculation was not possible. The small number of participants recruited means the results need to be interpreted with caution. Trends of resistant organisms and antibiotic prescribing reported in this study may therefore not be representative of actual patterns within the cohort of children with PBB and may have occurred by chance. Our results could be used to power a larger study.

Participants in the azithromycin groups were somewhat older than those in the comparison groups although this difference was not significant. Nasopharyngeal colonisation varies with age. With regards to *S. pneumoniae*, colonisation is inversely related to age and affected by numerous independent risk factors including ethnicity, day-care visits, family size, smoking, vaccinations and recent antibiotic use (Bogaert et al., 2004b; Rose et al., 2021). Colonisation rates increase over the few years of life, peak around 3 years and then decline with a stable prevalence observed after 10 years of age (Bogaert et al., 2004b). Up to 27-65% of children are carriers of S*. pneumoniae* (Abdullahi et al., 2012; Yahiaoui et al., 2016). Thus the reduced carriage of nasopharyngeal bacteria in the azithromycin group could in part be attributable to the older age of children within this cohort. The participants prescribed long-term azithromycin were likely to have had more severe disease than those in the comparison group given the fact long-term antibiotics were deemed necessary. They had also had more antibiotics prescribed at baseline although this difference was not significant when adjusting for the differences in age between the 2 groups, again showing the potential for age differences to confound the study.

These two studies were not designed to investigate the efficacy of azithromycin with regards to reducing exacerbations of PBB. It was presumed that courses of antibiotics prescribed for 2 or more weeks would be to treat exacerbations of PBB. This was confirmed by reviewing hospital records in prescriptions that were issued from the Children’s hospital. It was however not possible to confirm the diagnosis resulting in the prescription of a course of antibiotics for 2 or more weeks issued by a GP. Ethical approval was not sought to review full GP records, only prescription records. Participant antibiotic records may also not be complete as prescriptions could not be obtained from out of hours GP practices or emergency departments and hospital wards other than at SCH. Poor recall of courses of antibiotics during the study period further compounds the reliability of this outcome. An intention to treat (ITT) analysis (reviewed by (Gupta, 2011)) was used. ITT analysis (“once randomised, always analysed”) reflects the practical clinical scenario as it allows analysis of noncompliant patients and those with protocol deviations. ITT analysis gives an unbiased estimate of treatment effect, minimising type I error (false positive results) but it may increase the risk of type II (false negative) error. Because of the small sample size I did not undertake additional per protocol (PP) analysis (our protocol allowed starting antibiotics on clinical grounds after study commencement). Two participants in the comparison group in PBB study 2 were started on long-term antibiotics prophylaxis due to frequent exacerbations. Azithromycin was used in one case and in the other co-trimoxazole as azithromycin was not tolerated. One of these participants required the most number of acute courses of antibiotics during the study (five courses). This patient was analysed as being in the comparison group and hence increased the number of additional antibiotics in this setting despite taking prophylaxis (albeit not azithromycin) – since the number of ‘rescue’ antibiotics did not differ significantly between the 2 groups the ITT analysis is unlikely to have affected this outcome.

The initial decision to investigate isolates for azithromycin resistance only in PBB studies 1 and 2, was based on a focussed approach anticipating that azithromycin-resistant bacteria would be present if exposed to long-term azithromycin. PBB study 1 was a pilot study with limited funding and hence only 1 sensitivity test could be supported. We recognised at the outset however, that this approach imposed a limitation on the studies. Participants were exposed to high numbers of acute antibiotics courses both before and during the study. The majority of these were beta-lactams, and as such could have contributed to the presence of non-macrolide antibiotic resistance. We subsequently obtained funding to carry out extended phenotypic susceptibility testing to a broader range of antibiotics. The workflow for the processing of laboratory samples at STH was not optimal given that 6 samples did not have initial azithromycin susceptibility testing performed and 1 swab was lost. Some samples did not get stored as planned after initial testing. Part of the issue was that I was unable to access the laboratory to undertake the studies myself due to COVID restrictions on staff numbers within the laboratory space; this meant that a number of very busy staff with multiple other tasks undertook this work only when time permitted, rather than a single dedicated individual. This highlights an area for future study protocols to be addressed in order to ensure the appropriate processing of all study samples.

### Impact of the COVID pandemic

Due to the COVID pandemic, a number of unanticipated challenges arose, causing substantial disruption to the PBB study 2 design. Firstly, the planned number of nasopharyngeal swabs at specific time points could not be collected due to national lockdown restrictions. This resulted in a smaller number of DNPS being collected, and samples that were planned to have been taken whilst children were actively on long-term azithromycin could not be obtained. There were longer periods of time between contact with the participating families that meant family engagement with the study reduced and information regarding acute antibiotics, adherence to azithromycin and exacerbations of PBB was not as detailed. The lockdown restrictions, school and nursey closures, shielding requirements and general isolation of individual families resulted in less respiratory viruses and changes in the prescribing of both acute and long-term antibiotics (discussed in section 4.6.3) as well as potentially less transmission of nasopharyngeal bacteria to (and from) study participants. These factors may have also increased the differences between the 2 groups given those on long-term azithromycin were potentially seen as more vulnerable by parents and potentially had stricter isolation than those in the comparison group. This may reduce the generalisability of our results to children with PBB who are not subjected to periods of isolation. The STH laboratory had significant delays in processing the extended phenotypic culture results due to staff shortages with COVID as well as prioritising COVID related samples. Given the significant delays, there was insufficient time for me to perform PCRs for a range of antibiotic resistance genes, and the samples were instead sent for genome sequencing at MicrobesNG. This did provide more data, however only the S. pneumoniae samples (a subset of these isolates) could be analysed due to the increased cost of sequencing.

The ideal methodology to obtain definitive data would be to conduct an adequately powered randomised placebo-controlled trial to ensure homogeneity of the azithromycin and control groups. It was not thought that parents would accept a placebo and placebo medications are expensive to formulate. Informally, the majority of parents report they feel they have come to the end of all treatment options by the time prophylactic antibiotics are discussed and this would make it difficult to enrol patients into a trial of with a placebo or no prophylactic therapy arm. Data from observational studies may however inform future larger trials and multi-site trials might enable sufficient recruitment for a randomised study with a placebo group.

# **Chapter 5. Azithromycin and the nasopharyngeal microbiome**

## Introduction

Long-term azithromycin prophylaxis has been used for many years to reduce pulmonary exacerbations in cystic fibrosis and non-cystic fibrosis bronchiectasis (section 1.5). There is increasing interest in the use of long-term azithromycin as an immunomodulatory as well as antimicrobial agent, and more recent trials have reviewed its efficacy in primary ciliary dyskinesia, primary immune deficiencies, asthma, HIV and non-respiratory indications such as nephrotic syndrome and inflammatory bowel diseases with varying results (Ferrand et al., 2020; Gibson et al., 2019; Kobbernagel et al., 2020; Levine et al., 2019; Sawires et al., 2019). A current RCT (the PARROT study) is investigating the use of long term azithromycin (1 year) in children with neurological impairment to reduce hospital admissions with chest infections. In PBB there is little evidence to date to support its use.

Previous studies have reported on the development of macrolide resistance following long-term macrolide exposure (Abotsi et al., 2022), yet few have reported the effects on the respiratory microbiota. Adult studies have reported alterations in the composition of airway microbiota in asthma and non-cystic fibrosis bronchiectasis following long-term macrolide exposure (Rogers et al., 2014; Slater et al., 2014). The airway microbiota in children and adults with the same respiratory disease however differ (van der Gast et al., 2014). It is therefore imperative that research into the paediatric microbiota is carried out. This is particularly important during periods where the microbiota is developing such as in preschool children.

Pertinent questions that might be considered include whether baseline microbiota composition can identify those that will respond best to azithromycin in order to personalise treatment and whether potential alterations within the respiratory microbiota influence future exacerbations whilst taking long term azithromycin or on cessation (Acosta et al., 2021). The impact on other microbiota such as the gut would be important to consider yet outside the remit of this thesis.

The results presented from PBB studies 1 and 2 so far have focussed on the effects of long-term azithromycin on antibiotic resistance. PBB study 1 was a feasibility study and the resulting samples were not retained for microbiome analysis. To assess the broader impact of long-term azithromycin use in PBB, we also aimed to investigate the effects of prophylaxis on the nasopharyngeal microbiota.

I hypothesised that the diversity of bacteria residing within the nasopharynxes of children exposed to long-term azithromycin would be reduced compared to a comparison group not exposed to long-term antibiotics.

The primary objectives for the study were:

* To compare the nasopharyngeal microbiota composition of children with PBB taking long-term prophylactic azithromycin with an unexposed comparison group of children with PBB, through the use of 16s rRNA analysis.
* To investigate differences between the nasopharyngeal composition at the beginning and end of exacerbations of PBB

## Microbiome results

A total of 100 DNPS were collected in PBB study 2, 54 from participants in the comparison group and 46 from participants in the azithromycin group. Of these, 7 were taken around the times of pulmonary exacerbations and the others at regular intervals over the study period (although disruption due to COVID-19 restrictions affected the timing of samples and duration of prophylaxis). All the DNPS were frozen following bacterial culture. At the end of the sample collection, all the swabs were transported on dry ice to Professor Bogaert’s laboratory in Edinburgh, where DNA was extracted. Generation of a pooled PCR amplicon library was created through amplification of the V4 hypervariable region of the 16S rRNA gene by Professor Bogaert’s team. Sequencing was then performed at the Edinburgh genomics facility in order to characterise the nasopharyngeal microbiota. I undertook the following analyses with support from Professor Bogaert’s group.

### Quality control of 16S rRNA sequencing data using ‘decontam’ in R

The ‘phyloseq’ package in R was used to visualise the bacterial composition of the positive and negative control samples (2 positive mock community samples and 16 negative controls - 2 PCR blanks, 4 transport medium samples and 10 blanks containing relevant reagents). The mock community was produced by technicians at the University of Utrecht, Netherlands (collaborators with Professor Bogaert’s group). Selected bacteria are grown, DNA isolated and then pooled in specific proportions. The two PCR blanks were removed (see appendix 10 for bacterial composition details of control samples). One PCR blank contained *Haemophilus spp* and was removed (to ensure that an important respiratory bacterial species was not subsequently interpreted as a contaminant during the subsequent steps designating contaminant sequences). In total 7 samples and 4 control samples were removed prior to running ‘decontam’: 4 samples had insufficient DNA to proceed with further processing, 1 sample failed the ‘DADA2’ quality control (which models and corrects Illumina-sequenced amplicon errors), 1 duplicate sample and 1 sample with a low read count of 243 which was not thought to be able to reliably inform on the microbiota composition. Of the 4 controls samples removed, 2 were positive mock communities and 2 were PCR blanks.

Figure 5‑1 shows a flow chart of the quality control process with the number of nasopharyngeal samples that were initially processed through to the number of samples and taxa used in the final analysis.

‘Decontam’ was executed using the “combined” function in order to identify potential contaminants. This function uses the principle that contaminant sequences are likely to have a higher prevalence in negative controls than true samples and the fact that the frequency of contaminant sequences is expected to be inversely proportional to the DNA concentration (contaminating DNA will make up a larger fraction of the total DNA in samples with very little DNA). The generated list of contaminants was compared to a list of known potential contaminating genera (Salter et al., 2014). A selection of sequences were visualised to ensure the plots correlated to contaminants or true samples (Figure 5‑2). Following these 2 steps, the listed contaminants were removed from the samples set.

Finally, ultra-rare taxa that may only have been represented by a few reads in a couple of samples were removed as they are not thought to represent biologically meaningful bacteria. Only ASVs with a relative abundance of at least 0.1% in at least 2 samples were kept as suggested by Subramanian et al (Subramanian et al., 2014). A total of 2807 taxa were removed leaving 299 taxa to be used for the subsequent analysis. Given the total number of swabs obtained, the time interval between swab collection and the number of participants involved, the removal of 2807 out of 3106 taxa would is in keeping with a project of this size (Personal communication from PhD student, Justyna Binkowska and her team at the Centre for inflammation Research, University of Edinburgh). The filtering of rare taxa, that have a low contribution to the signal, can remove more than 70% of taxa thus reducing the complexity of microbiome data but retaining their integrity in downstream analysis (Cao et al., 2021).

**Figure 5‑1:** Sample processing pathway

*A flow chart showing the number of samples initially processed and the number removed during the quality control process*

*\*2 positive mock community samples and 16 negative controls (2 PCR blanks, 4 transport medium samples and 10 ISO\_blanks containing reagents)*

Chart, scatter chart

Description automatically generated

**Figure 5‑2:** ‘decontam’ output to visualise non-contaminant sequences and contaminant sequences

*Frequency of 9 Amplicon Sequence Variants (ASVs) in relationship to the DNA concentration of the samples. The 9 ASVs have been chosen in order to demonstrate the difference between contaminant ASVs and non-contaminant ASVs. The dashed black line shows the model of a noncontaminant sequence for which frequency is expected to be independent of the input DNA concentration. The red lines represent the model of a contaminant sequence for which the frequency is expected to be inversely proportional to the input DNA concentration. Caulobacter\_38, Curvibacter\_16, Pseudomonas\_31 and Thermus\_36 are all inversely proportional to the DNA concentration and identified as contaminants. The other ASVs are not contaminants as they are not inversely proportional to the DNA concentration.*

### Microbial composition of nasopharyngeal microbiota

There are different ways to determine the origin of a sequence following 16s rRNA targeted microbiome sequencing. Operational Taxonomic Unit (OTU) clustering is one metagenomic analysis approach that categorises bacteria based on sequence similarity, whereby each cluster represents a taxonomic unit of species or genus. ASV analysis is a more recent approach that determines the exact sequences recovered from high throughput gene marker (such as 16s rRNA). Rather than clustering, an error model is used to determine the probability of sequence errors. ASVs infer true biological origin as opposed to a clustering approach and is the method we have used in this study.

A descriptive overview of the microbial composition of all the DNPS can be seen in the stacked bar charts in Figure 5‑3 and Figure 5‑4. The 15 most abundant Amplicon Sequence Variants (ASVs) in each selection of samples are presented. The naming system of the ASVs indicates firstly the genus or species if this was able to be identified, i.e. Moraxella \_catarrhalis, or Moraxella (species) when the species could not be matched. Secondly, there is a numerical value following the taxonomy level. This reports how abundant that particular genus/species is across all the study samples.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Time between swabs | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | C9 | C10 | C11 | C12 | C13 | C14 | C15 |
| 1st-2nd | Co1w | A1w | A1w  Co4W  Co4W | A1w  P10d | Co2w  A1w | Co2w | No | A1w | A1w  Co4w | Co6w  Az1d  CoPx | None | Az2w | None | A1w | T1w |
| 2nd-3rd | None | None | Co2W | None | None | Co2w | Co1w  Az3m | None | Azm  Left study | CoPx | None | None | None | Co1w | None |
| 3rd-4th | None | F1w | None | None | None | Co2w | C1wx4  Az1m | None | Left study | CoPx | None | Cl2w  Co3d | None | None | None |

A picture containing text, screenshot, cabinet

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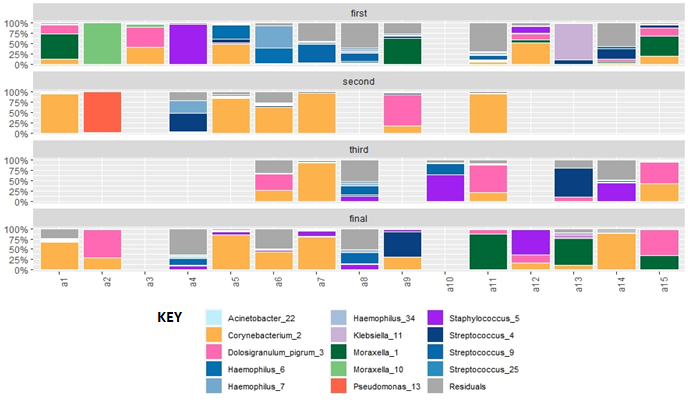
**Key**

|  |  |  |  |
| --- | --- | --- | --- |
| **Abbreviation** | **Name of antibiotic** | **Abbreviation** | **Name of antibiotic** |
| A | Amoxicillin | P | Penicillin V |
| Az | Azithromycin | T | Trimethoprim |
| Co | Co-amoxiclav | 3d | 3 days duration |
| CoPx | Co-amoxiclav prophylaxis | 1w | 1-week duration |
| F | Flucloxacillin | 1m | 1-month duration |

**Figure 5‑3**: Microbial composition of DNPS from the comparison group.

*The microbial composition of the top 15 most abundant taxa in the nasopharyngeal swabs collected from children in the comparison group. Baseline swabs are denoted by “first”. The 3 subsequent swabs taken over the study period are presented underneath in columns for each participant. Blank cells are present as some swabs were unable to be collected during the COVID-19 pandemic and 3 baseline swabs did not pass the quality control for 16s rRNA analysis. Swabs taken around exacerbations have been excluded.*

*The table below figure 5-3 shows the antibiotic exposure for each participant during the study period. C7 was started on long-term azithromycin. C10 commenced long term azithromycin but was unable to tolerate the taste and hence changed to co-trimoxazole after 1 dose. C9 started long term azithromycin however left the study before any additional swabs were taken.*



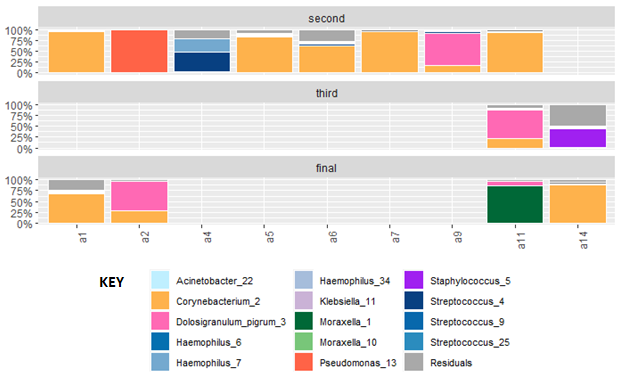
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Time of swab | A1 | A2 | A3 | A4 | A5 | A6 | A7 | A8 | A9 | A10 | A11 | A12 | A13 | A14 | A15 |
| 1st-2nd | None | None | ? | None | C0  1w+  IV | Clari  2w | None | None | None | None | None | None | None | None | Co2w |
| 2nd-3rd | None | None | LS | None | None | None | None | None | None | None | A1w | A1w | None | Co1w | None |
| 3rd-final | None | None | LS | A1w | None | None | Co2w  Co2w | None | None | Co2w | A1w  P10d | ? | AZM 3d | Pen  10d | Co2w  Co2w  Co4w |

|  |  |  |  |
| --- | --- | --- | --- |
| **Abbreviation** | **Name of antibiotic** | **Abbreviation** | **Name of antibiotic** |
| A | Amoxicillin | 3d | 3 days duration |
| Az | Azithromycin | 1w | 1 week duration |
| Clari | Clarithromycin | 1m | 1-month duration |
| Co | Co-amoxiclav | Ls | Left study |
| P | Penicillin | ? | Incomplete data |

**Figure 5‑4**: Microbial composition of DNPS from the azithromycin group

*The microbial composition of the top 15 most abundant taxa in the nasopharyngeal swabs collected from children in the azithromycin group. Baseline swabs are denoted by “first”. The 3 subsequent swabs taken over the study period are presented underneath in columns for each participant. Blank cells are present as some swabs were unable to be collected during the COVID-19 pandemic and 1 baseline swabs did not pass the quality control for 16s rRNA analysis. Swabs taken around exacerbations have been excluded. The table below figure 5-4 shows the antibiotic exposure for each participant during the study period. A3 left the study before their ethical approval was obtained to review the GP records and A12 did not consent to contacting the GP for medication records.*

At baseline there was a mixed microbial composition within the comparison group however by the study end, there was a trend towards greater dominance of *Moraxella* *spp.* and *Dolsigranulum pignum.* The noticeable outlier is participant C10, where a move to a *Staphylococcus*-dominant pattern is seen; this participant was commenced on long-term co-trimoxazole shortly after the commencement of the study for recurrent PBB and intolerance of azithromycin. Participant C7 was commenced on long-term azithromycin after the second swab and had the highest number of acute courses of antibiotics prescribed. In this participant there is a move towards a *Dolsigranulum pignum* dominant pattern seen. In the azithromycin group the composition was again relatively varied amongst participants at baseline, however by the second swab whilst on azithromycin there was a trend towards a predominance of *Corynebacterium species* that persists to the study end. This is seen more clearly in Figure 5‑5 showing only swabs collected whilst children were taking azithromycin at that point in time. As discussed in section 4.3.3, the duration of azithromycin exposure varied amongst participants, see Table 34 for details. The median duration of azithromycin exposure was 12 months (IQR 7.25-12 months), longer than the expected 6 month exposure over the winter months. Extending the study duration meant that the study period spanned 2 winters resulting in some participants having multiple exposures to long-term azithromycin. The time from cessation of azithromycin to the final swab also varied, median 0.9 months (IQR 0.1-6.4 months), again documented in more detail in Table 34.



**Figure 5‑5**: Microbial composition of the swabs taken whilst on azithromycin

*The microbial composition of the top 15 most abundant taxa in the nasopharyngeal swabs collected from children taking azithromycin at the time of swab collection. All other cells are left blank.*

**Table 34:** Details of azithromycin exposure and time to final swab following exposure in PBB study 2

|  |  |  |  |
| --- | --- | --- | --- |
| Participant number in the azithromycin group | Number of episodes on azithromycin | Duration of episodes for each participant (months) | Time off azithromycin before collection of the final swab for each participant\* |
| 1, 2, 5 | 1 | 12, 12, 12 | 0, 0, 3 weeks |
| 4, 6, 10, 13 | 1 | 5, 7.5, 7, 3 | 7m, 4.5m 8m, 14m |
| 11 | 1 | 17 | 0 |
| 15 | 1 | 2 | 13m but on co-trimoxazole |
| 7, 8, 9, 12 | 2 | 8+4, 6+6, 6+8, 8+8 | 1w, 1m, 1m, 3w |
| 14 | 3 | 6+6+0.25 | 0 - on AZM for 1 week pre swab |

*\* 0 = swab taken whilst still on azithromycin, w= weeks, m = months, 8+4 = 8 months with a break then 4 months*

*(Participant 3 = lost to follow up)*

### Differential abundance testing

Differential abundance testing, using the “metagenomeseq” model enables comparison of defined groups to see if differences in ASV relative abundance are significant. On analysis of the baseline swabs, *Moraxella\_lincolnii*\_19 was in lower abundance in the azithromycin group compared to the comparison group whereas *Moraxella\_*23 (*Moraxella* genus identified but not the species) was in higher abundance in the azithromycin group (Table 35 - A)

For the second set of swabs that were collected when children were still actively taking azithromycin, there was a significantly lower abundance of 4 ASVs seen in the azithromycin group that included *Moraxella*\_12 (See Table 35 - B).

On analysis of the final swabs, the largest logarithmic, statistically significant changes in ASV abundance seen in the azithromycin group compared to the comparison group, were a lower abundance of *Moraxella*\_1 and a higher abundance of *Corynebacterium*\_18. Table 35 - C, shows the three ASVs that were of a lower abundance in the azithromycin group and the 4 ASVs, with the largest logarithmic change that were of a higher abundance in the azithromycin group compared to the comparison group.

**Table 35**: Differential abundance testing between active and comparison groups at baseline, second and final sets of swabs

|  |  |  |
| --- | --- | --- |
| ASV | Logarithm of fold change | Adjusted p value |
| *Moraxella\_lincolnii*\_19 | -8.412139 | 0.001 |
| *Stenotrophomonas*\_47 | -7.294903 | <0.001 |
| *Moraxella*\_52 | -6.850686 | <0.001 |
| *Massilia*\_144 | 8.523 | <0.001 |
| *Haemophilus*\_8 | 9.105 | <0.001 |
| *Moraxella*\_23 | 11.946 | 0.001 |

1. Baseline swab

|  |  |  |
| --- | --- | --- |
| ASV | Logarithm of fold change | Adjusted p value |
| *Enterococcus*\_30 | -13.078 | 0.013 |
| *Corynebacterium*\_33 | -12.262 | 0.013 |
| *Moraxella*\_12 | -9.794 | 0.013 |
| *Haemopilus*\_56 | -8.603 | 0.023 |
| *Acinetobacter*\_44 | 1.354 | 0.087 |

1. Second set of swabs

|  |  |  |
| --- | --- | --- |
| ASV | Logarithm of fold change | Adjusted p value |
| *Moraxella*\_1 | -5.149 | 0.001 |
| *Dolosigranulum\_pigrum*\_3 | -3.594 | 0.004 |
| *Haemophilus*\_7 | -2.839 | 0.001 |
| *Actinobacillus*\_141 | 7.227 | 0.001 |
| *Corynebacterium*\_21 | 7.463 | 0.005 |
| *Porphyromonas*\_121 | 9.407 | <0.001 |
| *Corynebacterium*\_18 | 12.639 | <0.001 |

1. Final set of swabs

**Key**

Lower abundance taxa in the azithromycin group compared to the comparison group

Higher abundance taxa in the azithromycin group compared to the comparison group

### Alpha diversity of nasopharyngeal swabs

Alpha diversity is a measure of ‘within sample’ diversity. The Shannon index is a metric used to calculate alpha diversity, taking into account the sample richness (number of species) and evenness (relative abundance) of species within a community. Box and whisker plots of the Shannon indices for all the nasopharyngeal swabs are shown in Figure 5‑6. At baseline there was no significant difference in the Shannon index between the azithromycin and comparison group (Figure 5‑6 and Table 37). The diversity within samples collected whilst children were taking azithromycin during the second set of swabs was significantly (p=0.02) lower than those in the comparison group as determined using Fisher’s test (Figure 5-6 and Table 38). By the final swabs when 11 out of the 15 participants were no longer taking azithromycin, there was again no significant difference in alpha diversity between the azithromycin and comparison group.

|  |  |
| --- | --- |
| Chart, box and whisker chart  Description automatically generated   1. Alpha diversity of swabs taken over the study period in the comparison group. | Chart, box and whisker chart  Description automatically generated   1. Alpha diversity of swabs taken over the study period in the azithromycin group. |

**Figure 5‑6:** Alpha diversity of swabs taken from participants in both the azithromycin and comparison groups over the study period

*Shannon indexes have been plotted as a measure of alpha diversity for all the background swabs in both groups (exacerbation swabs excluded).* ***A)*** *shows the alpha diversity of the swabs in the comparison group and* ***B)*** *alpha diversity of the swabs in the azithromycin group. The x-axis represents the time of each round of swabs i.e., first, second, third and final rounds of swab collection.*

**Table 38**: Mean Shannon indexes for each of the 4 sets of swabs

|  |  |  |  |
| --- | --- | --- | --- |
|  | Mean Shannon index  Azithromycin group | Mean Shannon index  Comparison group | T test: p value |
| Baseline swabs | 1.436 | 1.167 | p = 0.375 |
| Second set of swabs | 0.625 | 1.712 | p = 0.020 |
| Third set of swabs | 1.482 | 1.012 | p = 0.221 |
| Final set of swabs | 1.332 | 0.996 | p = 0.219 |

### Beta diversity of nasopharyngeal swabs

Beta diversity is a measure of ‘between sample’ diversity. It quantifies differences in the microbial composition between samples. In order to assess this, a dissimilarity matrix is calculated from the ASV table. The composition of each sample is compared to all the other samples and assigned a numerically value. The Bray Curtis dissimilarity measure has been used to calculate this value. If two sites share all the same species at the same abundance, a numerically value of 0 is assigned whereas if two samples have completely difference species abundances, a value of 1 is assigned. Non-metric Multidimensional Scaling (NMDS) allowed for visualisation of how dissimilar samples are. Samples that are more similar to one another are represented by points that are closer together on the plot.

The mode of delivery, method of infant feeding and parental smoking are known factors that may influence the respiratory microbiota (Biesbroek et al., 2014; Pattenden et al., 2006; Reyman et al., 2019). In the azithromycin exposed group, 73% were delivered vaginally, 47% breast fed and 20% exposed to parental smoking, this compared respectively to 87%, 53% and 27% in the comparison group. We therefore investigated whether these exposures had a significant impact on beta diversity. At baseline there was no significant difference in beta diversity between the azithromycin and comparison group with regards to mode of delivery, method of infant feeding or presence of parental smoking (See appendix 11 for NMDS plots).

Figure 5‑7 shows the dissimilarity of the samples according to the time point at which they were taken. Only for the final set of swabs collected did being in the azithromycin group (versus the comparison group) significantly contribute to microbiota dissimilarity as assessed by permutational multivariate analysis of variance (azithromycin use calculated to account for 14 % of the variance in the microbiota composition), PERMANOVA model; R2=0.14, p=0.0049.

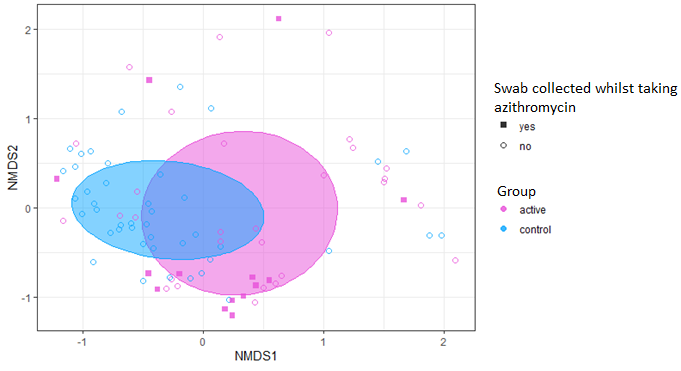
|  |  |
| --- | --- |
| Chart, bubble chart  Description automatically generated   1. Baseline swabs: R2 = 0.041, p= 0.391 | Chart, bubble chart  Description automatically generated   1. Second set of swabs: R2 = 0.093, p=0.096 |
| Chart, bubble chart  Description automatically generated   1. Third set of swabs: R2 = 0.093, p=0.153 | Chart, bubble chart  Description automatically generated   1. Final set of swabs: R2 = 0.141, p= 0.004 |

**Figure 5‑7:** Non-metric multi-dimensional scaling of beta diversity for DNPS collected from children in both comparison and azithromycin group

Azithromycin group Comparison group

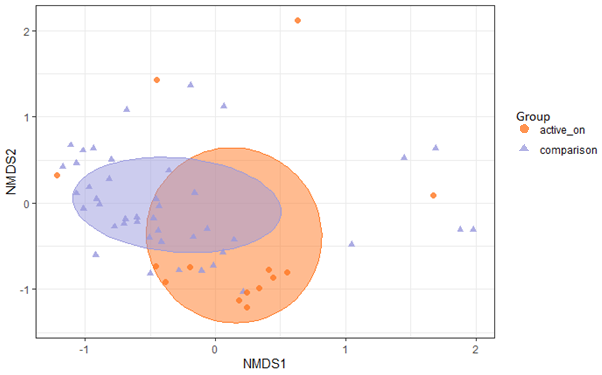
*Beta diversity has been calculated using the Bray-Curtis dissimilarity measure and plotted using Non-Metric Multidimensional Scaling (NMDS). Coloured plots indicate samples taken from participants either in the comparison (blue) or azithromycin group (orange).* ***A-D*** *show swabs taken at different time points over the study period. Significant dissimilarity was only found between the 2 groups in the final set of swabs taken.*

Active treatment with azithromycin (at the time of the swab) had a statistically significant effect on microbiota composition when compared to all individuals (both in the azithromycin group and comparison group) not currently taking azithromycin (PERMANOVA, R2=0.026, p= 0.01), Figure 5‑8. Pairwise models were used to further delineate this difference. Azithromycin treatment showed a more striking effect on the microbiota composition when those actively taking azithromycin at the time of swab collection were compared only to those individuals in the comparison group, R=0.079, p= 0. 001, Figure 5‑9. When those in the azithromycin group, actively taking azithromycin, were compared to individuals in the azithromycin group not on treatment at the time of swab collection, active treatment with azithromycin trended towards differences in the microbiota composition yet did not reach statistical significance; R= 0.04, p=0.085, Figure 5‑10.



**Figure 5‑8:** Non-metric multi-dimensional scaling of beta diversity for DNPS collected from children taking azithromycin at the time of the swab collection

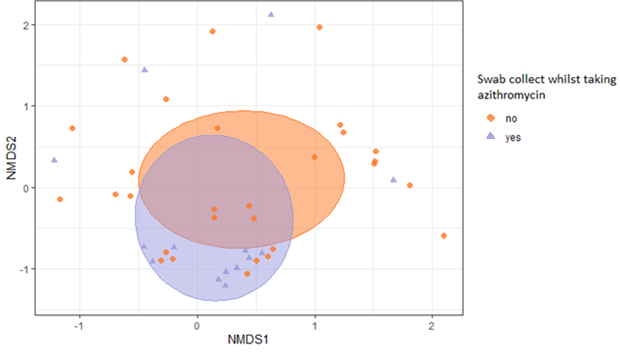
*Beta diversity has been calculated using the Bray-Curtis dissimilarity measure and plotted using Non-Metric Multidimensional Scaling (NMDS). Coloured plots indicate samples taken from participants in the comparison group, in the azithromycin group when actively taking azithromycin or in the azithromycin group when not actively taking azithromycin. Active treatment with azithromycin had a statistically significant effect on microbiota composition when compared to all other participants.* ***PERMANOVA; R2=0.026, p = 0.01***



**Figure 5‑9**: Non-metric multi-dimensional scaling of beta diversity: Those actively taking azithromycin compared to the comparison group

*Beta diversity has been calculated using the Bray-Curtis dissimilarity measure and plotted using Non-Metric Multidimensional Scaling (NMDS). Coloured plots indicate samples taken from participants in the azithromycin group, actively taking azithromycin at the time of swab collection, compared to those in the comparison group. Azithromycin treatment had a significant effect on the microbiota composition when those actively taking azithromycin at the time of swab collection were compared only to those individuals in the comparison group.*

***PERMANOVA;* R=0.079, p= 0. 001**



**Figure 5‑10**: Non-metric multi-dimensional scaling of beta diversity: Azithromycin group analysis

*Beta diversity has been calculated using the Bray-Curtis dissimilarity measure and plotted using Non-Metric Multidimensional Scaling (NMDS). Coloured plots indicate samples taken from participants in the azithromycin group, actively taking azithromycin at the time of swab collection, compared to those in the azithromycin group not taking azithromycin at the time of swab collection. Azithromycin treatment did not have a statistically significant difference on the microbiota composition when compared to those in the azithromycin group not exposed to azithromycin at the time of swab collection.*

***PERMANOVA;* R= 0.04, p=0.085**

## Discussion – Nasopharyngeal microbiota

This prospective observational study describes the impact of long-term azithromycin use on the nasopharyngeal microbiome in children with PBB compared to a cohort of children with PBB not exposed to long-term azithromycin. We hypothesised that the use of long-term azithromycin (6 months exposure over the winter months) would be associated with reduced diversity of the nasopharyngeal microbiome in this cohort of children. We did find a significant reduction in alpha diversity of the second set of nasopharyngeal swabs from children in the azithromycin group (who were actively taking azithromycin at the time of the second swab) when compared to the comparison group. This difference however resolved by the end of the study when the majority of children were no longer taking azithromycin. Due to the COVID-19 pandemic, the duration of azithromycin was extended past 6 months in some cases hence some participants were still actively taking azithromycin when collecting the final swabs (discussed in section 5.3.1 below). However, at the end of the study there was a significant difference in beta diversity between the two study groups. There was also a significant difference in beta diversity between children who were actively taking azithromycin at the time of swabbing (at any time throughout the study) versus children who were not taking azithromycin at the time of swabbing (incorporating the children from the comparison group AND those in the azithromycin group who had discontinued azithromycin)

At baseline, there was no significant difference between the groups with regards to alpha or beta diversity. Samples from both groups were often dominated by one or two ASVs. The majority included *Moraxella*, *Haemophilus* and *Corynebacterium* which are well documented to reside within the nasopharynxes of both healthy children and those with respiratory disease (Marsh et al., 2016; Raita et al., 2021; Sakwinska et al., 2014). Differential abundance testing did show significantly higher abundance of some ASVs in the azithromycin group when compared to the comparison group. The majority of these were however not in the top 15 most abundant ASVs across all samples but were still relatively highly abundant. The importance of these changes is unclear. There were however few significant changes of rarely abundant ASVs indicating that there was unlikely to be a high proportion of false-positives. On analysis of the second set of swabs, visualisation of the stacked bar charts suggested that a difference in *Corynbacterium*\_2 may have been expected. The difference may however not have been large enough to be statistically significant given the small sample size combined with the fact that only one sample (C5) had a predominance of this ASV. Multiple methods can be implemented when analysing differential abundance with associated strengths and weaknesses. However these methods do not take into account the compositional structure of the microbiome data (Lin et al., 2020). We utilised the “metagenomeseq” tool accepting that it has been reported to be associated with a higher false discover rate (Lin et al., 2020). Traditional indices of alpha diversity are also subject to advantages and disadvantages. It is not often possible to sample entire microbial environments such as the upper respiratory tract. As such smaller samples are collected from a large environment and inferences drawn. Willis suggests that samples in microbiome experiments do not faithfully represent the entire microbial community (Willis, 2019b). In order to draw meaningful conclusions when comparing microbial communities, a measurement error model should be used to adjust for the uncertainty of alpha diversity (Willis, 2019b). At present, such potential measurement errors are not routinely accounted for in microbiome studies. The Shannon index is widely used in microbiome analysis and was used in this study. It is acknowledged that this index may under-estimate true alpha diversity (Lande, 1996) but is less likely to give a false positive result.

Although having similar groups at baseline is a strength of the study in terms of assessing the impact of azithromycin, it does not suggest that stratification based upon the baseline nasopharyngeal microbiome, is able to differentiate children with PBB suffering from recurrent exacerbations that may require long-term azithromycin. Participants entering the azithromycin group in this study were required to have at least a 3-month wash period following any previous use of long-term antibiotics, however there were no exclusion criteria for the use of recent acute courses of antibiotics. This may have impacted upon the baseline microbiome given those in the azithromycin group had been prescribed slightly more courses of antibiotics than those in the comparison group and completed the last course of antibiotics slightly more recently (although not a significant difference between groups). Adult CF patients, demonstrating reduced lung function decline following long-term azithromycin and classified as responders, were also not found to have significant differences in their baseline sputum microbiota compared to non-responders. However, those that did respond and were naïve to azithromycin, were found to have a response-associated community profile on beta diversity analysis with sputum enriched with *Stenotrophomonas*, *Megasphaera* and *Abiotrophia* (Acosta et al., 2021). This was not reflected in our study, which is perhaps not surprising given PBB is a very different condition from CF in terms of pathogenesis, structural lung disease and lifetime treatment burden.

Few studies have analysed the impact of long-term antibiotics on the paediatric respiratory microbiome. An observational study in 32 children with CF (mean age 4.7months) reported that reduced bacterial diversity in the upper and lower airways was strongly associated with the use of prophylactic antibiotics (daily co-amoxiclav) and a younger age at sampling. Prophylactic antibiotics had however only been administered for half a month (+/- 0.9 month). The limitations of an observational study were noted in terms of compliance to antibiotics regimens, groups located in different countries and age (Pittman et al., 2017). The results of our study are in keeping with the above study. We have reported a significant reduction in the Shannon index whilst participants were actively taking long-term azithromycin compared to the comparison group exposed to intermittent courses of antibiotics. This difference had resolved by the study end at 20 months, at which time many of the participants had ceased the azithromycin, some of them several months previously. Similarly to Pittman, we did not find a reduction in the Shannon index in the comparison group exposed to intermittent antibiotics. We also found a significant difference in beta diversity of the nasopharyngeal microbiota whilst actively taking azithromycin (as opposed to being placed in the azithromycin group) compared to those in an unexposed comparison group. These changes persisted at the study end, 12-20 months, when the majority of children had stopped taking long-term azithromycin. The duration between cessation of azithromycin and the final swab did however vary considerably due to the COVID pandemic and is discussed further in section 5.3.1.

In the comparison group, the alpha diversity increased at the second swab sampling. The reason for this is unclear. Around the time of the second swab collection however, the COVID pandemic was beginning. Advice regarding social distancing, wearing of face masks and isolation were being introduced before the formal national lock down. These factors may have had an impact on the diversity of the nasopharyngeal microbiome in this group of children. As discussed in section 1.6.1, few studies have looked at the microbiome of children with PBB. Compared to healthy controls, children with PBB have been found to a dominance of *Haemophilus* and *Neisseria* of bronchoscopic protected brushings and a non-significant increase in *Moraxella* and *Streptococcus spp* (Cuthbertson et al., 2017). Across both groups in our study we found *Streptococcus spp*, *Moraxella spp* and *Haemophilus spp* in the top 15 ASVs however *Neisseria spp* did not feature. The finding of PBB microbiota profiles on BAL dominated by respiratory pathogens was also reported by (Marsh et al., 2019). They also described a further group with a diverse microbiota profile associated with *Prevotella spp.* *Prevotella spp* were not found to be in abundance in either of our 2 groups. The differences may relate to the fact that the demographics of the groups were quite different – the patients studies by Marsh et al. were an Australian cohort and were also younger than our cohort (mean age 1.7 years) and with less prior antibiotic exposure. Although similarities exit between the upper and lower respiratory tract microbiota, there are also differences. Marsh et al compared upper airway samples (nasopharyngeal and oropharyngeal) with BALF from a single lobe and reported that upper airway samples were a reliable surrogate in 69% of children with either PBB, idiopathic bronchiectasis or that were healthy (Marsh et al., 2016).

### Limitations of the microbiome study

As with the other two studies looking at the microbiome in PBB (Cuthbertson et al., 2017; Marsh et al., 2019), our numbers were small (<30 participants in each group) and the true variability of the microbiota may not have been fully sampled. Sampling the nasopharynx does not fully represent the microbiota of the lower airway. Given that one of the main concerns in PBB is that of the development of bronchiectasis, permanent damage to the lower airways, it would be important to understand the changes in the microbiota related to azithromycin within this specific part of the airway. However, given the practical difficulties in obtaining sequential lower airway samples in children, gathering information from the nasopharynx has allowed for longitudinal sampling of the microbiota from the same environmental niche. This would otherwise not have been possible from the lower airways.

Ideally samples would have been frozen at -80⁰C immediately after collection. This was not practically possible as samples were collected whilst children were in their homes. Samples were placed in transport medium which is designed to prevent bacterial overgrowth however this may have occurred.

The observational design of the study had inherent issues with the inability to control the duration of treatment. The participants in the comparison group were not deemed to require long-term azithromycin and thus may have had unrecorded differences other than potently having less severe disease with regards to the frequency of exacerbations. It would also have been valuable to have had a healthy control group, as well as the comparison group of children with PBB, to look at the background nasopharyngeal microbiota in children not exposed to frequent courses of antibiotics.

### Impact of the COVID pandemic

Due to the COVID pandemic, many swabs could not be collected due to lock down restrictions. This not only impacted on the sample size but also on the ability to conduct some of the intended statistical analysis. We were not able to run the PERMANOVA model on the full set of swabs as some the participants only had 1 sample for analysis and the total sample sizes for the second and third round of swabs were not large enough to run the model. As discussed in section 4.6.3, the mean duration of long-term azithromycin exposure was much longer than anticipated due to the COVID-19 pandemic. The study duration had to be extended to 20 months in order to obtain the final swabs. This resulted in the study period spanning 2 winters and hence some participants were restarted on azithromycin and had 2 episodes of azithromycin exposure. These factors meant that the final swabs were not all taken following 4-6 months cessation of azithromycin as planned. Given that a number of final swabs were collected whilst participants were still actively taking azithromycin or had only stopped taking it 3-4 weeks before the swabs were collected, a lasting impact of azithromycin may have influenced the microbiome of the final swabs. Finally, the number of swabs collected around exacerbations was too small to have any meaningful results to inform whether alterations in the nasopharyngeal microbiota relate to PBB exacerbations.

Despite these limitations we were able to conclude that azithromycin prophylaxis is associated with changes in the microbiome over time within individuals. Alpha diversity changes were reversible on treatment cessation yet differences in beta diversity persisted between azithromycin exposed and unexposed participants at the study end, 12-20 months.

# **Chapter 6. Overall discussion and future work**

## Overall discussion

In this thesis I firstly undertook a qualitative interview study investigating the parental experience of having a child prescribed prophylactic antibiotics. Following this I studied two prospective observational of children with PBB. In both of these studies I determined the effect of long-term azithromycin on antibiotic resistance in bacteria residing within the nasopharynx, and in the second I additionally investigated the effects of long-term azithromycin on the nasopharyngeal microbiota of children with PBB. The main findings of these studies were:

1. The overriding factor influencing parental decisions about the uptake of antibiotic prophylaxis, was wanting their child to be well now. The main concern voiced by parents was often that of antibiotics resistance. I have proposed a behavioural model that describes the phases families cycle through when a child is commenced on prophylactic antibiotics.
2. Fewer bacterial pathogenic species were isolated from swabs collected from those in the azithromycin group.
3. Baseline azithromycin resistance was higher than expected in both an azithromycin exposed group of children with PBB and a mostly macrolide-unexposed comparison group of children with PBB. This was predominantly driven by azithromycin resistant *S. pneumoniae*. During the studies, children exposed to azithromycin did not develop more azithromycin resistance compared to the comparison group.
4. The majority of the azithromycin resistance in *S. pneumoniae* was low-level (MICs up to eight times the EUCAST break point) and was not associated with mutations on genome sequencing.
5. We identified a major discrepancy between azithromycin and erythromycin resistance in the *S. pneumoniae* isolates. Only bacteria with high level azithromycin resistance were also erythromycin resistant and these carried mutations in known macrolide resistance genes.
6. Analysis of the nasopharyngeal microbiota revealed that those actively taking azithromycin had a significant reduction in alpha diversity. This resolved by the study end, when the majority of children had stopped taking azithromycin. Actively taking azithromycin also significantly diminished beta diversity, compared to those in the comparison group, and this persisted at the study end, when the majority of participants had stopped taking azithromycin.

We hypothesised that psychological factors would influence parents’ decisions relating to the uptake of prophylactic antibiotics and have successfully furthered the understanding of how parents view having a child prescribed long-term antibiotics.

The main barrier to recruitment to the interview study was the time-commitment; despite this I achieved the recruitment target, and conducted sufficient interviews to allow saturation to be reached. No adverse impact on the children or families participating was identified. The study findings were broadly consistent those from previous studies related to antibiotic use in children, and enabled us to construct a behavioural model to describe the phases parents go through once their child is prescribed antibiotic prophylaxis as fully discussed in Chapter 3. I presented the study findings to parents and the feedback was that the model did indeed reflect their experiences. The parents who attended were relieved to hear that other families had had similar experiences to themselves. We hope that this model will enable us to improve our discussions with parents whose children are being considered for prophylactic therapy, perhaps via the design of better patient information sheets. It is not sufficient for clinicians to prescribe long-term antibiotics with the expectation that parents will adhere. This “standard” approach neglects to appreciate the complexities of the interplay between health, psychology and behaviour. Neither does it take into account the potential problems and difficulties families face when administering long-term medication. We need to holistically address the parental experience when prescribing long-term antibiotic prophylaxis by (i) practically preparing families and supporting them in preparing the antibiotics (ii) breaking down barriers that may prevent their active involvement with engaging in consultations and (iii) improving confidence in assessing symptoms of acute respiratory illness. We must be mindful of potential scientific misconceptions, frequent parental concerns as well as detrimental social norms, and attempt to address these when discussing antibiotic prophylaxis. This study suggests that time and effort invested in these areas may facilitate medication adherence, reduce the anxieties many parents experience, and improve the relationships parents have with the medical professionals involved with their children’s care.

Although many of our more general findings reflect those of previous studies, it has highlighted more specific factors, such as the potentially negative impact of computer-based systems on the doctor-patient interaction, and also revealed the fact that the majority of the antibiotic supplied to the patients is being discarded into the drains/sewage systems. The indicative cost for the oral suspension is just over £4, but increasing use of azithromycin prophylaxis in paediatric practice does suggest a possible significant cost to the NHS in addition to the presence of antibiotic in the environment.

The second hypothesis I addressed was that children with PBB exposed to long-term azithromycin would develop macrolide resistant airway flora. As discussed (Sections 1.7 and 4.1), there is little or no evidence to support the use of long-term azithromycin in children with PBB and no information regarding efficacy or impact on antimicrobial resistance. We successfully recruited the planned number of participants to both observational studies and obtained serial nasopharyngeal swabs for analysis, although in the second study the timing of the swabs was disrupted by the COVID-19 pandemic.

The threat of antimicrobial resistance is increasing (WHO, 2020). Baseline azithromycin resistance in this population of children with PBB was surprisingly higher than expected in both the comparison group (never exposed to long-term azithromycin) and azithromycin group (some previously exposed to azithromycin). The majority was low level resistance observed in *S. pneumoniae* isolates. In a cohort of children exposed to multiple or prolonged courses of antibiotics, antibiotic resistance may become an increasingly significant clinical issue. Our second hypothesis that children exposed to azithromycin over several months would develop more azithromycin resistant airway flora compared to a comparison group was however not supported by the data I collected. This finding is not in keeping with some previous studies in conditions such as CF; it might reflect the different nature of the underlying respiratory condition, the higher than expected background resistance, or have been confounded by the fact that fewer bacteria being cultured in the azithromycin exposed group. The study may however offer some reassurance to those with PBB commencing long-term azithromycin in South Yorkshire and to the clinicians prescribing this therapy. Given the main ‘future’ fear articulated by parents in our qualitative study was the risk of antimicrobial resistance, our findings may inform discussions about commencing prophylaxis in this setting.

Obtaining microbiological samples from BAL in all children with PBB to make a diagnosis is not practical given the associated risks. It is possible that obtaining other microbiological samples such as nasopharyngeal samples might guide the treatment of acute episodes of PBB but this was not specifically addressed in our study. There is however a need to explore the nature of the low-level but potentially clinically significant azithromycin resistance we observed and to determine whether it is specific to PBB or a problem of the wider paediatric community in this geographical area. Children are adept at transmitting their respiratory flora to those around them, and the impact of establishing increased macrolide resistance in *S. pneumoniae* in the adult population would be significant; for example in penicillin-allergic patients, macrolide monotherapy is a standard treatment option for mild community-acquired pneumonia (*S pneumonia*e is the commonest cause of this condition) and may not be effective for the majority of isolates we have detected. Whilst clarithromycin is the most commonly used macrolide in the setting of adult pneumonia, azithromycin is also used in this and other acute infections.

The identification of a major and unexpected discrepancy between erythromycin and azithromycin resistance in the *S. pneumoniae* isolates is also a concerning finding. As such the UK HSA is keen to collaborate with us to investigate this issue further. It will be important to determine whether this discrepancy is explained by the technicalities of susceptibility testing, or whether it represents a physiological difference in the way *S. pneumoniae* handles different macrolides. At present our results do not support the current standard of using erythromycin to infer resistance to other macrolides.

My final hypothesis was that children with PBB exposed to long-term azithromycin would display reduced diversity of bacterial communities in the nasopharynx. The field of microbiome analysis is rapidly expanding with newer sequencing technologies and analysis techniques. Although I did not demonstrate significant baseline differences in the nasopharyngeal microbiota between the two study groups in terms of the need for azithromycin. I identified a temporary reduction in alpha diversity whilst taking azithromycin and a more prolonged effect (still present at 20 months) on beta diversity. These perturbations may have as yet unknown impacts on respiratory health. Extrapolation from studies on the gut microbiome would suggest that dysbiosis of the respiratory microbiome may have detrimental consequence, and requires further research, however establishing causality (as opposed to association) in studies of the microbiota is challenging. Longer studies monitoring changes within the respiratory microbiome or metabolome and larger study populations may however improve our understanding of this complex ecological niche, and allow us to consider interventions that may protect the natural microbiota or even restore a ‘healthy’ microbial flora. They may also inform clinical decision making with regards to the need for long-term antibiotics and identifying those at risk of developing bronchiectasis.

These complementary projects have developed links between Sheffield Children’s Hospital (Hardman, Shackley, Ungonna), the University of Sheffield Departments of Infection Immunity and Cardiovascular Disease (Condliffe, Darton, Suligoy), the Centre for Inflammation Research at the University of Edinburgh (Bogaert, Lusaretta Parga, Ruiz, Binkowska) and the School of Health and Related Research, Sheffield (Lee). The studies evolved over time, harnessing the varied skill sets of these investigators as required, with initial results influencing this evolution. They have also allowed me to develop my research skills and portfolio. Importantly, I had to obtain both ethical permission for all 3 studies, and obtain funding to support the work (only my salary was provided by my personal Bassetlaw Fellowship). A small grant was first obtained from Antibiotic Research UK to fund the pilot cohort (PBB study 1). Early data from this project enabled us to obtain funding from the Sir Halley Stewart Trust to support the study exploring the microbiome in PBB (PBB study 2). The higher than expected azithromycin resistance in the *S. pneumoniae* isolates meant that we were successful in an application to the Florey Institute to explore this in more detail by performing additional sensitivity testing and genomic sequencing. Throughout this iterative process, we have been responsive to the results and improved on the design of the studies.

Carrying out clinical research that involves invasive procedures in a paediatric population has unique ethical issues compared to studies in adults, as the child may have limited understanding as to what the study may entail and what the arguments for and against participation might be. Parents have responsibility to consent on behalf of their children, and as such must act in what they perceive as their child’s best interest. The threshold is therefore high for parents to allow their child to have a procedure that will cause the child pain. As we found in our recruitment, the majority of parents in the control group did not even respond to the text message asking whether they would like to take part. The precise reasons for this are not known, although it seems likely that they relate to potential for sampling to cause the child distress and the potential inconvenience. In fact, those parents who did participate in the initial pilot study (PBB Study 1) said the actual time commitment was minimal, and this was reflected in drawing up the Patient Information for PBB Study 2. We speculate that the invasiveness of sequential swab collection may have deterred some families. Those parents whose children were deemed severe enough to warrant antibiotic prophylaxis, may have had a lower procedural threshold, reflected in the higher uptake in this group. This might have been to gain more information about their child’s condition and potential adverse effects of antibiotic use, altruistic reasons to help others in a similar situation, or that they had been medicalised and were more used to their children having invasive procedures. Although our study was observational, this finding might suggest that a future randomised study might risk bias towards recruiting more severely affected children.

Ensuring continued engagement with families over the study period was challenging. Retainment of participants was good with only an 8% fall out across both studies despite the unexpected increased swab burden with a separate need for COVID-19 nasopharyngeal swabs during the study period. Many families did not remember to inform the research team of clinical changes or record compliance to azithromycin. Finding novel ways of interacting with study participants, ensuring they feel involved throughout and are updated regularly is important to ensure the high quality of data obtained. It remains to be seen whether the use of digital devices and apps will facilitate this.

Overall, participating in this research has equipped me with a broad range of transferable skills relevant to my development as a research-active clinician. These include undertaking a structured literature review, obtaining ethical permission and funding for my own studies, keeping a site file, qualitative interview skills, microbiological sampling and analysis, DNA extraction and learning basic programming for commonly used bioinformatic tools. I hope I will be able to employ these skills in future research projects.

## Future work

Protracted bacterial bronchitis remains one of the commonest diagnoses in children presenting to paediatric specialists with chronic cough yet there is still a paucity of published studies in this field. As we have reported, antibiotic resistance is an issue for this cohort of children exposed to multiple courses of antibiotics. It is however unclear whether these resistance patterns are isolated to children with PBB or perhaps as prevalent in healthy children within the local community. The use of long-term azithromycin trended towards a reduction in pulmonary exacerbations compared to those not exposed to long-term AZM. Yet breakthrough exacerbations still occurred requiring rescue antibiotics. Long-term azithromycin use does seem to reduce diversity within the nasopharyngeal microbiome. The implications of this change and repercussions for other microbiota such as those that reside in the gut, are not yet fully understood. As such, the results of these studies have led to consideration of further associated projects.

### Further projects explored to date

An appropriately powered and designed randomised, double blind, placebo-controlled study might seem to be the ideal way of determining the efficacy of long-term azithromycin in children with PBB with regards to reducing the number of pulmonary exacerbations and development of bronchiectasis, as well as delineating the impact of the intervention on antimicrobial resistance and dysbiosis. I have discussed such a study with both the respiratory team and with the parents involved in the studies reported in this thesis, and in general it emerged that parents of more severely affected children would not be willing to opt for a trial that might result in their child receiving placebo; this might introduce bias into such a study and prohibit or deter recruitment in those most at risk. The first project I have therefore explored, with the paediatric infectious diseases and respiratory teams at SCH, is to determine the optimal dosing regimen for long-term azithromycin in PBB. Recent evidence from adult studies have demonstrated efficacy of long-term azithromycin using low dose regimens. To date only two clinical trials have compared different dosing regimens in this children in this field:

1. Daily low dose azithromycin compared to high dose azithromycin (5mg/kg/dose vs 15mg/kg/dose) in children with CF (Kabra et al., 2010).
2. Daily (250mg) vs once weekly (1200mg) azithromycin in children and adults with CF (McCormack et al., 2007).

It is not known whether a low dose regimen would be as efficacious as the standard dosing regimen of 10mg/kg/ dose thrice weekly as recommended in the British National Formulary for children. A lower dose may have less impact on the microbiome and resistome as well as reducing the occurrence of direct side effects. In order to develop this idea further, I drafted the first sections of a multi-centre, double blind, randomised comparison clinical trial protocol to test the hypothesis that low dose long-term azithromycin (5mg/kg/dose thrice weekly) is not inferior to usual dose long-term azithromycin (10mg/kg/dose thrice weekly) in reducing pulmonary exacerbations and has less impact on the nasopharyngeal and intestinal microbiome and resistomes (see appendix 12). In order to ensure the study design was appropriate and for statistical advice, I met with the NIHR research design team. A non-inferior trial would be the design of choice, however, it would require 800-900 participants. The primary outcome chosen was the best option. The use of a different primary outcome and study design to answer the clinical question would compromise the credibility of the study on completion. A study of this magnitude would require multi-centre participation and a large grant would need to be obtained to fund such an endeavour. In order to be competitive to obtain such funding, a number of uncertainties would have to be first addressed:

1. Does low dose long-term azithromycin reduce the impact on the microbiome and thus potentially providing a basis for superiority?
2. What is the potential efficacy of low dose long-term azithromycin?
3. What is the acceptability of low dose long-term azithromycin to parents?

In order to address some of these uncertainties, I am exploring a pilot 4 centre randomised, controlled trial to obtain preliminary data on long term 5mg/kg/dose vs 10mg/kg/dose azithromycin in PBB. I also propose to investigate the impact of long-term azithromycin on the nasopharyngeal and stool microbiome/ resistomes. In collaboration with the gastroenterology team at Sheffield Children’s Hospital we will also look at the impact of 2 weeks of lactulose following cessation of long-term azithromycin to see if the gut microbiome is restored quicker in those exposed to lactulose. Lactulose is reported to have prebiotic properties and there is emerging evidence that its use may re-establish the paediatric gut microbiome faster than a control group following azithromycin exposure (Nikolaou et al., 2020).

The resistant bacterial isolates found residing within children’s nasopharynxes contribute to a pool of resistance genes that may circulate to a wider community of humans and bacteria through transmission via siblings, nurseries and schools. A further avenue of this study aims to look at the resistome of parents and siblings in order to assess the potential transfer of resistance genes within the family environment.

A second avenue I have recently explored with the respiratory team was setting up a BMedSci project to collect nasopharyngeal swabs from healthy preschool children with no previous antibiotic exposure. We aimed to assess the resistance patterns of the nasopharyngeal bacterial flora and compare to the results we found in children with PBB. Our bid was however unsuccessful on that occasion.

Our results from PBB study 1 have been already been presented to the funders (Antibiotic Research UK) in the form of an oral presentation at their national conference, as a poster discussion at the annual conference of the European Society for Infectious Diseases and the findings used as preliminary data to support a major grant application (Health Protection Research Unit in Antimicrobial Resistance). This bid was unfortunately ultimately unsuccessful although it was one of only 4 such applications to be shortlisted.

The qualitative study has been published in the Archives of Disease in Childhood and the results presented to a group of parents whose children have been prescribed long term azithromycin, as a poster at the annual conference of the European Society for Infectious Diseases and oral presentations to the local infectious disease team and at the local hospital research grand round.

### Future research that may arise from this work

To continue the qualitative aspects of our research, we would like to validate our behavioural model in other cohorts of parents whose children are prescribed long-term prophylactic antibiotics such as those with cystic fibrosis. The data relating to specific themes and subthemes could now be used in surveys to look at the perceptions of a larger cohort of parents with relation to their views and experiences of prophylactic antibiotics.

It is not known whether the high baseline azithromycin resistance found in the *S. pneumoniae* isolates is particular to the cohort of children with PBB exposed to multiple courses of antibiotics or whether this represents the background azithromycin resistance levels in the wider paediatric population. One approach we have considered to assess this is to analyse the nasopharyngeal aspirates taken from children with viral bronchiolitis. This is an extremely common lower respiratory infection, with 1 in 3 children developing bronchiolitis in the first year of life and 2-3% of these requiring hospitalisation. Nasopharyngeal aspirates are frequently taken from children with bronchiolitis in order to cohort them on hospital wards with regards to the virus detected. These samples are usually only processed for viral respiratory PCRs. They could however be cultured for potential bacterial respiratory pathogens and the isolates tested for azithromycin susceptibility. This will be explored next winter, as testing this year was confounded by rapid testing for COVID with nasal swabs.

As noted, the discrepancy between erythromycin and azithromycin susceptibility in *S. pneumoniae* has clinical implications of potential treatment failure and promotion of antibiotic resistance. In collaboration with the Health Security Agency, the frozen *S. pneumoniae* isolates will be re-cultured and re-tested to azithromycin and erythromycin using a variety of different brands of E-test strips. Further analysis into the possible effects of CO2 during culture may also be conducted, as well as testing other macrolides such as clarithromycin. To investigate the mechanism(s) underlying this finding, a project could be undertaken to look at the intracellular concentrations of different macrolides in *S. pneumoniae*. Examining the movement of intracellular macrolides may allow insight into how this species handles different macrolides in terms of bacterial permeability and use of efflux pumps. Additional investigations might include assessing the bacterial transcriptome, for example looking at the impact of stress response but also looking at the role of other genes in an unbiased fashion. The hypothetical protein we identified in the highly resistant *S. pneumoniae* isolates with varying macrolide resistance genes also warrants further research. Investigating the role, location and frequency of this protein may improve the knowledge of other mechanisms that promote high level *S. pneumoniae* macrolide resistance.

The use of antimicrobials must be appropriate and balanced to ensure adequate treatment of clinical conditions yet to minimise both side-effects for the individual and population as a whole. Some of the potential side effects such as the development of antibiotic resistance are known but others such as the longer term effects of the microbiome disruption are still to be fully understood. It is hoped that our continuing research will allow further insights into this issue.

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# **Appendices**

## Appendix 1: Specific cough pointers

**Specific cough pointers to be identified on clinical assessment of chronic cough include**:

Symptoms: chest pain, history suggestive of inhaled foreign body, dyspnoea, exertional dyspnoea, haemoptysis, failure to thrive, feeding difficulties (including vomiting or choking), cardiac or neurodevelopmental abnormalities, recurrent sinopulmonary infections, immunodeficiency or epidemiological risk factors for tuberculosis.

Signs: respiratory distress, digital clubbing, chest wall deformity or auscultatory crackles. Investigations: chest radiograph changes other than peri-hilar changes or lung function abnormalities.

## Appendix 2: Abbreviated SCH guidelines for the management of PBB

Diagnosis and Management of Children with PBB (Kansra et al. 2018)

Reference: 1852v1

Written by: Sonal Kansra

Peer reviewer: Alison Scott

Approved: July 2018

Review Due: February 2021

**Diagnosis and Management**

**PBB clinical**

Children with chronic wet cough (> 4 weeks), in absence of any specific pointers of significant lung disease (see appendix 2).

X-rays show minor changes (bronchial wall thickening) only

These children should be designated as PBB (clinical) and treated with 2 weeks of antibiotics covering common respiratory pathogens Streptococcus pneumoniae, Haemophilus influenzae and Moraxella catarrhalis. Usually this will be Co-amoxiclav.

For most children a 2 week course will be needed, a further 2 weeks can be prescribed if the child has improved significantly but is not completely cough free. (Only a minority will need longer courses and have high index of suspicion in these for more severe disease i.e. CSLD or bronchiectasis).

For children who do not respond to 4 weeks of antibiotic treatment or have more than one episode, further investigations including baseline immunology (Full Blood Count, Immunoglobulin levels and vaccine responses to HiB, Tetanus and Prevenar serotypes) and sweat test is recommended. Referral to tertiary Respiratory consultants should be considered and bronchoscopy discussed.

**PBB (Bronchoscopy/microbiology confirmed)**

**Treatment**

Children with bronchoscopic evidence of PBB (Purulent secretions, heavy growth of typical bacteria, neutrophilia) should be treated with 4 weeks of antibiotics, with antibiotic choice guided by culture and sensitivity results.

If the bronchoscopy shows significant inflammation or purulent secretions, then IV antibiotics will be commenced on consultant decision only. Recurrences are common and it is reasonable to wait for 2 weeks to see if these are related to a new viral infection. Recurrences should be treated with 2 weeks of antibiotics and it will be useful to do a cough swab for culture and sensitivities and a NPA or viral throat swab for respiratory viruses at the onset.

**Prophylaxis**

Antibiotic prophylaxis should be discussed for children who have more than 3 recurrences in 12 months.

Azithromycin (10mg/kg OD M/W/F) should be first line for prophylaxis because its use has robust evidence in CSLD and bronchiectasis and in cystic fibrosis. The dosing is easy, it is well tolerated and has less interactions and cardiac toxicity as compared to other macrolides

Septrin second choice (Use age appropriate dose once daily)

Co-amoxiclav, nebulised colistin and cephalosporins should be used as prophylaxis on consultant advice only.

## Study information leaflets

### Interview study

**The parental experience of having a child prescribed prophylactic antibiotic**

**Contents**

**We invite you to take part in a research study**

1. Why are we doing the study?
2. What do you need to know about the study?
3. Why are you being asked to take part?
4. What will I need to do if I take part?
5. Possible risks.
6. More information about taking part.

* Before you decide whether to take part, it is important for you to understand why the research is being done and what it will involve.
* Please take time to read the following information carefully. Discuss it with friends and relatives if you wish.
* You are free to decide whether or not to take part in this trial. If you choose not to take part, this will not affect the care that you or your child receives from their doctor.

**Important things you need to know**

* This research project will explore the thoughts and concerns parents may have whose children are prescribed long term preventative (prophylactic) antibiotics.
* This leaflet provides information regarding the details of the study
* If you would like more information about taking part in the study, please contact a member of the research team.

**Why are we doing the study?**

Many children are prescribed prophylactic antibiotics for a variety of medical problems. This is to try and keep them well. Parents or guardians are usually the people who give their children the prescribed antibiotics or remind them to take them. This is not always an easy task. We know parental views regarding antibiotics are important to understand. Previous studies have found that the public in general do not always have a good understanding of antibiotics. They often don’t fully appreciate the risks and benefits.

There is little information about what parents think about long-term antibiotics. This is an important area to explore. If we understand what parents experience and understand their thoughts regarding prophylactic antibiotics, it may improve the way medical professionals work together with families in these situations. Issues or views may arise in the interviews that had not previously been considered by medical professionals. These will be taken forward by the research team and studied further.

**This study aims to:**

1. Explore the parental experience of having a child who is prescribed long-term prophylactic antibiotics.

**What you need to know about the study?**

We aim to interview twelve parents whose children are prescribed prophylactic antibiotics to reduce the frequency of respiratory tract infections. Each of the twelve parents will be interviewed once by Dr Simon Hardman on an individual basis.

**The Interviews**

The interviews will be held at Sheffield Children’s Hospital or your home depending on which is easier for you. They will take place in the autumn of 2018. The discussion will be on what it means to you having a child on regular antibiotics. Interviews will typically last between 45 and 60 minutes. All material from the interviews will be anonymised. All interviews will be audio-recorded so that the discussions can be analysed at a later date. A quiet room is therefore required. For taking part in the study you will receive ten pounds to cover any travel expenses and as a token of appreciation.

**Why are you being asked to take part in the study?**

This is an opportunity to help with a research project in order to gather new information about parental views. You will have the time to express your views which is not always possible in an outpatient appointment. You may learn more about antibiotics. It is unlikely that you will directly benefit from the study but if significant results are found, it may influence how we discuss long term antibiotics with parents and their families in the future.

**What will I need to do if I would like to take part in the study?**

Dr Simon Hardman from the research team will contact you over the next week by telephone to see if you would like to take part. If you read the information about the study, and decide you would like to take part, a time and place will be arranged for you to be interviewed.

You will need to sign a consent form. This form confirms that you understand why the study is being carried out as well as the risks and benefits. You will have the opportunity at this point to ask any further questions.

**What are the possible risks?**

It is not anticipated that upsetting or distressing subjects will be discussed during the interviews thus minimizing any risks. The interviewer will be non-judgmental. There are no other risks involved.

**More information about the study**

All information regarding the interviews will be kept on a confidential secure database.

You will be invited to a meeting at the end of the study where the results will be presented and you will have the opportunity to discuss these. The final results will be published in a medical journal and presented at a variety of medical meetings. All data will be anonymised.

The study has been reviewed by the Health Research authority. Ethical approval has been obtained.

This is a research project funded by the immunology department at Sheffield Children’s NHS Foundation Trust. It is a collaborative project between the University of Sheffield and the Sheffield Children’s NHS Foundation Trust. At any before or during the interview, you may withdraw from the study.

### PBB Study 1

**PARENT/LEGAL GUARDIAN INFORMATION SHEET**

**Study title**: Antibiotics to prevent chest infections

We would like to invite you and your child to take part in our research study. Before you decide we would like you to understand why the research is being done and what it would involve. **One of our team will go through the information sheet with you and answer any questions you have.** Talk to others about the study if you wish.

Part 1 tells you the purpose of this study and what will happen to you and your child if you take part.

Part 2 gives you more detailed information about the conduct of the study.

Ask us if there is anything that is not clear.

**Part 1** – **To give you first thoughts about the project**

**What is the purpose of the study?**

Many children are under the care of the respiratory team at Sheffield Children’s Hospital because they have a persistent wet cough and a condition called protracted bacterial bronchitis (PBB). This is defined as a wet cough for more than 4 weeks, no other detectable underlying lung problem and a response to 2 weeks of antibiotics. Some of these children get recurrent episodes of PBB requiring multiple courses of antibiotics. Recurrent bacterial infections may cause permanent damage to the breathing tubes (airways). This condition is called bronchiectasis. Children who are thought to be at risk of developing bronchiectasis are often prescribed low dose, regular preventative antibiotics (especially over the winter months). This is to try and reduce the number of infections. The antibiotic often used is called azithromycin.

Antibiotics are important medicines used to treat bacterial infections. One of the problems with taking antibiotics for a long time is that the bacteria get used to them, adapt and become resistant so that the antibiotics no longer work. Sometimes when the antibiotics are stopped, the resistant bacteria go away.

We intend to look at whether giving children regular low dose antibiotics to prevent chest infections over the winter causes antibiotic resistance.

To do this we will take samples of the bacteria that live up everyone’s noses and see if they develop resistance to the antibiotic azithromycin in those children taking it regularly over winter. This study will run for a total of 12 months to enable us to see if the resistance persists or goes away once the azithromycin is stopped in the Spring.

This research study is a pilot project. This means it is a small scale study in order to obtain preliminary data and assess the feasibility of carrying out such a project. Following this, it is hoped we will organise a full-scale larger research study addressing the same question.

**Why have we been invited?**

This is an opportunity to help with a research project in order to gather new information about how we treat children with protracted bacterial bronchitis.

Your child has been chosen because they have the condition PBB and are under the care of the respiratory or immunology teams at Sheffield Children’s Hospital.

There will be two groups within the study. Your child will be part of one of the two groups. The allocated group will depend on whether your child is prescribed azithromycin by the respiratory team as part of your child’s standard care. This study does not influence the respiratory team as to whether your child is prescribed azithromycin or not. The decision to prescribe azithromycin is based on the child’s clinical symptoms and needs.

* The first group of children will selected because they are being prescribed azithromycin as part of their standard over the winter. This is because they are thought to be at risk of developing bronchiectasis. This is the Study group.
* The second group of children will be selected because they are not being prescribed azithromycin over the winter as it is not required. If your child is in this group and over the study period, the respiratory team feel your child needs azithromycin, your child will be prescribed azithromycin and will still be able to continue with the study. This group will be the control group.

In total we aim to recruit 20 children, ten in each group. At the end of the study we will compare the groups to see if there are any differences.

**Do we have to take part?**

No, you do not have to take part in the study. It is up to you and your child (wherever possible) to decide to join the study. We will describe the study and go through this information sheet. If you agree to take part, we will then ask you to sign a consent form. If your child is able to understand the research and is happy to take part and can write their name, they will be asked to sign an assent form with you, if they want to.

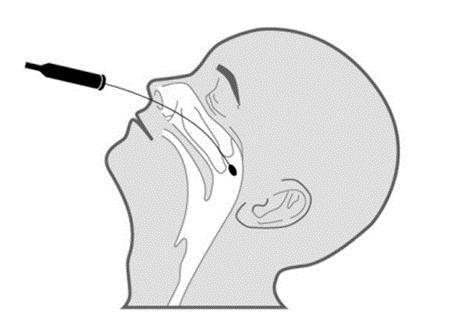
You will be given a copy of the information sheets and the signed consent and assent forms to keep for your records. You are free to withdraw at any time, without giving a reason. This would not affect the standard of care your child receives.

**What will happen to my child if we agree take part?**

If you agree to take part, your child will be involved in the research study for 12 months. It is an observational study where we will observe to see what happens but not change the management or care that your child would usually get.

Over the 12 months, they will have nasal swabs taken every 3 months (a total of 5 swabs). This is the way in which we will take samples of the bacteria up your child’s nose to see if they are becoming resistant to the antibiotic azithromycin.

To do this a small cotton-bud tipped stick is inserted up your child’s nostril. It is rotated to collect the sample and then removed. The process takes less than a few seconds. These will be taken either when you visit the hospital for a clinic or at your home depending on what is most convenient for you.



A diagram to show the cotton tipped swab being inserted up the nose to collect the bacteria

If information is found that could potentially influence how your child is treated, your clinical team will be informed as well as your GP.

Children in the group who take azithromycin will also be asked to fill in a medication diary for the 6 months they take azithromycin. They will be given a dairy and asked to tick a box if they remembered or forgot to take their medicine. It is expected that children will need parental supervision for this task. It is not a test and they will not be told off if they forget. Your honesty would be appreciated so that we can interpret the results accurately.

We will also look at your child’s medical records to review their past history and to see how many hospital admissions they have over the study period.

**What will we have to do?**

If you would like your child to take part in this research study, you will need to sign a consent form. Your child may be asked to sign too if they want. Following this a baseline nasal swab will be taken.

Dr Hardman will then contact you every 3 months to arrange a time and place for the next nasal swab to be taken. For those filling out the medication diaries, these will be reviewed when the nasal swabs are taken at 3 and 6 months.

**What are the possible disadvantages and risks of taking part?**

Taking nasal swabs can be an unpleasant experience but is short lived, less than a few seconds. Occasionally the nasal swab can damage the delicate blood vessels in the nose and cause a minor nose bleed. If this were to happen, we would need to press on your child’s nose for a few minutes to stop the bleeding. Every effort will be made to reduce the anxiety felt by some children during this procedure and a bravery sticker will be given to them afterwards. If at any time you or your child feels that the actual or perceived distress is too great, please don’t hesitate to tell the research doctor.

There will a time commitment for the swabs to be taken and for those filling in the medication diary. Otherwise there are no risks or disadvantages in taking part.

If your child is not prescribed azithromycin at the beginning of the study but the respiratory team feel they need it, being in this study will not stop them from getting it. This study does not affect the treatment your child gets.

**What are the possible benefits of taking part?**

It is unlikely that your child will directly benefit from the research during the study period. It is however hoped that this project will lead to future studies and potential changes in how we treat and manage children with protracted bacterial bronchitis.

**What happens when the research study stops?**

We will collect all the information together and we will decide if it is useful in telling us if the doctors can manage protracted bacterial bronchitis better in the future.

**What if there is a problem?**

Any complaint about the way you or your child have been dealt with during the study or any possible harm you or your child might suffer will be addressed. The detailed information on this is given in Part 2.

**Will my child’s taking part in the study be kept confidential?**

Yes. We will follow ethical and legal practice and all information about your child will be handled in confidence. The details are included in Part 2.

**This completes Part 1.**

**If the information in Part 1 has interested you and you are considering your child’s participation, please read the additional information in Part 2 before making any decision.**

**Part 2 of the information sheet**

**What if relevant new information becomes available?**

Sometimes we get new information about the treatment being studied. If this happens, someone from the research team will tell you and your child and discuss with you whether you want your child to continue in the study. If you decide not to carry on, arrangements will be made for your child’s care to continue. If you decide to continue in the study you may be asked to sign an agreement outlining the discussion.

**What will happen if we don’t want to carry on with the study?**

You can withdraw your child from the study at any point. If you would like to keep in contact with us to let us know their progress that can be arranged.

If you withdraw from the study, we will destroy all your child’s identifiable samples if you wish, but we will need to use the data collected up to their withdrawal.

**What if there is a problem?**

**Complaints**

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions.

If you remain unhappy and wish to complain formally, you can do this by contacting:

Patient Advice & Liaison Co-ordinator

Sheffield Children’s NHS Foundation Trust

Tel: 0114 271 7594

**Harm**

In the event that something does go wrong and your child is harmed during the research and this is due to someone’s negligence then you may have grounds for a legal action for compensation, but you may have to pay your legal costs. The normal NHS complaints mechanisms will still be available to you.

**Will my taking part in this study be kept confidential?**

All information which is collected about your child during the course of the research will be kept strictly confidential. Any information about your child which leaves the hospital will have their name and address removed so that they cannot be recognised from it. The research team members and the microbiology laboratory, where the nasal samples will be processed, will be the only people to have access to identifiable data. Once the study is complete all data will be kept for 5 years in a locked cupboard within the clinical research facility as part of the clinical governance procedures.

Our procedures for handling, processing, storage and destruction of data are compliant with the Data Protection Act 2018.

The nasal samples will be destroyed after they have been processed. The bacteria grown may however be used in other research studies. These bacterial samples will not contain any human tissue. They will be anonymised so that there is no link from the bacterial sample to your child.

We will also ask for permission to inform your family GP that your child will be taking part in the study.

Your child’s medical notes may also be looked at by other people within the hospital involved in the running and supervision of the study to check that it is being carried out correctly.

**What will happen to any samples my child gives?**

The samples taken from your child will be sent to the microbiology laboratory at Sheffield Teaching Hospitals. They will be labelled with a study number and your child’s unique Sheffield Children’s Hospital number. The samples will be stored until they have been processed in a freezer within the microbiology laboratory.

Once the nasal samples have been processed, they will be destroyed. The bacteria grown may be stored and transferred to the University of Sheffield for further research. These samples will not contain any human tissue and will be completely anonymised.

**What will happen to the results of the research study?**

When the study has finished we will present our findings to other researchers, and we will put the results in medical magazines and websites that researchers read. We would also like to put a brief summary on the hospital research website so that you will be able to read about our results too. This will be available at the end of the study, in February 2020*,* on [www.sheffieldchildrens.nhs.uk/research-and-innovation.htm](http://www.sheffieldchildrens.nhs.uk/research-and-innovation.htm). The results will be anonymous, which means that your child will not be able to be identified from them.

**Who is organising and funding the research?**

The research is being organised by Sheffield Children’s NHS Foundation Trust and paid for by the charity Antibiotic Research UK*.*

**Who has reviewed the study?**

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given a favourable opinion by the West of Scotland Research and Ethics Committee 3.

It has also been given approval by the Research Department to run at this hospital.

**General Data Protection Regulation Information**

Sheffield Children’s NHS Foundation Trust is the sponsor for this study based in the United Kingdom. We will be using information from you and/or your child in order to undertake this study and will act as the data controller for this study. This means that we are responsible for looking after your information and using it properly. Sheffield Children’s NHS Foundation Trust will keep identifiable information about you for 5 years after the study has finished, in some instances personal data maybe kept for longer where there is explicit consent in place.

You can find out more about how we use your information at the following link: https://www.sheffieldchildrens.nhs.uk/your-information/

Your rights to access, change or move your information are limited, as we need to manage your information in specific ways in order for the research to be reliable and accurate. If you withdraw from the study, we will keep the information about you that we have already obtained. To safeguard your rights, we will use the minimum personally-identifiable information possible.

Sheffield Children’s NHS Foundation Trust will use your name, hospital number and other identifiers to contact you about the research study, and make sure that relevant information about the study is recorded for your care, and to oversee the quality of the study. The only people at Sheffield Children’s NHS Foundation Trust who will have access to information that identifies you will be people who need to contact you regarding your participation in the study, or audit the data collection process. The people who analyse the information will not be able to identify you and will not be able to find out your name, NHS number, or contact details.

The following website provides information about how your information is used in research: https://www.hra.nhs.uk/information-about-patients/

Your information will only be used for the purpose of health and care research, and cannot be used to contact you or to affect your care. It will not be used to make decisions about future services available to you, such as insurance.

If you would like to find out more information regarding research at Sheffield Children’s NHS Foundation Trust then please follow the link:

<https://www.sheffieldchildrens.nhs.uk/research/>

**How can I find out more?**

If you would like to find know more about research in general, the Clinical Research Facility at this hospital has an Information for families section on its website www.sheffieldchildrens.nhs.uk/research-and-innovation.htm or you could contact the hospital Clinical Research Facility: Address given

If you would like to know more specific information about this research project, please contact the project co-ordinator: Details given

If you would like advice as to whether your child should participate you could contact the project team, or one of your child’s health care professionals.

If you have any concerns during the study, you should contact the project team.

**If I am interested in this study, what do I do next?**

If you have read the study information and would like further information or to participate, please call, text or email Dr Simon Hardman the project coordinator: Details given

Please leave your name and contact details. He will then call you to answer any further questions or arrange to meet up so that you can sign a consent form. If you child is old enough, they will be asked if they want to sign an assent form and the first swabs will be taken. A suggested time frame for contacting Dr Hardman after reading the information would be between 1 and 7 days if you intend to do so.

**If you and your child decide to take part in this study, you will be given this information sheet and signed consent and assent forms to keep.**

**Thank you for taking the time to read this information sheet.**

### PBB study 2

**PARENT/LEGAL GUARDIAN INFORMATION SHEET**

**Study title:** **Antibiotics to prevent chest infections:**

**Changes in bacterial communities and resistance**

We would like to invite you and your child to take part in our research study. Before you decide we would like you to understand why the research is being done and what it would involve. One of our team will go through the information sheet with you and answer any questions you have. Talk to others about the study if you wish.

Part 1: Tells you the purpose of this study and what will happen to you and your child

if you take part.

Part 2: Gives you more detailed information about the conduct of the study.

Ask us if there is anything that is not clear.

**Part 1 – To give you first thoughts about the project**

**1. What is the purpose of the study?**

Many children are under the care of specialist teams at Sheffield Children’s Hospital because they have a persistent wet cough and a condition called protracted bacterial bronchitis (PBB).

PBB definition:

* A wet cough for more than 4 weeks
* No other detectable underlying lung problem
* Response to 2 weeks of antibiotics.

Some children get recurrent episodes of PBB requiring multiple courses of antibiotics. Recurrent bacterial infections may cause permanent damage to the breathing tubes (airways). This condition is called bronchiectasis. Children who are thought to be at risk of developing bronchiectasis are often prescribed low dose, regular preventative antibiotics (especially over the winter months). This is to try and reduce the number of infections. The antibiotic often used is called azithromycin.

Antibiotics are important medicines used to treat bacterial infections. One of the problems with taking antibiotics for a long time is that the bacteria get used to them and become resistant so that the antibiotics no longer work. Sometimes when the antibiotics are stopped, the resistant bacteria go away.

Until recently the lungs were thought to be sterile. We now know there are organisms (microbiome) that live in the lungs and help keep them healthy. There are similar organisms living in the nasal passages. These microbiomes may be adversely effected by antibiotics.

In this study, we intend to look at how children respond to taking regular low dose antibiotics in terms of:

* Potential development of antibiotic resistance.
* Changes that may develop within the microbiome.
* Bacteria and viruses associated with recurrent episodes of PBB.

To do this we will take samples of the organisms that live up everyone’s noses. We will use nasal samples because it is difficult to get samples from the lungs. The microbiome in the nose can reflect what is happening in the lungs.

This study will run for a total of 12 months to enable us to see if resistance and microbiome changes persist or go away once the azithromycin is stopped given most children are prescribed it only over the winter months.

This research study is a pilot project. This means it is a small scale study in order to obtain preliminary data and assess the feasibility of carrying out such a project. Following this, it is hoped we will organise a full-scale larger research study addressing a similar question.

**2. Why have we been invited?**

This is an opportunity to help with a research project in order to gather new information about how we treat children with PBB. Your child has been chosen because they have the condition PBB.

There will be two groups in the study. Your child will be in one of the two groups. The allocated group will depend on whether your child is prescribed azithromycin by the respiratory or immunology teams as part of your child’s standard care.

Group 1: The Study Group

Children will be selected because they are being prescribed azithromycin as part of their standard over the winter.

Group 2: The Comparison Group

Children will be selected because they are not being prescribed azithromycin over the winter. If your child is in this group and over the study period needs azithromycin, your child will be prescribed azithromycin and can still continue with the study.

This study does not influence the respiratory and immunology teams as to whether your child is prescribed azithromycin or not. The decision to prescribe azithromycin is based on the child’s clinical symptoms and needs.

In total we aim to recruit 30 children, fifteen in each group. At the end of the study we will compare the groups to see if there are any differences.

No, you do not have to take part in the study. It is up to you and your child (wherever possible) to decide to join the study. We will describe the study and go through this information sheet. If you agree to take part, we will then ask you to sign a consent form.

**3. Do we have to take part?**

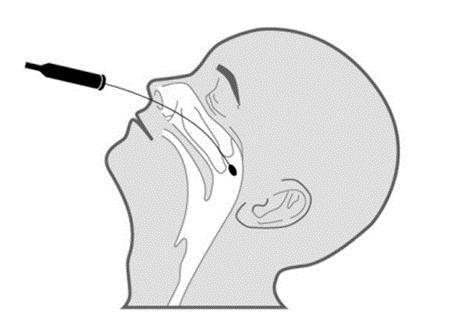
You will be given a copy of the information sheets and the signed consent form to keep for your records. You are free to withdraw at any time, without giving a reason. This would not affect the standard of care your child receives.

If you agree to take part, your child will be involved in the research study for 12 months. It is an observational study where we will observe to see what happens but not change the management or care that your child would usually get.

**4. What will happen to my child if we agree to take part?**

Over the 12 months, all the children will have nasal swabs taken every 4 months (a total of 4 swabs). This is the way in which we will take samples of the bacteria up your child’s nose.

To do this a small cotton-bud tipped stick is inserted up your child’s nostril. It is rotated to collect the sample and then removed. The process takes less than a few seconds. These will be taken either when you visit the hospital for a clinic or at your home depending on what is most convenient for you.



A diagram to show the cotton tipped swab being inserted up the nose to collect the bacteria

**5. What will we have to do?**

If your child has an episode of PBB during the study period and requires 2 weeks of antibiotics, we will take 2 nasal swabs before the antibiotics are started. One for bacteria and one for viruses. We will ask you to contact us to let us know if your child has a recurrent episode.

At the end of the exacerbation we would like to take 1 further nasal swab for bacteria. This is optional. You may decide your child is now recovering and you don’t want the swab to be taken as they have had lots of investigations.

Families of children taking azithromycin will also be asked to fill in a medication diary whilst they take the antibiotic. This will involve ticking a box if you remembered or forgot to take the Azithromycin. It is expected that children will need parental supervision for this task. It is not a test and they will not be told off if they forget. Your honesty would be appreciated so that we can interpret the results accurately.

We will also look at your child’s medical records to review their past history and to see how many hospital admissions they have over the study period. If information is found that could potentially influence how your child is treated, your clinical team will be informed.

If you would like your child to take part in this research study, you will need to sign a consent form. Following this a baseline nasal swab will be taken.

Dr Hardman will contact you every 4 months to arrange a time and place for the next nasal swab to be taken. For those filling out the medication diaries, these will be reviewed when the nasal swabs are taken.

If your child has a recurrent episode of PBB and is prescribed 2 or more weeks of antibiotics by the hospital team or through your GP, we would like you to contact Dr Hardman so he can arrange for 2 nasal swabs to be taken before starting the antibiotics.

**6. What are the possible disadvantages and risks of taking part?**

Taking nasal swabs can be an unpleasant experience but is short lived, less than a few seconds. Occasionally the nasal swab can damage the delicate blood vessels in the nose and cause a minor nose bleed. If this were to happen, we would need to press on your child’s nose for a few minutes to stop the bleeding.

Every effort will be made to reduce the anxiety felt by some children during this procedure and a bravery sticker will be given to them afterwards. If at any time you or your child feels that the actual or perceived distress is too great, please don’t hesitate to tell the research doctor.

There will a time commitment for the swabs to be taken and for those filling in the medication diary. Otherwise there are no risks or disadvantages in taking part.

If your child is not prescribed azithromycin at the beginning of the study but is prescribed it during the study period, being in this study will not stop them from getting it. This study does not affect the treatment your child gets.

**7. What are the possible benefits of taking part?**

It is unlikely that your child will directly benefit from the research during the study period. It is however hoped that this project will lead to future studies and potential changes in how we treat and manage children with protracted bacterial bronchitis.

**8. What happens when the research study stops?**

We will collect all the information together and decide if it is useful in telling us whether doctors can manage PBB better in the future.

**9. What if there is a problem?**

Any complaint about the way you or your child have been dealt with during the study or any possible harm you or your child might suffer will be addressed. The detailed information on this is given in Part 2.

**10. Will my child’s taking part in the study be kept confidential?**

Yes. We will follow ethical and legal practice and all information about your child will be handled in confidence. The details are included in Part 2.

**This completes Part 1.**

**If the information in Part 1 has interested you and you are considering your child’s participation, please read the additional information in Part 2 before making any decision.**

**Part 2 of the information sheet**

**What if relevant new information becomes available?**

Sometimes we get new information about the treatment being studied. If this happens, someone from the research team will tell you and your child and discuss with you whether you want your child to continue in the study. If you decide not to carry on, arrangements will be made for your child’s care to continue. If you decide to continue in the study you may be asked to sign an agreement outlining the discussion.

**What will happen if we don’t want to carry on with the study?**

You can withdraw your child from the study at any point. If you would like to keep in contact with us to let us know their progress that can be arranged. If you withdraw from the study, we will destroy all your child’s identifiable samples if you wish, but we will need to use the data collected up to their withdrawal.

**What if there is a problem?**

Complaints

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions.

Name: Simon Hardman

Title: Doctor

Hospital/Department: Sheffield Children’s Hospital

If you remain unhappy and wish to complain formally, you can do this by contacting:

Patient Advice & Liaison Co-ordinator

Sheffield Children’s NHS Foundation Trust

**Harm**

In the event that something does go wrong and your child is harmed during the research and this is due to someone’s negligence then you may have grounds for a legal action for compensation, but you may have to pay your legal costs. The normal NHS complaints mechanisms will still be available to you.

**Will my taking part in this study be kept confidential?**

All information which is collected about your child during the course of the research will be kept strictly confidential. Any information about your child which leaves the hospital will have their name and address removed so that they cannot be recognised from it. The research team members and the microbiology laboratory, where the nasal samples will be processed, will be the only people to have access to identifiable data. Once the study is complete all data will be kept for 5 years in a locked cupboard within the clinical research facility as part of the clinical governance procedures.

Our procedures for handling, processing, storage and destruction of data are compliant with the Data Protection Act 2018. The nasal samples will be destroyed after they have been processed. The bacteria grown may however be used in other research studies. These bacterial samples will not contain any human tissue. They will be anonymised so that there is no link from the bacterial sample to your child. We will also ask for permission to inform your family GP that your child will be taking part in the study. Your child’s medical notes may also be looked at by other people within the hospital involved in the running and supervision of the study to check that it is being carried out correctly.

**What will happen to any samples my child gives?**

The samples taken from your child will be sent to the microbiology laboratory at Sheffield Teaching Hospitals. They will be labelled with a study number and your child’s unique Sheffield Children’s Hospital number. The samples will then be transferred to the university of Sheffield Microbiology laboratory for further processing. Your child’s details will not be on these samples, only sample identification numbers. The final analysis will take place in an external laboratory run by the company Genewiz. Once the nasal samples have been processed, they will be destroyed. The bacteria grown may be stored and transferred to the University of Sheffield for further research. These samples will not contain any human tissue and will be completely anonymised.

**What will happen to the results of the research study?**

When the study has finished we will present our findings to other researchers, and we will put the results in medical magazines and websites that researchers read. We would also like to put a brief summary on the hospital research website so that you will be able to read about our results too. This will be available at the end of the study, in April 2021*,* on [www.sheffieldchildrens.nhs.uk/research-and-innovation.htm](http://www.sheffieldchildrens.nhs.uk/research-and-innovation.htm).

The results will be anonymous, which means that your child will not be able to be identified from them.

Who is organising and funding the research?

The research is being organised by the University of Sheffield and Sheffield Children’s NHS Foundation Trust. It is being funded by the Sir Halley Stewart Trust.

**Who has reviewed the study?**

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given a favourable opinion by the XXXXX Research and Ethics Committee. It has also been given approval by the Research Department to run at this hospital.

**General Data Protection Regulation Information**

Sheffield Children’s NHS Foundation Trust is the sponsor for this study based in the United Kingdom. We will be using information from you and/or your child in order to undertake this study and will act as the data controller for this study. This means that we are responsible for looking after your information and using it properly. Sheffield Children’s NHS Foundation Trust will keep identifiable information about you for 5 years after the study has finished, in some instances personal data maybe kept for longer where there is explicit consent in place.

You can find out more about how we use your information at the following link: https://www.sheffieldchildrens.nhs.uk/your-information/

Your rights to access, change or move your information are limited, as we need to manage your information in specific ways in order for the research to be reliable and accurate. If you withdraw from the study, we will keep the information about you that we have already obtained. To safeguard your rights, we will use the minimum personally-identifiable information possible.

Sheffield Children’s NHS Foundation Trust will use your name, hospital number and other identifiers to contact you about the research study, and make sure that relevant information about the study is recorded for your care, and to oversee the quality of the study. The only people at Sheffield Children’s NHS Foundation Trust who will have access to information that identifies you will be people who need to contact you regarding your participation in the study, or audit the data collection process. The people who analyse the information will not be able to identify you and will not be able to find out your name, NHS number, or contact details.

The following website provides information about how your information is used in research: https://www.hra.nhs.uk/information-about-patients/

Your information will only be used for the purpose of health and care research, and cannot be used to contact you or to affect your care. It will not be used to make decisions about future services available to you, such as insurance.

If you would like to find out more information regarding research at Sheffield Children’s NHS Foundation Trust, then please follow the link:

<https://www.sheffieldchildrens.nhs.uk/research/>

**How can I find out more?**

If you would like to find know more about research in general, the Clinical Research Facility at this hospital has an Information for families section on its website www.sheffieldchildrens.nhs.uk/research-and-innovation.htm or you could contact the hospital Clinical Research Facility:

Sheffield Children’s NHS Foundation Trust

If you would like to know more specific information about this research project, please contact the project co-ordinator:

Name: Dr Simon Hardman

If you would like advice as to whether your child should participate you could contact the project team, or one of your child’s health care professionals.

If you have any concerns during the study, you should contact the project team.

**If I am interested in this study, what do I do next?**

Dr Simon Hardman will text you 24-48 hours after you have received the information leaflet to see if you are interested. If you read the study information and would like further information or to participate, please reply to the text message, call or email Dr Simon Hardman the project coordinator. A suggested time frame for contacting Dr Hardman after reading the information would be between 1 and 7 days if you intend to do so.

Contact details for Dr S Hardman: Given

Please leave your name and contact details. He will then call you to answer any further questions or arrange to meet up so that you can sign a consent form. If your child is old enough, they will be asked if they want to sign an assent form and the first swabs will be taken.

If you and your child decide to take part in this study, you will be given this information sheet and signed consent and assent forms to keep.

Thank you for taking the time to read this information sheet.

## Appendix 4: Interview questionnaire

**Semi-structured prompts for the phenomenological interviews**

**Primary Questions:**

1. What does it mean to you to have a child who has had to take regular antibiotics?
2. What is your experience of getting your child to take their medication?

**Prompts:**

1. **What are antibiotics used for?**
   1. Have you any concerns about antibiotic use in your child? What are your concerns? Some people may be concerned, can you think of any reasons why?
   2. What benefits/harms do you see of your child being on preventative antibiotics?
   3. What is your experience of dealing with episodes of fever when your child is already on preventative antibiotics?
   4. When do you think it is appropriate or inappropriate for antibiotics to be given.
   5. How serious do you consider chest infections to be in your child? What sort of impact does it have on their life?
   6. What does it mean to you if antibiotics are not prescribed by your doctor when you request or think your child should have a course of antibiotics?
   7. What do you do with antibiotics that you haven’t used?
   8. Are there any harms in taking antibiotics for common colds? If so, what might these be?
   9. Are you aware of any friendly bacteria? If so, can you explain where you have heard about them and where they might live.
   10. What was explained when starting prophylaxis
2. **Antibiotic resistance.**
   1. When you hear the phrase “antibiotic resistance”, what comes to mind?
   2. Can you tell me in your own words what antibiotic resistance is?
   3. What do you understand by the term antibiotic resistance/MRSA/ Superbug
   4. Where do you think these bacteria are found?
   5. Where have you heard about antibiotic resistance?
   6. What ideas have you got about how or why antibiotic resistance occurs?
   7. How do you think your child would get resistant bacteria?
   8. Have you ever been told your child has resistant bacteria? How did you feel about this? What did this mean for your child?
   9. Have friends or other family members had “resistant bacteria”? You did you react to this?
3. **Controlling antibiotic resistance.**
   1. Do you think there is a link between how we use antibiotics and antibiotic resistance? If so how? If not, why not?
   2. Is antibiotic resistance something you are concerned about for your child? If so what are your concerns? If not, why not?
   3. What do you think you can do about the problem?
   4. What do you think other people can do about the problem?
   5. How important do you think it is to take every dose of a course of antibiotic? Why do you think it is important to complete a course of antibiotics?
   6. In your view, is antibiotic resistance an issue that affects the community at large?
   7. What do you think can be done to manage antibiotic resistance?
   8. Can you think of things that you can do as an individual/ family that can help reduce antibiotic resistance?
4. **Compliance.**
   1. Getting children to take antibiotics three times a week can be difficult. What is your experience of getting your child to take their medication?
   2. Why do you think people take their antibiotics?
   3. Can you remember any times when your child didn’t complete the prescribed course of antibiotics? Why was that? Why do you think people don’t complete their courses?
   4. Are there things you do to remind you your child needs their medication on that day?
   5. Tell me about the practicalities of giving the medication?
   6. What things in your daily life make it difficult to give the antibiotics?
   7. What sorts of problems do you run into when collecting repeat prescriptions, if any?
   8. Have there been times when you have run out of antibiotics? Why do you think this happened?

When you are busy or away, how do you ensure that your child gets their antibiotics?

## Appendix 5: Bacterial culture and DNA extraction protocol (Qiagen, 2020)

**Protocol:** Pre-treatment for Gram-Positive Bacteria (reference the DNA easy Qiagen protocol)

This protocol is designed for purification of total DNA from Gram-positive bacteria and describes the preliminary harvesting of bacteria and incubation with lysozyme to lyse their cell walls before DNA purification.

1. Take 1-2 beads from the frozen streptococcus pneumoniae isolates and streak onto blood agar plates. Incubated overnight at 37°C in 5% CO2.
2. Select approximately 30-50 colonies and suspend in 1ml of phosphate-buffered saline solution (PBS). Harvest bacterial cells in a microcentrifuge tube by centrifuging for 5 minutes at 9,000g. Discard the supernatant.
3. Resuspend bacterial pellet in 180 µl enzymatic lysis buffer.

Enzymatic lysis buffer:

20mM Tris-Cl, pH 8.0

2mM sodium EDTA

1.2% Triton X-100

20mg/ml lysozyme

1. Incubate for 2 hours at 37°C.

(The original Qiagen protocol suggests a 30 minutes incubation period)

(After incubation, heat the heating block or water bath to 56°C if it is to be used for the incubation in step 6)

1. Add 25 µl Proteinase K and 200 µl Buffer AL (without ethanol). Mix by vortexing
2. Incubate at 56°C for 30 min.
3. Add 200 µl ethanol (96–100%) to the sample, and mix thoroughly by vortexing.
4. Pipet the mixture from step 3 into the DNeasy Mini spin column placed in a 2 ml collection tube (provided). Centrifuge at ≥6000 x g (8000 rpm) for 1 min. Discard flowthrough and collection tube.
5. Place the DNeasy Mini spin column in a new 2 ml collection tube (provided), add 500 µl Buffer AW1, and centrifuge for 1 min at ≥6000 x g (8000 rpm). Discard flow-through and collection tube.
6. Place the DNeasy Mini spin column in a new 2 ml collection tube (provided), add 500 µl Buffer AW2, and centrifuge for 3 min at 20,000 x g (14,000 rpm) to dry the DNeasy membrane. Discard flow-through and collection tube.
7. Place the DNeasy Mini spin column in a clean 1.5 ml or 2 ml microcentrifuge tube (not provided), and pipet 100 µl Buffer AE directly onto the DNeasy membrane. Incubate at room temperature for 1min, and then centrifuge for 1 min at ≥ 6000 x g (8000 rpm) to elute

## Appendix 6: Further explanation of the phases of the behavioural model

**Explanation:** The first phase of stability begins with parental acceptance of antibiotic prophylaxis after weighing up the associated risks and benefits. A desire to normalise prophylaxis ensues in order to address social norms of not wanting their child to stand out and to make life as easy as possible. This is achieved by establishing a routine and minimising environmental disruptions.

Following this parents may rationalise their decisions if exacerbation frequency reduces. Previous concerns may be suppressed, a sense of security develops and restrictions placed on their child’s activities may be lifted. Adherence may be promoted as prophylaxis is seen as vital or complacency may develop.

Episodes of acute illness inevitably occur. These induce fear that their child will deteriorate and prophylaxis has failed. An objective response follows drawing on previous experiences to assess and manage the situation at home. Barriers need to be overcome if assessment by a health care professional is felt necessary: social norms of wasting people’s time, being seen as “paranoid” and practical aspects of making appointments. Determining the threshold for assessment is difficult for parents but potential drivers are their perceived severity of symptoms or fear of deterioration/ end organ damage.

After the acute illness, parents reflect on their parenting response and outcome of the assessment depending on whether treatment was given or not. This may influence their assessment and expectations of future illnesses as well as reflecting on the efficacy of the antibiotic prophylaxis. A period of stability ensues once more and parents reweigh the associated balance of risk.

## Appendix 7: Final draft of published journal article

**Title: The parental experience of prophylactic antibiotics (PEPPA)**

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Keywords:

paediatrics, qualitative research, parents, antibiotic prophylaxis, respiratory therapy

Word count: 2500

**Abstract**

**Background and objectives**

Long-term prophylactic antibiotics are often used to prevent bacterial infections. However, supporting evidence for this is not always robust. Including parents in decisions relating to medication is key to medicines optimisation. Parental concern regarding medication is a major determinant of poor adherence. This study explores parental experiences of having a child prescribed prophylactic antibiotics and how that affects their antibiotic use behaviour.

**Methods**

We conducted a prospective single centre exploratory qualitative study at Sheffield Children’s Hospital. Through 15 interviews, involving 18 participants, we explored parental’ “lived experiences” and attitudes towards azithromycin prophylaxis prescribed for various respiratory conditions. Thematic analysis was conducted.

**Results**

The overriding factor influencing parental decisions about the uptake of antibiotic prophylaxis, is wanting their child to be well now. The main concern voiced by parents is that of antibiotic resistance given their children are high users of antibiotics. This is however seen as a problem for the future, not the present. Preparing families adequately helps prevent practical difficulties relating to medication. Facilitating “normalisation” of prophylaxis through daily routines and minimising disruption to the family environment may reduce parental anxiety, promote adherence and result in easing of potential restrictions to the child’s daily activities.

**Conclusion**

Grounded in our deeper understanding, we propose a behavioural model that describes phases parents go through whilst having a child on prophylactic antibiotics. Time invested in holistically addressing the parental experience and having an awareness of potential issues parents face, may facilitate medication adherence, reduce anxieties and improve doctor-parent relationships.

**The parental experience of prophylactic antibiotics (PEPPA)**

**Background**

Long-term prophylactic antibiotics are often used to prevent serious bacterial infections and their sequelae in susceptible populations. Evidence-based guidelines for antibiotic prophylaxis exist for conditions such as HIV and sickle cell disease (Panel on Opportunistic infections in HIV., 2019; Rankine-Mullings et al., 2017). However, in other conditions the supporting evidence is less robust (Bush, 2019). Azithromycin prophylaxis is often used in children with recurrent respiratory tract infections due to its antibiotic and anti-inflammatory effects. It is recommended for conditions such as bronchiectasis (T Hill et al., 2019) and protracted bacterial bronchitis (Gilchrist, 2019). The benefits of antibiotic prophylaxis need to be weighed against potential risks of antibiotic resistance and medication side-effects. Ideally, this trade-off should be discussed with parents although it is unknown how often this occurs.

Including parents and children in decisions relating to medicines is key to medication adherence (Trivedi, 2017). Indeed, parental concerns regarding medication contribute to poor adherence, which is a significant problem in chronic paediatric illnesses (Conn et al., 2005; McGrady et al., 2013). Parental knowledge and beliefs are essential considerations when changes in patterns of antibiotic prescribing are required (Andrews, Thompson, Buckley, Heneghan, Deyo, et al., 2012).

However, very few studies have investigated parental perceptions of antibiotic prophylaxis. Parents may see antibiotic prophylaxis as beneficial (Diorio et al., 2012; Elliott et al., 2001), but this may not necessarily result in good adherence (Elliott et al., 2001). Parental concerns about antibiotic resistance were perceived to be a community problem rather than an issue affecting their children (Diorio et al., 2012). This study seeks to understand the parental experience of having a child prescribed prophylactic antibiotics and how that affects their antibiotic use behaviour.

**Methods**

**Participants and procedure**

We conducted a prospective, single-centre, exploratory qualitative study at Sheffield Children’s Hospital (SCH) with parents or guardians whose children attended the paediatric respiratory and immunology outpatient clinics. All participants gave written informed consent. Travel expenses were covered but no other incentive to participate was offered.

Parents of children aged 2-10 years who had been taking oral azithromycin prophylaxis for at least 3-months to prevent lower respiratory tract infections, were invited to participate.

Recruitment took place between 1st September 2018 and 31st April 2019 using a purposive sampling method to capture a breadth of views based on the ages of parent and child, parental education, ethnicity and severity of the child’s condition (Figure 1). Theoretical saturation was anticipated to occur between 12 and 20 interviews [12].

**Diagram

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**Interviews**

Face-to-face interviews were conducted by SH, at SCH or the family home, depending on parental convenience. Semi-structured interviews were conducted using an interview questionnaire based on topics of interest to the research question and themes previously identified in the literature relating to parents’ perceptions of acute antibiotics (Appendix 4).

Interviews focused on parents’ “lived experiences” of having a child who had been prescribed prophylactic azithromycin. Parents were encouraged to fully describe their thoughts, feelings and views regarding prophylactic antibiotic use. An iterative approach was taken building on emerging themes from previous interviews.

Interviews were recorded using an encrypted digital voice recorder and field notes taken of interesting observations.

**Analysis**

All interviews were transcribed verbatim and standard thematic analysis conducted (J Smith, 2009). Transcripts were coded by SH and reviewed independently by a second researcher (AL). A framework of subthemes was developed and iteratively adapted following each interview. We focused on finding recurrent, unusual, collective and opposing subthemes, in order to understand how parents experienced the events they described, why they had made certain decisions and factors influencing their behaviour.

Three themes and associated subthemes were identified and agreed on through a continuous process of refinement and discussion between researchers. Background medical and social information was used to contextualise the meanings of comments.

Ethical approval was granted by North West - Haydock Research Ethics Committee (Reference 18/NW/0579)

**Results**

18 parents took part in the study through 15 interviews. The participants’ characteristics are summarised in Table 1. Six interviews were conducted at SCH and nine in the participant’s home. The average interview time was 45 minutes (30-70 minutes range). From these interviews, three main themes emerged as described below (See table 2 with supporting quotes in table 3).



|  |  |  |  |
| --- | --- | --- | --- |
| **Main Theme** | Subtheme | Codes | Referenced quotes in Table 3 |
| **Decision making regarding prophylaxis** | Determinants of active involvement | Self-belief | 1.1 P10, P6 |
| Health literacy | 1.3 P1 |
| Impact on parent | 1.1 P8 |
| Passive Barriers | Social norms | 1.2 P9, P2  1.3 P6 |
| Environmental | 1.2 P1 |
| Influencing factors | Perceptions of prophylaxis | 1.6 P6, P14 |
| Health beliefs | 1.4 P3, P4, P9  1.5 P12, P2 |
| Impact on quality of life | 1.4 P8 |
| Understanding of science | 1.5 P10 |
| Balancing risk | Benefits | 1.6 P3 |
| Present vs future | 1.5 P7 |
| Concerns | 1.6 P13 |
| **The context of prophylaxis within the family and their environment** | Normality | Establishing a routine | 2.1 P2 |
| Barriers | 2.1 P6 |
| Practicalities of medication | Preparedness | 2.1 P5  2.2 P14, P8 |
| Administering issues |  |
| Waste and disposal | 2.2 P15 |
| Adherence | Obstacles and difficulties | 2.2 P9, P10 |
| Initiatives |  |
| Impact on quality of life | 2.3 P2, P3, P4, P5 |
| **Response to acute illness whilst on prophylaxis** | Emotive reasoning | Immediate response | 3.1 P1 |
| Previous experiences | 3.1 P8 |
| Assessment | Threshold for consultation | 3.2 P11 |
| Importance of specific symptoms |  |
| Driving pressures to take action | 3.1 P1, P12 |
| Management | Misconceptions of terminology and concepts | 3.3 P2 |
| Expectations |  |
| Understanding of antibiotics | 3.3 P5 |

**Table 2: Themes identified in the analysis**

**Legend.**

Table 2 shows the framework of subthemes that developed from codes applied to the interview transcripts. A consensus of three main themes was then agreed upon. This was through discussions amongst the researchers with regards to interpretation of the findings consideration of the surrounding literature and ensuring the story line of the participants was conveyed. Supportive quotations from the interviews are referenced within table 2. These can be found in Table 3 (P=parent)

1. **Decision making regarding prophylaxis**

Parents took on both active and passive roles in medical consultations when making decisions regarding the use of antibiotic prophylaxis. Some parents had strong feelings of being experts of their children and wanted to more actively engage with consultants. [Table 3, 1.1]

Parents who described taking a more passive role reported feeling less informed. Barriers to engagement identified by these parents included chaotic consultation rooms, poor eye contact from the doctor, time pressures, social norms of politeness, not wanting to be made to feel like a “drama queen” and seeing the doctor as a higher authority. [Table 3, 1.2] These barriers led to unvoiced concerns that in turn meant parents sought increased support from health care professionals in the future.

Interestingly, three parents described a transition from passive to active involvement once prophylactic antibiotics were brought up in the consultation. Reasons behind this included perceived significant risks of antibiotic prophylaxis, a desire to improve their knowledge given the increase in severity of the situation and frustration at feeling uninformed. [Table 3, 1.3]

All parents hoped or expected antibiotic prophylaxis to reduce the number of respiratory tract infections, improve their child’s health and restore normality to their lives. Fear of a common cold progressing to a hospital admission and significant clinical deterioration was a recurrent concern. Parents talked about how their health beliefs of antibiotics sometimes conflicted with a decision to start long-term antibiotics. Such health beliefs included aversions to taking antibiotics as they hadn’t needed them in childhood, preferences for alternative medicine and fears about what antibiotics do to the body. [Table 3, 1.4]

The majority considered antibiotic resistance when deciding on antibiotic prophylaxis for their child. This was often described as “the body becoming immune to antibiotics”. Parents perceived their children to be at “high risk” for acquiring resistant organisms due to high antibiotic exposure. This however was not seen as an imminent threat for their children rather as a problem for when they were older. Medication side effects were less well appreciated. Many parents were influenced by media messages relating to antibiotic use but felt in a state of conflict as they believed their child needed antibiotics. [Table 3, 1.5]

Most parents reported that antibiotic prophylaxis was the only option left having exhausted all others. Other contributing factors included pressures relating to poor school attendance and competing demands from siblings and employers, due to multiple hospital admissions. The desire for their child to be well and have a better quality of life at that moment in time was overwhelmingly the most significant factor when deciding whether to try prophylactic antibiotics. [Table 3, 1.6]

1. **The context of prophylaxis within the family and their environment**

Parents described various ways in which they normalised their lives incorporating their child’s antibiotic prophylaxis. Establishing a routine was the main way of creating a sense of normality. This was often achieved using visual cues and electronic devices as reminders. A number of barriers were cited such as school holidays, when the established routine was interrupted and ensuring timely collection of prescriptions. Some parents admitted it was easy to forget to give the antibiotics. [Table 3, 2.1]

Parents often have to reconstitute the antibiotic powder at home. Whilst this negates the need for weekly pharmacy visits to collect pre-made suspension, many parents were not expecting to have to do this and found it somewhat daunting. They also found it difficult to get repeat prescriptions in a timely fashion which impacted on adherence. Two parents said that significant prescription difficulties made them want to stop prophylaxis. Many were concerned about the amount and cost of wasted antibiotics and were not aware of appropriate disposal methods. Some reported flushing surplus antibiotics down the toilet or sink. [Table 3, 2.2]

Most parents felt prophylaxis was beneficial in reducing infections, hospital admissions, or improved school attendance. Some reported they previously isolated their child from social situations that had potential for infection such as school or public transport. They viewed antibiotic prophylaxis as a protective measure which enabled their child to take part in more activities. A few parents described prophylaxis as a reminder that their child was susceptible and needed their activities restricted. [Table 3, 2.3]

1. **Response to acute illness whilst on prophylaxis**

Despite taking prophylactic azithromycin, most children had breakthrough respiratory illnesses. There was often an initial emotive response when parents heard their child starting to cough. This evoked fears of progression to a hospital admission. Combined with feelings of helplessness and futility of self-help measures, parents often felt driven to take action. [Table 3, 3.1]

Parents found it difficult to decide on the threshold for consulting a healthcare professional during acute illnesses. As well as looking for clinical cues, they also reported social norms and practicalities as barriers to consulting. These included, “not wanting to waste the doctor’s time” or being seen as a “paranoid mother” and difficulties making GP appointments.

The perceived severity of their child’s illness and susceptibility did not always correspond to that of the healthcare professional or underlying diagnosis. Parents of two children with bronchiectasis had higher thresholds for seeking medical attention and isolated their children less, despite having a more severe underlying diagnosis. [Table 3, 3.2]

Parents’ understanding of science, relating to antibiotics and infections, plays a role in determining their expectations for antibiotic prescribing during acute respiratory illnesses. All parents knew antibiotics should not be used for common colds. Despite some knowing viruses caused the common cold, the term “infection” was often misinterpreted as being severe and requiring antibiotics. Confusion arose when they were told their child had a virus but were given antibiotics for the “viral infection”. This reinforced the severity of the situation and started a cycle of expectations for future antibiotic courses. [Table 3, 3.3]

**Discussion**

The overriding factor influencing parental decisions about the uptake of antibiotic prophylaxis, is wanting their child to be well now. The main concern voiced by most parents is that of antibiotic resistance, but this is seen as a problem for the future, not the present.

Based on our findings, we propose a behavioural model describing phases parents’ cycle through once their child is prescribed antibiotic prophylaxis. The model illustrates how the key themes inter-relate (See figure 2 for model and explanation). An awareness of this cycle may help clinicians prepare families for prophylaxis, pre-empt potential difficulties and focus on areas to improve the family’s experience.

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Parents have the responsibility of making medical decisions on behalf of their children. Those parents actively participating in decisions have feelings of security and control over the situation and are more convinced the treatment prescribed is correct (Boland et al., 2017). In comparison, those passively taking part are reported to have post-consultation decisional conflict and feel powerless and insecure (Boland et al., 2017). Parents may not always want to be actively involved in decisions. Their desire to be involved may change with time, knowledge, the gravity of the decision and their emotional response (Aarthun et al., 2014). Those parents, supported by health care professionals, who acknowledge the importance of the parents’ contributions, have a positive experience and are more likely to become involved in future decisions (Aarthun et al., 2018).

From our study, clinicians should be mindful of passive parents with unvoiced concerns when making decision regarding prophylaxis. Breaking down potential barriers that dissuade parents’ participation may help a transition to more active involvement. This includes acknowledging parents as experts in their own right, irrespective of heath literacy, focusing eye contact on parents rather than computer screens, providing adequate information and addressing social norms.

Parental understanding significantly impacts on decision making about their child’s health [17]. It is suggested that clearer explanations about antibiotic indications in terms of signs and symptoms and clarification of the severity of bacterial and viral infections would help reduce misconceptions about when antibiotics are required or not [16]. Our finding of a parental misconception of the word “infection” corroborates this literature.

Our study echoes findings previously reported of ongoing misconceptions relating to resistance, i.e. the body rather than the pathogen becoming resistant [14, 15], and confusion between viral and bacterial infections and hence the need for antibiotics [15, 16]. Previous reports suggest parents perceiving their children as low antibiotic users do not see antibiotic resistance as an issue for their children [16]. However, parents in our cohort see their children as high users of antibiotics, who are at risk of resistance (albeit in the future) and weighed this factor when making decisions about prophylaxis. This was not reported in the only previous study looking at perceptions of parents on an oncology unit [10] and is a novel observation.

Ensuring parents are prepared for prophylaxis is important. This requires that practical aspects of medication are covered and ways of medication normalisation are discussed. The aims are to facilitate adherence, acceptance and reduce anxiety. Unintentional effects of prophylaxis should be considered. Paradoxically, parental anxiety may increase with prophylaxis promoting a sense of susceptibility to infections resulting in childhood activities being further restricted. Preparatory counseling could be undertaken by a variety of health care professional including pharmacists.

Treatment adherence is challenging and a complex balancing act between competing concerns including parental beliefs, child resistance, preserving family relationships and promoting “normal life” for the family [17]. Remembering to administer medication was reported to be one of the biggest challenges [18] which was re-iterated in our cohort of parents.

Discussing thresholds for seeking medical assessment during acute illnesses is helpful. It enables parents to feel they are seeking assessment at appropriate times, alleviates anxieties and through good communication with general practitioners, raises awareness of suggested management plans. This in turn may break a cycle of expectations for future antibiotics.

Our study has limitations. The cohort of parents interviewed does not fully represent the diversity of families seen at SCH. Only three male parents and one non-white British family were interviewed. Non-English speaking parents were excluded as interpreters could not be funded. Further research is needed to explore the ethnic and cultural dimensions that may influence parents’ experiences. The parents interviewed may be a self-selecting group, choosing to participate in order to voice concerns or opinions. Parents who did not participate may potentially be different and have other diverse experiences. The interviewer was a paediatric registrar whose medical outlook may have biased the questions asked and interpretation of the parents’ comments. Finally, the proposed model is based on prophylaxis to prevent respiratory tract infections and may not be generalisable to other indications of antibiotic prophylaxis. The parental experience may differ where antibiotics are prescribed with multiple other medications, for example, as occurs in patients with cystic fibrosis.

**Conclusion**

It is not sufficient for clinicians to prescribe prophylactic antibiotics with the expectation that parents will adhere. This “current” approach does not take into account the complex interplay between health, psychology and behaviour. We need to address the parental experience holistically when prescribing long term antibiotic prophylaxis by preparing families and breaking down barriers that may prevent their active involvement in consultations. Time and effort invested in this we believe, would facilitate antibiotic adherence, reduce anxieties and improve relationships between parents and medical professionals.

**Acknowledgements**

We acknowledge the parents and careers who took part in the interviews and the specialist nurses who helped with the recruitment process.

**Funding Statement**

This research was supported by the Bassetlaw clinical research fellowship scheme, post reference no. 50059609, and the charity Antibiotic Research UK, ANTSRG 03/2018.

**Contribution Statement**

FS conceived the idea. All authors contributed to the study design. SH conducted the interviews. AL and SH conducted the initial analysis and developed the framework of themes. AC, FS and KU contributed to the final analysis. SH drafted the manuscript. All authors provided critical feedback and helped shape the manuscript.

**“What is already known on this topic”**

1. Parental knowledge and beliefs have been identified as essential factors to consider when changes in antibiotic prescribing are required.
2. Active parent involvement in decision making promotes feelings of security that the correct medication has been prescribed.
3. Parents feel that prophylactic antibiotic use is beneficial and are less concerned that antibiotic resistance may directly affect their children.

**“What this study adds”**

1. Parents feel antibiotic prophylaxis is beneficial but they are concerned about future antibiotic resistance affecting their children and running out of treatment options.
2. Improving preparation for antibiotic prophylaxis may help address issues of adherence, antibiotic resistance and reduce parental anxieties.
3. A behavioural model reflecting different phases parents go through with regards to antibiotic prophylaxis is proposed that could be used to enhance parental experience.

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**Table 3 Anonymised quotations (Parental consent specifically obtained)**

|  |  |
| --- | --- |
| Theme identification number | Quotations |
| 1.1 | “We all help to decide. I mean if we weren't doing our job as parents, keeping an eye on him, we wouldn't know he’s got that cold and things like that.” **(Parent 10)** |
| “They (doctors) are qualified. They think they know best. But they don't. They don't go through the nights of the child not sleeping because they can’t breathe, they do not know… they might know on a medical concept.” **(Parent 8)** |
| “They will ask, do you have any questions and how we feel about his medication. It does feel like a 2-way discussion. It’s that we are being passive.” **(Parent 6)** |
| 1.2 | “...the doctor tells me the plan is and as a parent I just go along and think he's the doctor, he knows best... I think me asking questions, I'm holding that doctor up, I don’t' want to bother him. It isn’t till I come home I think, is it doing him any good (prophylaxis)? Is it harming him?” **(Parent 9)** |
| “You can't always concentrate. Your toddler is sticking his hands in the orange bin with the gross stuff or dragging a car up the wall when you are trying to listen to really important information. It’s ever so difficult. **(Parent 1)** |
| ” I go into clinic and they (doctors) are sat at the computer typing away or writing. It is very rare they even give you eye contact. And you think they are under so much pressure and you are just another body. I feel I’m just another body, with another poorly child.” **(Parent 9)** |
| “I don't want to seem like a paranoid mum. I feel she has taken a turn for the worst when she is coughing and being sick. Believe me I’m not a drama queen” **(Parent 2)** |
| 1.3 | “We're in such a position now that we constantly ask questions. Not to be difficult but we want to know why. We had big questions about long term antibiotic use. You hear a lot in the press about the use of antibiotics and so we had a lot of questions regarding that. It was us asking rather than people proactively telling us anything.” **(Parent 1)** |
| “I think people perceived doctors being that higher authority that sometimes can't be questioned. That is not how it is in society these days. Doctors are saying you are the expert on your illness.” **(Parent 6)** |
| 1.4 | “I was brought up with natural medicines and homeopathy and it always worked. I hate antibiotics because I was brought up to hate them. But they have worked so I was grateful, but very uncomfortable with him having to be on them.” **(Parent 4)** |
| “Again I've mixed feelings. Because I think, is it natural to be on an antibiotic for all that long term?” **(Parent 9)** |
| “I was always very anti antibiotics. If I could get away without having them, I would. I mean your body can stop most things. **(Parent 3)** |
| “We won’t' go on holiday because we are fearful that it is going to be another hospital admission and we don't want to be in a strange place in a strange hospital. **(Parent 8)** |
| 1.5 | “You just don’t' want him to get something really bad when he is older and nothing (antibiotics) works.” **(Parent 7)** |
| “Well it would be a problem for him wouldn’t it when he is older.” **(Parent 14)** |
| “I mean I were brought up with you got antibiotics for pretty much anything. But I know nowadays they don't like it. As you get older and you really do need them, if you have them too often you are eventually immune to it..” **(Parent 12)** |
| “If you take antibiotics, your body can grow immune to it.” **(Parent 10)** |
| “It’s just a bit of scaremongering. It goes whichever way it wants to go, media in my opinion… They play it down a bit like antibiotics won't cure your cold and stuff. But for us, we’re not abusing antibiotics. It’s quite clearly she’s dependent upon them. We have to go with the specialists.” **(Parent 2)** |
| 1.6 | “I was a bit worried about how long is it going to be before they (prophylaxis) stop working. Because I know your body builds up a resistance towards them… I just thought about him being well now. I mean in years to come there might be more antibiotics available. Here and now I just wanted him to be as well as he can be. Like he were only at school 50% of the time. So he were behind on school work. I thought that it were the right thing to do to start him on the antibiotics.” **(Parent 13)** |
| “Well, I could have said no I don't want (prophylaxis) but there wasn't like… well if you don't want to do that we'll do this instead. So it was kind of like you can do this or you can't do this, but there was no like this is another option. “**(parent 14)** |
| “It (prophylaxis) felt as if it was something that was done almost as a last resort.” **(Parent 6)** |
| “Erm I just hated seeing her so ill all the time. I just wanted them to do something, I didn't care at the time what. I was happy for her to try them (prophylaxis) at that point. And I never thought I'd say that as I'm not an antibiotic fan. I never used to be but I am now.” **(Parent 3)** |
| 2.1 | “I was given this bag of medicines and it was you need to give it to him Monday Wednesday Friday and it freaked me out to be honest... I were terrified of forgetting. What if I forget just one day? He’s going to get ill. If I don’t give it to him on time, what if I give them to him late, will that make any difference to him? Could he get an infection because I've given him his antibiotics in the afternoon and not the morning?” **(Parent 5)** |
| “…it’s easy to forget it. I mean you go a week and actually no he’s not had it… Yeah we do find that he will start to be more unwell. Like we notice he just gets a bit more wheeze especially at night. Have we done it, have you done it? No, ahh that might be why.” **(Parent 6)** |
| “Remembering what day you are on when you are doing Monday, Wednesday, Friday, (can be difficult). Whereas if it’s part of your daily routine, yeah we just get up, brush our teeth, have our medicine, have our breakfast and mark it on the calendar which we all share. **(Parent 2)** |
| 2.2 | “The consultant explained it very well, what it was for, what they hoped it would do, and he would stop it. It was literally I had no idea it was a powder and I had to make it so that was a bit of a surprise.” **(Parent 14)** |
| I had nothing but hassle with this antibiotic. Just the simplest thing from getting it from the chemist because at the start they wouldn't give me dry powder to make it up myself. So I was like battling to and fro trying to get this antibiotic and even now, I didn’t have no medicine for him on the. So every month I've had to phone up the doctors and say, I've no medicine left for Monday. **(Parent 9)** |
| “On a personal side of things it were like, thank God we don't have to do that now (administer prophylaxis)... It’s a case of that little bit of relief now.” **(Parent 10)** |
| “The new antibiotics only last 5 days. You have to discard whatever is left…. I'm wasting antibiotics every week, which... That is NHS money. (Interviewer: What do you do with the antibiotics?) Well I just have to flush it away. We don't know how to dispose of it really.” **(Parent 15)** |
| They don't provide you with enough information when they give them (prophylaxis) you. They just say here, have some antibiotics. So I don't' know much about the antibiotics to be fair. **(Parent 8)** |
| 2.3 | “Like I said with the ball pools and things like that. If he wasn't on those antibiotics, he wouldn't set foot in the place. I'd be too scared of him picking something up. While ever he's on these antibiotics, I’ve got that little peace of mind that they are protecting him. If he came off them I’d think that would stop and the only place he'd go would be the garden..” **(Parent 5)** |
| “She now does swimming lessons whereas I couldn't let her do that before because I'd be worried in case she picked more bugs up.” **(Parent 3)** |
| “I throw her in at everything. When we are outside, she can do mucky play. I don't treat her any differently (being on prophylaxis)” **(Parent 2)** |
| We haven’t been swimming but yeah in terms of mixing with people, I feel more comfortable that I know we have got that cover. But if someone has got a cough, don't cough on him but he has got the antibiotics. Yeah so I guess it gives you some kind of reassurance that he’s not completely on his own. **(Parent 4)** |
| 3.1 | “Even if he just does 2 coughs in his sleep, you go, here we go again. You know and I try and put it into perspective because I think well… We always get through it... So yeah when he starts with a cough. I always expect the worse. We'll end up in hospital he’ll go onto oxygen.” **(Parent 1)** |
| “Back to chest infections and in and out of hospital. It’s a destruction of her life that she doesn't need. A common cold can quite easily turn into a severe chest infection. It is just stress. Stress for her, stress for me and progression from normal common colds to chest infections. It is just so drastic.” **(Parent 8)** |
| “But a lot of the time it is viral and I know that antibiotics won't help but you feel like you are doing something. And I know that... but as a parent you want to be doing something.” **(Parent 1)** |
| “(giving acute courses of antibiotics) you feel like you are doing something. I mean when kids get poorly you feel pretty helpless. Cough syrups don’t' work. You can't give them decongestants. So you can pretty much just give them paracetamol and hope it works.” **(Parent 12)** |
| 3.2 | “Initially we were told; when she has a temperature it might be that she has a worse infection so get her checked out. She gets temperatures less times than not. So that is not a very good gauge for us. Then we were told if her cough is lasting for a couple of weeks, go and get it checked out. And then we were told actually 2 weeks is too long to be waiting. So because of our not wanting her to be on antibiotics too much, coupled with her not getting high temperatures, we haven’t been going and getting extra antibiotics that much. So, I think that is why we have been told, we need to lower our threshold… So I guess the more it happens, the more I’m getting to know when she might need to have some extra help and when not.” **(Parent 11)** |
| “It’s a bit stressful because it is difficult to get a GP appointment and I know it sounds awful but to fit it in. To try and fit it in, phoning up at 08:00 in the morning…I don't necessarily feel like I'm doing a good job at managing it.” **(Parent 11)** |
| 3.3 | “You can get other bacterial infections like hand foot and mouth, that was going round school and I kept him at home…They ask people not to take antibiotics for a cold because they don't work.There’s nothing you can do its gonna run its course unless it does change and you get an infection then you can have some antibiotics.” **(Parent 5)** |
| “There’s quite a few bugs out there like viruses and stuff that can be beaten by the human body but obviously when it’s an infection it needs treating by antibiotics.” **(Parent 2)** |

Legend

Quotations in this table have been taken directly from the interview transcripts. They have been selected to support the themes developed in the final analysis as reported in table 2 and described in detail within the results section.

## Appendix 8: BAL bacterial culture results from participants in PBB study 1 and 2

### BAL results of participants with a microbiological diagnosis of PBB – Study 1 PBB

Bacterial isolates grown from bronchoalveolar lavage (BAL) in those with a microbiological diagnosis of PBB in PBB study 1

|  |  |  |
| --- | --- | --- |
| Bacterial isolate growth on BAL | **Control**  Microbiological diagnosis N = 5 | **Azithromycin**  Microbiological diagnosis N = 6 |
| *H. influenzae* | 3 | 4 |
| *S. pneumoniae* | 3 | 1 |
| *M. catarrhalis* | 3 | 1 |
| *H. parainfluenzae* | 0 | 2 |

### BAL results of participants with a microbiological diagnosis of PBB – Study PBB 2

Bacterial isolates grown from bronchoalveolar lavage (BAL) in those with a microbiological diagnosis of PBB in PBB study 2

|  |  |  |
| --- | --- | --- |
| Bacterial isolate growth on BAL | **Control**  Microbiological diagnosis N=8 | **Azithromycin**  Microbiological diagnosis N=14 |
| *H. influenzae* | 5 | 12 |
| *S. pneumoniae* | 2 | 3 |
| *M. catarrhalis* | 3 | 2 |

## Appendix 9: PBB study 1: MIC data

*All bacterial isolates were tested for azithromycin susceptibility using E-test strips. The EUCAST breakpoints were used to determine susceptibility or resistance. The dotted red line indicates the breakpoint for each bacteria.*

* *M. catarrhalis: >0.5 mg/L*
* *S. aureus: > 2 mg/L*

## Appendix 10: Quality control using ‘decontam’ in R

The following graphs were generated during the quality control process in R using the packages ‘phyloseq’ and ‘decontam’. First the raw reads were to ensure that the samples reads were higher than the controls,

### Raw reads in relation to sample type

Chart

Description automatically generated

*A graph to show the number of raw reads per sample in relation to the 4 sample types.*

### Microbial composition of all negative controls

A picture containing chart

Description automatically generated

*A graph to show the raw read count and relative abundance of the top 15 ASVs in the negative controls. Blank\_526\_1a and \_1b were PCR blanks and removed.*

### Microbial composition of the mock communities

Chart, bar chart

Description automatically generated

*A graph to show the relative abundance of the top 15 ASVs in the two positive control mock communities with known community compositions.*

### Microbial composition of the Iso\_blank controls

Chart

Description automatically generated

*LA graph to show the raw read count and relative abundance of the top 15 most abundant ASVs in the 10 negative ISO\_blank controls containing reagents aliquoted during the DNA extraction*

### Microbial composition of the nasopharyngeal swab transport medium blanks

Chart

Description automatically generated with medium confidence

*A graph to show the microbial composition and raw read count of the 4 nasopharyngeal transport medium controls that did not contain any samples or reagents. Only the top 15 most abundant ASVs are shown.*

## Appendix 11: Additional NMDS plots

Baseline comparisons

Neither being in the active vs comparison group nor the mode of delivery were significant when tested in multivariable PERMANOVA model; R2= 0.04, p= 0.37 and R2= 0.04, p=0.47 respectively. A univariant PERMANOVA model was run in order to ensure that there was not a large effect of the azithromycin treatment masking the effect of the mode of delivery when in a multivariable model. This was however not the case; R=0.04, p=0.53.

Chart, bubble chart

Description automatically generated

*Beta diversity calculated using the Bray-Curtis dissimilarity measure and plotted using Non-Metric Multidimensional Scaling (NMDS). Coloured plots indicate the baseline samples taken from participants either in the comparison or azithromycin group. The square or cross represents whether the participant was delivered vaginal delivery or not.*

Neither being in the active vs comparison group nor being breast fed or not were significant when tested in multivariable PERMANOVA model; R2= 0.04, p= 0.39 and R2= 0.05, p=0.30 respectively. A univariant PERMANOVA model was run in order to ensure that there was not a large effect of the azithromycin treatment masking the effect of the mode of delivery when in a multivariable model. This was however not the case; R=0.05, p=0.26.

Chart, bubble chart

Description automatically generated

*Beta diversity calculated using the Bray-Curtis dissimilarity measure and plotted using Non-Metric Multidimensional Scaling (NMDS). Coloured plots indicate the baseline samples taken from participants either in the comparison or azithromycin group. The square or cross represents whether there was breast fed or not.*

Neither being in the active vs comparison group nor exposure to parental smoking were significant when tested in multivariable PERMANOVA model; R2= 0.04, p= 0.42 and R2= 0.03, p=0.75 respectively. A univariant PERMANOVA model was run in order to ensure that there was not a large effect of the azithromycin treatment masking the effect of the mode of delivery when in a multivariable model. This was however not the case; R=0.03, p=0.76.

Chart, bubble chart

Description automatically generated

*Beta diversity calculated using the Bray-Curtis dissimilarity measure and plotted using Non-Metric Multidimensional Scaling (NMDS). Coloured plots indicate the baseline samples taken from participants either in the comparison or azithromycin group. The square or cross represents whether there was parental smoking or not.*

## Appendix 12: Draft of clinical trial protocol for future study



**TRIAL FULL TITLE –** Long-term high vs low dose azithromycin in children with protracted bacterial bronchitis: a randomised controlled trial

**TRIAL SHORT TITLE** – Long-term azithromycin dosing in PBB

**DATE AND VERSION NUMBER** – 31/05/2022 Version 1

**LAY SUMMARY**

Chronic wet cough in preschool children is very common and may be due to bacterial or viral infections. In practice it is difficult to tell them apart; Viral infections usually get better on their own and do not respond to antibiotics. Recurrent bacterial chest infections may however cause permanent damage to the breathing tubes (airways). This condition is known as bronchiectasis and predisposes to infection, hence reducing the number of infections is important. Children who are at risk of developing bronchiectasis, are often prescribed long-term ‘prophylactic’ antibiotics (especially over the winter months) to try and reduce the number of infections and thus the risk of airway scarring. In particular, there is increasing use of a strong antibiotic called Azithromycin. The use of long-term antibiotics is however associated with various side effects. These include not only the potential side effects directly relating to the antibiotic (e.g. diarrhoea) but also promotion of antibiotic resistance and the destruction of colonising beneficial bacterial populations that reside within us particularly in the gut and airways (the microbiome). Results from adult studies investigating the effects of long-term azithromycin to prevent bronchiectasis and respiratory tract infections have found azithromycin to be efficacious at low doses. In children the optimal dosing regimen has not been studied. Preliminary results from studies have shown that lactulose (a medication often used for constipation) may restore changes to the gut microbiome following long term antibiotic use.

We hypothesis firstly that using low-dose long-term azithromycin is as effective as the usual dose long-term azithromycin. We postulate that a low dose regimen would have less detrimental effects on the microbiome and reduce the risk of harbouring antibiotic resistance. Our second hypothesis is that administering 2 weeks of lactulose on stopping long term azithromycin will restore any changes to the gut microbiome quicker than a control group not exposed to lactulose.

We aim to:

1. Investigate whether low dose Azithromycin (5mg/kg/dose thrice weekly) is as effective as the usual dose of Azithromycin (10mg/kg/dose thrice weekly) long-term in reducing pulmonary exacerbations
2. Assess the impact of the above dosing regimens on the nasopharyngeal and intestinal microbiome, and antibiotic resistance patterns
3. Investigate whether 2 weeks of oral lactulose of stopping long term azithromycin facilitates restoration of the gut microbiome

**1.0 BACKGROUND**

Chronic cough in children is extremely common with a prevalence in the region of 9% (Leonardi et al., 2002). It is a cause of multiple medical consultations and has a negative impact on quality of life. Protracted bacterial bronchitis (PBB) is diagnosed in up to 40% of children presenting to paediatric respiratory specialists with chronic cough (Marchant et al., 2006). PBB is defined as wet cough for >4 weeks with no other detectable chronic respiratory disease and a perceptible response to 14 days of oral antibiotics (Kantar et al., 2017) it has been associated with neutrophilic inflammation on bronchoalveolar lavage (Chang et al., 2006) and with positive bacterial cultures, particularly Haemophilus influenzae (Marchant et al., 2006). It is most often described in children under the age of 6 years and because of the challenges of invasive sampling in this age group, the diagnosis is usually made on clinical grounds.

Many children with PBB continue to have pulmonary exacerbations characterised by increased cough, volume of sputum and associated with new chest signs such as crackles. These recurrent episodes may be due to bacterial or viral causes but in children it is often difficult to tell them apart. In practice, these episodes are usually treated with at least 2 weeks of antibiotics. Although the natural history is unclear, recurrent PBB (>3 episodes per year) has been reported to be predictive of the future diagnosis of bronchiectasis (Goyal et al., 2014) (Wurzel et al., 2015), which confers lifelong susceptibility to respiratory infection and increased risk of premature death (Quint et al., 2016).

Randomised controlled trials have shown that long-term therapy (3 months to 2 years) with the macrolide antibiotic azithromycin, can reduce the frequency of pulmonary exacerbation in paediatric Primary Ciliary Dyskinesia, Cystic Fibrosis (CF), non-cystic fibrosis bronchiectasis and HIV associated chronic lung disease (Ferrand et al., 2020; Kobbernagel et al., 2020; Southern et al., 2012; Valery et al., 2013). The effects on other outcomes such as lung function are less clear (Ferrand et al., 2020) (Wong et al., 2012). It is thought that its efficacy in these chronic inflammatory diseases is due to the immunomodulatory effects of azithromycin rather than a predominant antimicrobial effect. Although the exact mechanism of action of azithromycin in each condition is uncertain, substantial evidence exists to suggest that inhibition of neutrophilic inflammation and macrophage activation play a significant role (Zimmermann et al., 2018). With increasing supportive evidence for the use of azithromycin in other inflammatory lung conditions, 10mg/kg/dose thrice weekly Azithromycin over the winter months is recommended for those with PBB experiencing more than 3 pulmonary exacerbations a year (Gilchrist, 2019). There is however limited supporting evidence for long term azithromycin use in PBB.

The optimal paediatric dosing for long term azithromycin is unknown. Its long tissue half-life (average 48-96 hours) and larger volume of distribution, allows for extended interval dosing as opposed to daily dosing(Lode, 1991). The BNFc advises a weight dependant thrice weekly dosing regimen of 10mg/kg/dose in cystic fibrosis with chronic pseudomonas infection. Guidelines for the management of primary immune deficiencies recommend 10mg/kg weekly or 5mg/kg alternate days to prevent bacterial respiratory tract infections in children (Bonilla 2015). In clinical trials, a variety of regimens have been used including daily, thrice weekly and once weekly dosing using doses between 5-30mg/kg/dose depending on the intervals between doses.

In children, there are just 2 RCTs comparing dosing regimens, neither of which reported any differences in primary outcomes. The first compared 6 months of daily low dose to high dose Azithromycin (5mg/kg/dose *vs*. 15mg/kg/dose) in children with CF. No difference in pulmonary exacerbation frequency was seen whilst on treatment nor the distribution of organisms found on cough swabs during exacerbations (Kabra et al., 2010). The second study included children and adults with CF and investigated 6 months of once daily (250mg) *vs*. once weekly (1200mg) Azithromycin. No differences were seen in intravenous antibiotic rates or hospitalisation for pulmonary indications (McCormack et al., 2007). Studies in adults with asthma and primary antibody deficiencies have also reported reduced pulmonary exacerbations with low dose regimens of 250mg Azithromycin thrice weekly (Brusselle et al., 2013); Milito et al., 2019).

Both standard 3 day courses and longer course of up to 2 years of azithromycin have been associated with an increase in macrolide phenotypic resistance and resistance genes in intestinal and respiratory flora (Hare et al., 2015; Malhotra-Kumar et al., 2007; Taylor et al., 2019; Wei et al., 2018). The implications of harbouring a reservoir of resistance genes are both important for the individual who may develop future infections with resistant organisms but also to the community if such genes are transmitted between individuals. More recently, culture-independent techniques have allowed analysis of the impact of antimicrobials on the diverse microbial communities that reside within anatomical niches such as the intestinal and respiratory tracts (microbiota) and resistance genes (resistome).

Standard courses of azithromycin have been associated with a short-term reduction in paediatric gut microbial richness and diversity although these changes were no longer evident at 1-3 years follow up (Doan et al., 2017; Parker et al., 2017; Wei et al., 2018). A distinct microbiota composition has been reported in children exposed to macrolides within the previous 6 months with reduced abundance of Actinobacteria (*Bifidobacterium*) and increased relative abundance of gram-negative organisms from Bacteroidetes and Proteobacteria phyla (Korpela et al., 2016). By disrupting the microbial community, antibiotics also alter production of microbial metabolites, short chain fatty acids (SCFAs), which are known to have anti-inflammatory properties as well as being involved in energy balance, metabolism and modulation of adipose and liver tissue (den Besten et al., 2013). Early childhood antibiotic exposure particularly under the age of 2 years, when the adult microbiome has not been fully established, has been associated with an increased risk of obesity, asthma and allergy (Langdon et al., 2016; Pascal et al., 2018). The extent, impact and duration of changes to the microbiome and resistome following long-term Azithromycin exposure is not fully understood.

Lactulose, a synthetic disaccharide often used to treat constipation, is reported to have prebiotic properties and the ability to promote beneficial bacterial growth specifically those producing SCFAs (Cardelle-Cobas et al., 2009). Emerging evidence suggests that concurrent lactulose (20mg/kg/dose) following exposure to 3 days of azithromycin may re-establish the paediatric gut microbiome faster, compared to a control group who were not administered lactulose (Nikolaou et al., 2020). This raises the question of whether Lactulose is able to reduce the detrimental effects of longer term Azithromycin on the gut microbiome in children.

This randomised controlled trial in children with PBB firstly aims to compare thrice weekly low (5mg/kg/dose) and standard (10mg/kg/dose) dose long-term Azithromycin regimens. The primary outcome will be the number of pulmonary exacerbations over the study period and secondary outcomes will include gut microbiome and resistome analysis. Secondly, the effects of lactulose in re-establishing the gut microbiome following antibiotic exposure, will be investigated. If a low dose regimen is found to be as effective as reducing pulmonary exacerbations compared to the standard regimen, which has less impact on the microbiome and resistome, a larger RCT will be performed to inform future guideline development as to the optimal dosing of Azithromycin in paediatric PBB.

**2.0 TRIAL OBJECTIVES AND PURPOSE**

This is a feasibility trial with the purpose of testing the hypothesis that low dose long-term azithromycin (5mg/kg/dose thrice weekly) is not inferior to usual dose long-term azithromycin (10mg/kg/dose thrice weekly) in reducing pulmonary exacerbations and has less impact on the nasopharyngeal and intestinal microbiome and resistome. We aim to collect preliminary data on the efficacy and safety of low dose azithromycin (AZM) prophylaxis before proceeding with a larger non-inferiority randomised controlled trial. The following objectives include the core set of outcomes identified for clinical trials involving children with PBB (Gilchrist 2020).

**Primary objectives**

**First primary objective**

|  |  |  |
| --- | --- | --- |
| Primary outcome | Primary outcome measure | Time points of evaluation |
| To determine whether 6 months of low dose AZM is not inferior to usual dose AZM in reducing the number of pulmonary exacerbations | The mean difference between trial arms in the number of pulmonary exacerbations\* experienced during the study intervention period | At 2, 4 and 6 months |

\*Pulmonary exacerbations will be defined in 2 ways:

1) PBB exacerbation (PPBE) as defined in the Sheffield children’s hospital guideline (appendix 2)

* 4 week history of persistent wet cough
* No new cough pointers\*\* identified on clinical assessment to suggest another cause of chronic cough
* Treatment with 2-4 weeks of oral antibiotics

\*\*Specific cough pointers Symptoms: chest pain, history suggestive of inhaled foreign body, dyspnoea, exertional dyspnoea, haemoptysis, failure to thrive, feeding difficulties (including vomiting or choking), cardiac or neurodevelopmental abnormalities, recurrent sinopulmonary infections, immunodeficiency or epidemiological risk factors for tuberculosis.

Signs: respiratory distress, digital clubbing, chest wall deformity or auscultatory crackles. Investigations: chest radiograph changes other than peri-hilar changes or lung function abnormalities

1. Protocol defined lower respiratory tract infection (PDLRTI) based on definitions used for children without PBB.

48 hours of 2 or more of the following symptoms and signs:

* Temperature over 38oC
* Fast or increased work of breathing
* Increased cough
* Increased sputum volume or colour intensity
* New changes on a chest radiograph or chest auscultation (crackles/crepitations or bronchial breath sounds)
* The need for respiratory support or oxygen

Second primary objective

|  |  |  |
| --- | --- | --- |
| Primary outcome | Primary outcome measure | Time points of evaluation |
| To determine whether 2 weeks of lactulose, on cessation of long-term azithromycin use, re-establishes the gut microbiome faster than a lactulose unexposed control group | The difference in stool microbiota between study arms during the lactulose intervention period. | At baseline\*, then 3,6, 6.5\*\* 9, 12 and 18 months |

\*Baseline samples would be taken before the first dose of AZM was given.

\*\*This stool sample will be taken at the end of the 2 week period of lactulose administration.

**Secondary objectives**

|  |  |  |
| --- | --- | --- |
| Secondary objectives | Secondary outcome measure | Time points of evaluation |
| To determine whether 6 months of low dose Azithromycin compared to usual dose AZM is not inferior with regards to :   1. Cough resolution 2. Parent-proxy reporting of the child’s quality of life 3. Cumulative antibiotic treatment whilst on the study medication 4. Hospital admissions 5. Development of bronchiectasis 6. Tolerability and adherence 7. The number of pulmonary exacerbations outside of the intervention period | Change in (weekly) cough scores recorded in a cough score diary  Change in child and parent quality of life  Additional number of acute courses of antibiotics prescriptions (parent record)  Number of acute hospital admissions for respiratory deterioration  Diagnosis of bronchiectasis based on chest HRCT findings  Parent documented adherence diary and volumes of returned surplus study medications  Differences in the number of pulmonary exacerbations (as described in the primary outcome) | Baseline, 3, 6, 9, 12, months  Baseline, 3, 6, 9, 12 months Health related QoL questionnaire  3,6,9,12, months   * Parent records * GP/ hospital prescription record review at 6 and 12 months18 months   Baseline, 6, 12, months  Baseline, 6 , 12 months  1, 3 ,6, 6.5 months  3,6, 12, 18 months for Azithromycin |
| To compare the impact of azithromycin on gut and respiratory microbiota and antimicrobial resistance | Nasopharyngeal swabs for culture/ standard antimicrobial susceptibility testing and microbiome analysis.  Stool microbiome and resistome analysis | Baseline, 3, 6, 12 months  Baseline, 3,6, 6.5,9,12 months |

1. **TRIAL DESIGN**

We will carry out a single centre prospective, double blind randomised comparison trial in children with a clinical or microbiological diagnosis of protracted bacterial bronchitis. The study will compromise of 2 key phases. The first investigating azithromycin and the second lactulose. A total of 90 children will be recruited.

**Phase 1**

We will carry out a multi-centre, randomised double blind comparison trial in children with a clinical or microbiological diagnosis of protracted bacterial bronchitis. There will be three study groups: Two active groups and one control group (see diagram 1). A total of 30 children will be recruited to each of the groups.

Participants with PBB will be randomised to one of two azithromycin dosing regimens in a 1:1 ratio. Group 1 will receive the standard paediatric azithromycin dose of 10mg/kg/day thrice weekly, as recommended in the British National Formulary for children with infection in cystic fibrosis. Group 2 will receive a low dose regimen of 5mg/kg/dose thrice weekly. Both groups will be prescribed the study drug for 6 months. Group 3 will consist of healthy age-matched controls who meet the inclusion and exclusion criteria. All participants will remain in the trial for 18 months.

**Phase 2**

Participants in groups 1 and 2 will be randomised, on a 1:1 ratio, to receive either two weeks of daily lactulose at a dose of 1ml (<15 kg) or 2ml (>15kg) or no lactulose.

**Diagram 1: Flow chart of participant recruitment and randomisation**

Potential Patients identified

Informed consent obtained

Phase 1: 0-6 months

**Group 3**

Healthy Controls: (n=30)

**Children with PBB**

Requiring long term azithromycin

Remain in study for 18 months

First Randomisation

**Group 2**

Azithromycin

5mg/kg/dose thrice weekly

(n=30)

**Group 1**

Azithromycin 10mg/kg/dose thrice weekly

(n=30)

Phase 2:

6-6.5 months, or on cessation of azithromycin

Second randomisation

**Group 4**

Lactulose daily for 2 weeks

(n=30)

**Group 5**

No Lactulose

(n=30)

Remain in study for 18 months

**Selection and withdrawal of participants**

We will recruit children, and their guardians under the care of the following hospital trusts:

1. Sheffield’s Children’s Hospital Foundation Trust
2. Centres 2-4

Children with PBB (Groups 1-2) will be recruited who are under the care of the Paediatric Respiratory team. Healthy controls (Group 3) will be recruited from fracture clinics, audiology or ophthalmology clinics.

**Inclusion criteria groups 1 and 2**

* Children aged between 6 months and 5 years (inclusive) at randomisation
* Written informed consent from the guardian
* Guardian has a good understanding of the English language and able to communicate in English
* Clinical or microbiological diagnosis of protracted bacterial bronchitis\*
* Previously prescribed long-term antibiotics and undergone at least a 13 week “washout” period”
* Have received at least 2 courses of oral antibiotics to treat LRTIs over the preceding 52 weeks

**Inclusion criteria Group 3 – healthy controls**

* Age matched to within 12 months of groups 1 and 2
* Sex matched 1:1 (male: female) to participants in group 1 and 2
* Written informed consent from the guardian
* Guardian has a good understanding of the English language and able to communicate in English
* Not on medication that could impact the respiratory or gastrointestinal systems (including for example anti-reflux medication, laxatives, probiotics, antibiotics for last 1 year). No known GI disease/condition

**Exclusion criteria group 1-3**

* Diagnosis of cystic fibrosis or ongoing investigations
* Children prescribed long-term antibiotics for other reasons i.e. UTI
* Not registered with a GP
* 3 monthly Intravenous antibiotics
* Known contra-indication to using or hypersensitivity to macrolide antibiotics (e.g. Prolonged QTc or concurrent prescribing of QTc prolonging medication
* Known severe hepatic disease
* Recruitment to another investigational medicinal product (IMP) trial and continuing to use the IMP
* Penicillin allergy
* Excluded from randomisation into phase 2 if already prescribed laxatives, on a food exclusion diet or taking probiotics.

Children recruited to Groups 1 and 2 will receive 6 months of azithromycin. The decision to start long-term azithromycin will be made by the treating respiratory doctor who is managing the child. Children must have had a least 2 exacerbations of PBB over the preceding year to be considered for long term azithromycin. Due to the fact that children will be treated as per the standard best practice by the respiratory team, we acknowledge that possible changes to their long-term antibiotic prophylaxis may be made if second line prophylaxis is deemed necessary.

To avoid confounding issues, participants will not be recruited if they are already participating in another IMP trial. Where participants involved in this study wish to consider recruitment to another trial, and it is not thought that this would have a detrimental effect on this trial, the chief investigator will be consulted to assess whether this is possible.

**Subject recruitment**

Identification of potential participants may vary and could include the following methods:

1. Respiratory outpatient clinics
2. Bronchoscopy day case lists
3. Audiology, ophthalmology and fracture clinics

Participants will take part on a voluntary basis and no financial incentive will be given

**Initial screening**

A member of the research team will pre-screen respiratory clinic outpatient lists and bronchoscopy lists to identify those with a diagnosis of PBB or who have previously been prescribed long-term azithromycin for PBB. These patients will be highlighted to the clinical outpatient teams. The clinical team will approach families and inform them of the study. If they are interested in hearing more about the study they will be directed to a neighbouring room where they will be introduced to a research team member. Here they will have the opportunity to discuss the trial in more detail. At this point confirmation of a potentially eligible participant will take place by the research team member and the screening log completed. The appropriate Participant Information and Consent form(s) (PISCs) will be provided for parents or guardians with parental responsibility (from here on referred to as parents) as well as information booklets appropriate for the child’s age. The voluntary nature of the study as well as the risks and benefits will be emphasised. Families will be informed that it would be necessary to collect the first study samples before starting azithromycin if they intend to take part. All participants will be given a prescription for long term azithromycin at their clinic appointment. They will be instructed not to collect the prescription if they wish to enrol in the study.

Families will be contacted by text message after 24 hours of receiving the study information to see if they would like to participate. If they respond and would like to take part, a suitable date and place will be made to meet the family again. At this point, verbal consent will be obtained for randomisation to take place. This is so that the trial medication can be taken to the family when the researcher travels to meet the family again. During this meeting, written consent will be obtained, confirmation of randomisation and baseline, pre-treatment study samples collected. No samples or baseline assessments will be taken nor medication administered until written consent has been obtained.

If potential participants do not want to take part and do not respond, they will already have a prescription for long term azithromycin at the standard dose 10mg/kg/dose thrice weekly.

**Consent**

Due to the age criteria of eligible participants, all participants will require written consent by a guardian with parental responsibility. The guardian will ideally help introduce the study to the child in the presence of a research team member. Children may give assent and sign an assent form if developmentally able to do so. Parents/guardians can withdraw their consent at any time during the study.

**Eligibility**

Once written consent has been obtained, the eligibility criteria will be confirmed and baseline assessments can then be undertaken.

Baseline Assessments to be completed by the Research team

* Weight, height, BMI
* Demographics: Family smoking history, number of siblings living at home, day care/ school attendance.
* Medical history: Gestation at birth, mode of delivery, infant feeding/ breast feeding history, vaccination history, previous ventilation, allergy status.
* Previous antibiotic exposure: Emergency and GP attendances for antibiotics.
* Concomitant medication list
* Nasopharyngeal swab
* Stool sample

Baseline Assessments to be completed by the caregiver

The caregiver must complete the following baseline assessments

* Respiratory symptoms questionnaire. (Trinick et al., 2012)
* Quality of life questionnaire. EQ 5D Y, a child specific and age appropriate measure of health related quality of life (Fitriana et al., 2021)

**Randomisation**

A delegated medical practitioner will confirm eligibility prior to commencement of Phase 1. Randomisation to Groups 1 or 2 will take place when participants and the researcher are organising a time to physically meet. This will be based on verbal consent in order for the trial medication to be taken to the family. Participants will be randomised to receive either 10mg/kg/dose thrice weekly Azithromycin or 5mg/kg/dose of thrice weekly azithromycin on a 1:1 ratio. Treatment will commence on the following Monday, Wednesday or Friday dependant on the day of randomisation. The total duration of azithromycin will be 6 months. If further long term antibiotics are required, standard dosing azithromycin will be prescribed. Participants and researchers will otherwise remain blinded until the study end at 18 months

During the second randomisation in Phase 2, participants from groups 1 and 2, who have stopped long term antibiotics, will be randomised to receive a standard dose of daily lactulose for 2 weeks or no lactulose.

(Details of randomisation process to be confirmed)

**Blinding**

Phase 1 is a double blind trial so participants, clinicians and the research team will be blinded to treatment allocation. The study pharmacist will be the designated unblinding team member and be unblinded to treatment allocation. The pharmacist will not be involved in patient care apart from dispensing the treatment.

(Unblinding procedures yet to be written)

The decision to start long-term azithromycin will be made by the treating respiratory clinician. If participants are willing to consider the study, no prescription for azithromycin will be given during the clinic visit.

Phase 2 is randomised controlled trial. Participants will be randomised to either receive lactulose of not. No placebo will be administered to those not allocated to lactulose. Hence participants and the research team will not be blinded to the treatment allocation.

**Participant timeline, assessments and procedures** – yet to be completed

**Administration of intervention**

Following randomisation into phase 1, the trial medication will be dispensed. A member of the research team will take bottes of the trial medication to the family during the visit where written consent will be taken and baseline investigations/ samples collected.

Trial medication – Azithromycin

The Sheffield Children’s hospital pharmacy will provide bottles of azithromycin powder to be reconstituted on a weekly basis as is standard practice. Following randomisation, the trial medication will be dispensed to the research team member allocated to meet the family and obtain written consent. The researcher will take the medication to the family. Bottles of powdered antibiotic will be reconstituted by the family on a weekly basis as is the standard procedure. Information on how to reconstitute the antibiotics will be provided as is routine. Administration will be at home by the parent or carer for at least 6 months. All used bottles will be returned to the research centre at the 6 month visit.

(Details on how the different strengths of azithromycin will be made up are yet to be decided)

Trial medication – lactulose

Standard lactulose liquid will be used that can be dispensed by any pharmacy. Administration will be at home by the parent for 2 weeks. A prescription will be given to families so that they can obtain lactulose from their local pharmacy.

Timings for sample collection – As detailed in the secondary objectives table

**Sample analysis**

**Nasopharyngeal swab analysis**

Deep nasopharyngeal swabs will be sampled by using cotton swabs (Transwab, Liquid Amies medium) and transported to research centre and frozen at -80 ⁰C within 6 hours. Samples will then be sent to the UCL microbiology lab on dry ice where they will be stored at -80⁰C until batch processed. Firstly the swab medium will be culture for any growth of *S. pneumoniae, H. influenzae, S aureus* and *M. catarrhalis*. This will be recorded and confirmed by standard techniques. Minimum inhibitory concentrations (MICs will be determined for Azithromycin susceptibility in identified isolates using Etest strips and resistance determined according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Susceptibility to other antibiotics will be confirmed using disk diffusion methods as is the standard practice at NHS hospital laboratories. See table 7 and 8 for details of antibiotic susceptibilities to be tested for.

For each nasal swab sample, we will perform 16s V4 sequencing to characterise the bacterial community composition. DNA will be extracted using a chemical/ bead beater protocol within the microbiology laboratories at UCL under the supervision of Dr Albar. Long-read nanopore sequencing will be undertaken. Data will be analysed and visualised in “phyloseq” and “vegan” packages within R.

**Stool sample analysis and processing**

Sterile stool collection kits will be issued to families with instructions on how to collect and store the sample. Families will be asked to place the sample in a freezer until the research assistant is able to collect it within 24 hours. The samples will then be stored at -80⁰, sent to the UCL microbiology lab on dry ice and again stored at -80⁰C until batch processed. To characterise intestinal microbiota, DNA will be extracted from stool using Qiagen QIAamp Mini Kit, and from each extract the V4 16s rRNA gene region would be amplified. The DNA would then be purified and pooled before undertaking long read nanopore sequencing.

**Subject compliance**

Phase 1

Parents will be given a study booklet with diary to complete. This will involve ticking a box to say the study medication was given.

(We will explore the use of a study app that may make it easier for parents to use and remember.)

Phase 2

Parents will be given a study booklet with diary to complete. This will involve ticking a box to say the study medication was given.

(We will explore the use of a study app that may make it easier for parents to use and remember.)

Participants will be asked to send a photo of the completed chart to the research team with their phone.

**Other follow up assessments**

Parents will be asked to complete a proxy quality of life questionnaire (EQ-5D-Y) and respiratory symptom questionnaire.

The parent cough-specific quality of life questionnaire (PC-QOL) is validated 27 item questionnaire that can be used for evaluation and monitoring of treatment for cough dominant conditions (Newcombe et al., 2010). It uses a 7-point Likert-type scale to assess the level of parents’ feelings (15 items) and worry (12 items) related to their Child’s cough.

The Liverpool Respiratory Symptom Questionnaire (LRSQ) was developed for pre-school children with respiratory symptoms and CF (Powell et al., 2002; Trinick et al., 2012). It uses Likert scales, comprises 8 domains, takes <10 minutes to complete and elicits symptoms over a three-month period.