Comparison of cough plates and cough swabs for detecting respiratory pathogens in non-expectorating children with cystic fibrosis

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Master of Philosophy (MPhil)

December 2019

Keele University

Acknowledgements

Undertaking an intercalated masters has been an immensely rewarding experience. I am very grateful for the opportunity to spend a whole year dedicated to the area of medicine I love, paediatrics, and for the time to explore academic medicine. Completion of an additional year of studies has not been without its challenges and I could not have completed this thesis without the help and support of many people whom I would like to acknowledge.

First and foremost, I would like to thank my supervisor Dr Fran Gilchrist, who has supported and guided me throughout this year. Without his knowledge and mentorship, I would not have been able to complete this thesis. I can only aspire to be as compassionate and knowledgeable as him in my future career.

Secondly, I would like to thank my co-supervisor Dr Will Carroll for his generosity and support with my thesis whilst also supervising his own MPhil student. I am hugely grateful for all his input and enthusiasm, particularly with regards to statistics.

Dr Martyn Rees has been one of the key drivers behind this project and I am deeply thankful to him and his team for supporting me in collecting the data required for this project. I would also like to thank his secretary Rachel who made going through 5 years of notes as streamlined and straightforward as possible.

A large part of my masters, and the part I have most enjoyed, has been gaining a clinical knowledge of cystic fibrosis and respiratory microbiology. I am hugely grateful to all the clinical staff at Royal Stoke Hospital and Princess Royal Hospital Telford who shared their experience and knowledge with me over the last year. I would particularly like to thank Alice Alcock for facilitating my placement in microbiology and the paediatric CF team at Royal Stoke Hospital including: Dr Fran, Dr Will, Dr Ang, Dr James, Kelly, Siobhan, Sue, Zoe, Ruth, Lizzie and Daisy for

always making me feel welcome. I am also extremely grateful to all the children and their families for allowing me to learn so much from their experiences with cystic fibrosis.

I would also like to show my gratitude to the staff at Keele Medical School for allowing me to intercalate and for nominating me for the Wolfson Award. I am very grateful for receiving this award which has assisted me financially this year.

Finally, I would like to thank my family, friends and partner. I am so grateful to have a wonderful circle of friends, in particular Beth, Grace and James, who have been an endless source of moral support for me this year. I am immensely thankful to my loving family, especially my parents, for instilling in me a love of learning and the work ethic that have made every stage of my education possible. And lastly, I would like to thank my partner, Rob, for his unwavering encouragement and support. I am very lucky to have you all.

Motivation for undertaking this intercalated MPhil

When considering intercalating, I looked at numerous options to fit my interests but none of the options appealed as much as this project. I love paediatrics and am an aspiring paediatrician. I have always found working with children and their a fun and rewarding experience. I think paediatrics is one of the most versatile medical careers, with options to be a generalist and a specialist and to work in hospitals and the community. For these and many other reasons I want to become a paediatrician and wanted an intercalation that involved paediatrics.

As well as having an interest in paediatrics I have also really enjoyed microbiology and infectious diseases during my undergraduate medical studies. I hope to spend some of my future career working in paediatric global health and infectious diseases and so the microbiology element of this project also appealed to me.

During medical school I have been fortunate enough to have the opportunity to be involved with some small research projects including a lab-based summer studentship and a systematic review. As a student still exploring research, another appealing aspect of this project was that I have no experience of research involving clinical data and statistics and so I knew I would gain a lot of new skills from this project and have the opportunity to explore a new area of research.

Finally, I believe this MPhil has a great combination of research skills and clinical experience. I spent 1 week in microbiology and attended weekly cystic fibrosis clinics for the duration of this MPhil. The combination of research and clinical experience was very appealing and to my knowledge is unique to this MPhil.

Roles and Responsibilities

Bridget Kemball

I was responsible for development of the database and collecting all the data used in this thesis from Princess Royal Hospital Telford, liaising with Dr Martyn Rees for access to notes and electronic systems. I performed all statistical analysis used in this thesis with guidance on appropriate statistical tests and use of STATA from Dr Gilchrist, Dr Carroll and the Research and Development Department.

Dr Francis Gilchrist

Dr Gilchrist was the project supervisor and oversaw the whole project. He organised all clinical placements including microbiology and cystic fibrosis, liaised with Dr Rees to enable data collection and provided guidance and suggestions for all aspects of this project. He provided valuable guidance on appropriate statistical tests to meet the aims and objectives.

Dr William Carroll

Dr Carroll was the project co-supervisor and was instrumental in providing advice on appropriate data analysis, statistical testing and guidance in the use of STATA.

Dr Martyn Rees

Dr Rees enabled the collection of all data from patients at Shrewsbury and Telford Hospitals and has been collecting cough swabs and cough plates from all his CF patients for over a decade.

Research and Development Department Royal Stoke University Hospital

Discussion with the Research and Development team happened at several times during this project. They provided valuable comments on the database used, appropriate amount of data and some of the data analysis used in this thesis.

Alice Alcock

Alice oversaw my weeks placement in microbiology enabling me to learn about respiratory microbiology in a clinical setting.

Abbreviations

A

ARR - Absolute risk reduction

B

BAL – Bronchoscopic alveolar lavage

BMI – Body mass index

C

CF – Cystic fibrosis

CFLD – Cystic fibrosis related liver disease

CFRD – Cystic fibrosis related diabetes

CFTR – Cystic fibrosis transmembrane conductance regulator

CF SPID – Cystic fibrosis screen positive patients with an inconclusive diagnosis

CF-SpIT – Cystic fibrosis sputum induction trial

CF START – Cystic fibrosis anti-staphylococcal antibiotic prophylaxis trial

CI – Confidence Interval

CP – Cough plate(s)

CS – Cough swab(s)

D

DIOS – Distal intestinal obstruction syndrome

F

FEV_1 – Forced expiratory volume in 1 second

I

IT- Information technology

M

MDT – Multidisciplinary team

N

NHS – National Health Service

NICE – National Institute for Health and Care Excellence

NIPT - Non-invasive prenatal testing

NNS – Number needed to sample

O

OGTT – Oral glucose tolerance test

OR – Odds ratio

P

PEP - Positive expiratory pressure

PCR – Polymerase chain reaction

R

R&D – Research and Development

rhDNase - Recombinant human DNase

RSUH – Royal Stoke University Hospital

S

SATH – Shrewsbury and Telford Hospitals

T

TORPEDO-CF - Trial of Optimal Therapy for Pseudomonas Eradication in CF

U

UHNM- University hospital of North Midlands

UK – United Kingdom

US – United States

Abstract

Background

Cystic fibrosis (CF) is an autosomal recessive, multisystem disorder affecting ion transportation across epithelial membranes. Respiratory complications are the leading cause of morbidity and mortality and CF lung disease is characterised by repeated infections leading to inflammation and fibrosis of the lungs.

Early and accurate identification of respiratory pathogens is essential to enable timely and efficacious treatment of infections. This is ensured by obtaining microbiology samples at each clinic visit. The gold standard is sputum culture; however, many children are unable or unwilling to expectorate. In non-expectorating children, cough swabs (CS) or cough plates (CP) can be used. Previous studies comparing the performance of these two samples are conflicting.

Aims

The primary aim of this study is to compare yield of respiratory pathogens identified by CS and CP in non-expectorating children with CF.

Secondary objectives include comparing the types of organisms identified by CS and CP and assessing whether lung function, Body mass index (BMI) centile, age and gender affect whether a CS or CP is more likely to identify a pathogen.

Methods

Non-expectorating children with CF attending Shrewsbury and Telford NHS Trust provide CS and CP samples at each outpatient visit. The results of the paired cultures from November 2013-2018 were analysed. Samples positive for non-pathogenic organisms including yeast, candida and *Bacillus* species were excluded.

Results

We identified 663 paired CS and CP samples from 38 patients. Mean (SD) age was 9.9 (4.7) years. The CS and/or the CP was positive for a respiratory pathogen on 118 (18%) of the paired samples. This included 66 (10%) CS and 87 (13%) CP samples. Only 27 (23%) of the paired samples with a positive culture identified the same pathogen(s) on both CS and CP. Number needed to sample: in this sample 13 children needed both CS and CP samples to identify one additional respiratory pathogen compared to CS alone. McNemar's test (p=0.028) and the generalised estimating equation (p=0.020) suggest that the difference in CS and CP performance was statistically significant. However, the magnitude of this difference was so small whether it is clinically significant remains to be determined.

CS identified a greater variety of pathogens compared to CP, identifying 6 additional pathogens not cultured on CP. Lung function was the only significant predictor of both CS and CP positivity on multivariable analysis. BMI centile also had a statistically significant relationship with CP being positive for pathogens on multivariable analysis.

Conclusions

The rate of respiratory pathogen isolation was similar for CS and CP samples. Simultaneous use of CP and CS identified more pathogens than either sampling method alone. Further studies are required to investigate if similar improvements could be achieved by obtaining duplicate CS or CP samples.

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1.0 Literature Review

1.1 Background

Cystic fibrosis (CF) is an autosomal recessive condition causing defective ion transportation across epithelial surfaces throughout the body. It is a multisystem condition, but most significantly affects the lungs, pancreas, reproductive organs and gastrointestinal tract. CF is of great clinical relevance as it is the most common life limiting condition in Caucasian populations. A diagnosis of CF only a few decades ago was equated to a death sentence in a patient's early twenties; this has changed alongside the rapid growth of understanding around the condition. The significant improvement in prognosis has been determined by advances in both diagnostic tests and treatment options, a trend which continues today.

1.2 History of CF

Although understanding of the pathology and aetiology of CF has only occurred relatively recently, CF is far from a new entity. Descriptions thought to be linked to CF date back as far as the middle ages, where babies whose skin tasted salty were thought to be hexed and cursed with an early death (1). From the fifteenth century onwards, there are anecdotal reports of characteristic features of CF such as steatorrhoea, with the first autopsy thought to describe CF occurring in 1595 (2). It is even postulated that romantic composer, Frederic Chopin, may have had CF (3).

During the twentieth century, CF began to be recognised as a disease of both the lungs and pancreas. Dorothy Andersen's landmark paper in 1938 "Cystic fibrosis of the pancreas and its relation to coeliac disease" finally gave the disease of folklore a name (4). Andersen was a paediatric pathologist and identified CF by observing fibrocystic changes in the pancreas at post mortem. Andersen went onto describe the Mendelian inheritance of CF almost a decade later (5).

Other important landmarks in our understanding of CF include Gibson and Cooke's discovery of sweat testing in the 1950s as an alternative to invasive trypsinogen testing (6). Gibson and Cooke's discovery was prompted by di Sant'Agnese's recognition of abnormal salt loss in sweat of patients with CF (7). Di Sant'Agnese's work on electrolyte abnormalities followed observation of increased heat prostration of patients with CF at Columbia Hospital New York during a heatwave. Sweat testing is still considered the gold standard in diagnosing CF over half a century later (8).

The 1950s also saw great advancements in the treatment of CF. Physiotherapy began to be used in the United Kingdom (UK) as an important component of routine CF care (9). Physiotherapy was introduced by Dr Winifred Young to CF patients from the time of diagnosis, at her clinic at the Queen Elizabeth Hospital in London, although not all physicians at the time were convinced of the value of physiotherapy (10).

Physiotherapy was also adopted as part of a more holistic approach to CF care pioneered by LeRoy Matthews in the United States (US). His prophylactic regimen included regular microbiological testing, which guided targeted antibiotic treatment, nebulised treatment and the use of mucolytics and decongestants (11). Much of his practice is still commonplace in CF clinics today. Another advancement in treatment in the 1950s was the introduction of a high fat diet with oral pancreatic enzyme supplementation. This approach, instigated by Dr Douglas Croizer, was contrary to previous dietary advice given to patients with CF (12). However, his findings of improved nutritional status and survival when adopting a high fat, high calorie diet in CF still hold true today and are another cornerstone of CF management. Further advances in the CF diet occurred in the UK, where Dr Archie Norman's unit at Great Ormond Street Hospital provided evidence that pancreatic enzyme supplementation was beneficial for children with CF (13).

The UK and the US were not the only countries to make strides in CF care in the 1950s. In 1953, Dr Charlotte Anderson started a CF clinic in Melbourne. Dr Anderson contributed to knowledge surrounding both coeliac disease and CF including the use of bronchodilators in CF (14). She went on to become Professor of Paediatrics in Birmingham from 1968 to 1980 (10).

Advances in CF treatment continued in the 1960s and 1970s. Dr Margaret Mearns was a pioneer of CF care, and demonstrated that prompt and effective use of antibiotics significantly improved respiratory outcomes in children with CF (15). She also cautioned the use of prophylactic anti-staphylococcal antibiotics as she felt they may lead to increased infections with *Pseudomonas aeruginosa* (16), an opinion that is still debated today (17).

Decade after decade, understanding of CF has continued to grow (2). The 1980s saw the identification of the most common genetic mutation which causes CF (18–20), lung transplantation in patients with advanced CF (21) and the creation of the newborn screening test for CF in 1983 (22), although this was not to be adopted throughout the UK until 2007. Multidisciplinary CF specialist care also began to be developed in the UK, with Dr James Littlewood's centre at Leeds being an example of this (10). Littlewood and his team contributed greatly to CF knowledge, including research on colomycin (23), nutrition (24) and many other elements of CF care (10). Littlewood went on to play numerous important roles in the CF Trust, including being Chairman of the Trust from 2003 to 2011 (10).

Other notable discoveries in CF management include the introduction of nebulised antibiotics and mucoactive agents, both of which were developed in the 1990s. Recombinant human DNase (rhDNase) is a mucoactive agent. It reduces the viscosity of sputum in patients with CF, improving efficacy of physiotherapy as more secretions can be shifted (25). Use of rhDNase has been shown to reduce frequency of pulmonary exacerbations and improve lung function (26). Nebulised hypertonic saline, another mucoactive medication, was also trialled as a therapy for CF in the 1980s (27) and 90s (28). Hypertonic saline aids liquefication of mucus, increases

mucociliary clearance (29), and has been shown to improve lung function in patients with CF (30).

Alongside physiotherapy and mucoactive agents, antibiotics form an integral part of CF pulmonary care. Inhaled tobramycin was first trialled in 1999 as an intermittent treatment for CF patients with chronic *Pseudomonas aeruginosa* infection (31). Colistimethate sodium had previously been used in Europe to treat *P. aeruginosa* infection. Tobramycin showed an increase in lung function, reduced density of *P. aeruginosa* infection and reduced hospital admissions when compared to placebo. Inhaled tobramycin also showed no detectable ototoxic or nephrotoxic side effects. Colistin, tobramycin and aztreonam are now all used as nebulised antibiotics in patients with CF. Nebulised anti-pseudomonal antibiotics are also used in the eradication of new growths of *P. aeruginosa.* They can be used independently of or in conjunction with oral or intravenous antibiotics to eradicate *P. aeruginosa* and prevent chronic infection and consequent airway remodelling.

Research into genes therapies began in the 1990s and 2000s. In 2011 and 2012, CF transmembrane conductance regulator (CFTR) modulators, a treatment option for patients with specific mutations were developed. Advances in treatment continue to be discovered, with clinical trials in 2018 for triple therapy now showing promise (32,33). Figure 1 summarises some of the key landmarks in CF history since 1938.

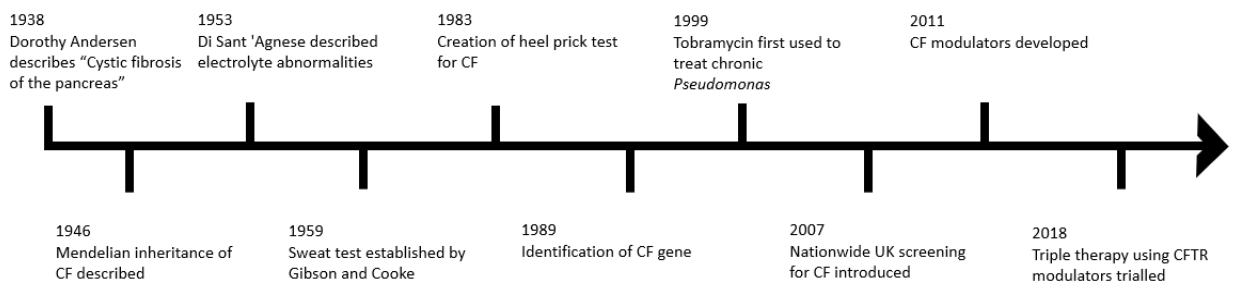

Figure 1- A timeline (not to scale) detailing some of the important landmarks in CF history

1.3 Epidemiology of CF

In 2017, over 10,400 patients were living with CF in the UK (34). Over half of those living in the UK with CF are adults, a reflection on the increasing life expectancy with CF. It has been shown that 1 in 25 Northern Europeans carry one of the many genes associated with CF and there is an incidence of CF occurring in 1 in every 2,500-3,500 births in Northern Europe (35).

1.4 Genetics and Classification of CF

In the 1980s, one of the greatest achievements of CF research occurred in the discovery of the causative gene for CF. The first major breakthrough was in 1985, when Tsui and colleagues identified the locus of the CF gene to be on the long arm of chromosome 7 (36). In 1989, the long-awaited discovery of the CFTR gene which causes CF occurred, alongside discovery of the most common mutation: Phe508del. This gene is present in approximately 70% of patients in Northern Europe with CF (18–20). The Phe508del gene is less common elsewhere. The gene itself is known as the CFTR gene as the protein product of this gene is key in transport of chloride and sodium ions across epithelial cell membranes.

Over 2000 documented mutations of the CFTR gene have now been isolated (37). Due to this large genotypical variance there is also great heterogeneity in the clinical features of patients with CF. Classification systems have been developed to anticipate the clinical outcome based on the genotype of the patient and, in the age of gene targeted therapies, help to guide treatment choices.

The first classification system was developed by Welsh and Smith in 1993 (38), who initially proposed four classes. Since then the classification system includes six classes (39). See Table 1.1 and Figure 2.

Table 1.1 - The six-class classification of CF

Class	Genetic Defect	Functional Consequence	Common mutations
I	Nonsense, frameshift and large deletions	Little or no functional CFTR produced	GF42X, R553X, W1282X, R1162X
II	Some missense mutations and in-frame deletions	Prevent CFTR transportation to the cell surface resulting in few functional CFTR's.	Phe508del, N1303K, R1066C
III	Missense mutations leading to defective regulation of the CFTR channel.	Gating defect – reduced opening probability of CFTR channel.	G551D, G178R, G551S, G1349D
IV	Missense mutations leading to defective conductance through the CFTR channel.	Impede conductance of ions through the channel.	R117H, R334W, R347P, R1070W
V	Promoter or splicing abnormalities that do not alter the CFTR protein but significantly reduce its abundance.	Reduced amounts of normal CFTR protein.	A455E, D565G, 2789+5G->A
VI	A variety of mutations which increase the turnover of CFTR protein.	Normal CFTR protein is produced but it is unstable and degraded quickly.	120Δ23, N287Y

It is important to note that Phe508del, the most common mutation, produces more severe disease than some mutations. Classes I to III typically produce more severe disease whereas classes IV to VI typically have a milder phenotype (40).

Although identifying an individual's genotype is important, it is common for children with the same genotype to have very different clinical progression. It is not only the CFTR mutation that affects outcome but there is also a major role for environmental factors and gene modifiers (41,42). Therefore, clinicians cannot predict with confidence the course of disease based solely on genotype.

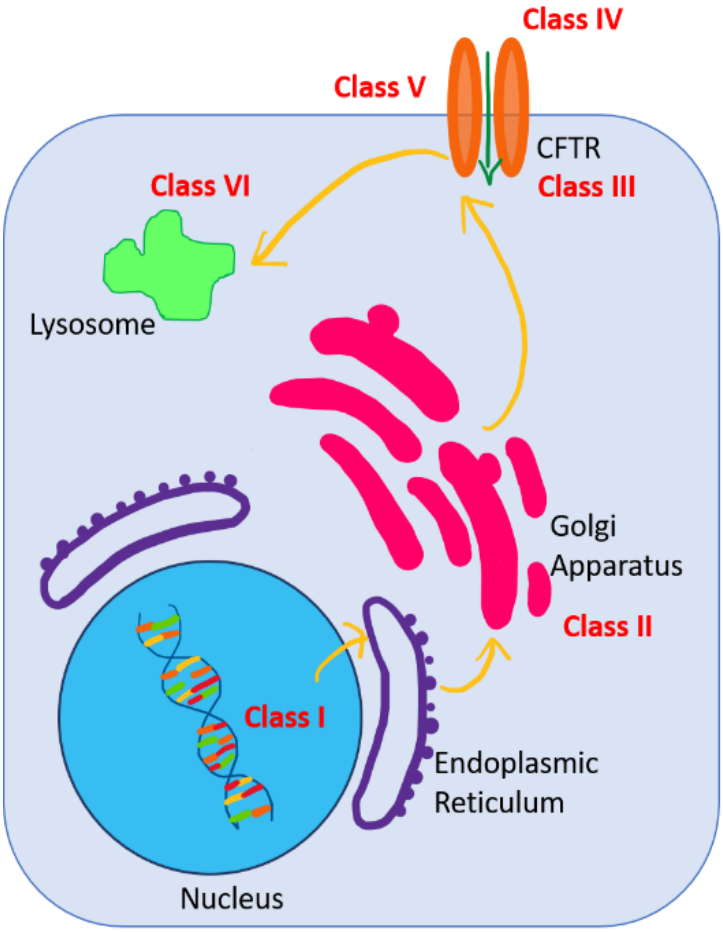

Figure 2 - A graphic to demonstrate the different classes of CFTR mutation.

1.5 Pathogenesis of CF

Regardless of the individual gene mutations, the pathogenesis of CF is broadly the same; there is loss of function of the CFTR protein. The CFTR protein is an ion channel present in epithelial surfaces throughout the body (43). In simplistic terms, the CFTR was initially thought of as a chloride ion channel and its absence or dysfunction therefore affects the transportation of chloride ions across epithelial surfaces. This explains the high levels of chloride ions present in sweat, that form the basis of CF sweat test.

The CFTR channel may also be involved in chloride-bicarbonate exchange. This is important as alterations in bicarbonate concentration have an impact on several important physiological elements: pH, mucin unfolding, innate immunity and gastric acid neutralisation (44). The

transport of chloride ions through CFTR has synergistic effects on ion transportation through other channels, particularly sodium transportation (43).

The culmination of this alteration in ion transportation is the presence of viscous mucus in both the airways and gastrointestinal tract in patients with CF. This is responsible for many of the clinical feature seen in CF.

1.6 Clinical Picture

1.6.1 Respiratory

Although CF is a multisystem disorder, respiratory symptoms usually dominate the clinical picture. Respiratory complications are by far the leading cause of morbidity and mortality. The care of patients with CF is usually coordinated by respiratory physicians. CF lung disease is characterised by recurrent respiratory infections leading to chronic inflammation and parenchymal damage causing progressive respiratory failure.

Changes to respiratory secretions and reduced mucociliary clearance lead to an increased susceptibility to infection. Typical organisms include: *Haemophilus influenzae, Staphylococcus aureus, Burkholderia cepacia* complex and most significantly *Pseudomonas aeruginosa.* Infection with *P. aeruginosa* is of particular clinical relevance as it may lead to chronic infection and has worse prognostic implications. Further details on respiratory infections, their detection, management and clinical significance will be discussed in chapter 2.

1.6.2 Pancreatic

In the same way that viscous secretions affect the respiratory system, so can they affect the pancreas. CFTR is abundantly expressed on pancreatic acinar cells (45) and is responsible for the transport of chloride and bicarbonate to maintain an alkaline fluid in a healthy pancreas. The absence of CFTR in the pancreas results in a high concentration of protein and low volume of fluid making it more viscous (46), this is known as mucoviscidosis. The small ducts of the

pancreas become blocked and plugged with the viscous fluid in utero, and over time this leads on to large duct obstruction. Obstruction is followed by destruction, inflammation, fibrosis and fatty deposits within the pancreas (47).

The progressive destruction of pancreatic tissue in the majority of patients with CF leads to pancreatic exocrine insufficiency. Consequently, insufficient levels of digestive enzymes reach the gastrointestinal tract, causing malabsorption especially affecting the absorption of fats and fat-soluble vitamins – vitamins A, D, E and K. Clinically, malabsorption can manifest as steatorrhoea, failure to maintain length and weight centiles and in severe cases may lead to malnutrition.

However, not all patients with CF go on to develop pancreatic insufficiency. Approximately 15% continue to produce sufficient enzymes to allow for normal digestion. Faecal elastase testing is used to screen patients with CF for pancreatic insufficiency (48). Pancreatic status has been linked to genotype (19,49) with more severe mutations (classes I-III) more frequently exhibiting insufficiency and milder mutations (Classes IV to VI) typically being pancreatic sufficient. It is important to note that even though a patient may be clinically pancreatic sufficient, damage to the pancreas is still occurring and despite sufficiency remain at an increased risk of developing pancreatitis (50).

1.6.3 Endocrine

As well as causing destruction of the exocrine pancreas, over time CF can also impede the function of the endocrine pancreas due to destruction of the islets of Langerhans which produce insulin. This contributes to the development CF related diabetes (CFRD). Abnormalities in insulin resistance and glucose metabolism are also contributing factors. CFRD develops insidiously and many patients may be asymptomatic at presentation. Presenting features can include: polyuria, polydipsia, weight loss or failure to maintain or gain weight, poor growth, delayed or slowed progression of puberty and decline in lung function (51).

CFRD is a separate entity to type I and type II diabetes mellitus, although it shares features with both types. As CF prognosis has improved, so too has the prevalence of CFRD. Prevalence increases significantly with age and 40-50% of adults with CF have CFRD (52). Females appear more susceptible to CFRD at a younger age (53). Individuals who are homozygous Phe508del are at an increased risk for CFRD, showing that some CF genotypes have a higher risk of CFRD than others (54). Screening for CFRD via oral glucose tolerance testing (OGTT), is usually performed annually from the age of 10 as part of their annual review (55,56). Detection of CFRD is not only important in optimising control of blood glucose but also for optimising lung function, as patients with CFRD are also more likely to have poor respiratory function (57).

CFRD is the most common endocrine abnormality associated with CF, but additional endocrine complications include bone disease and male hypogonadism. The causes of CF-related bone disease are multi-factorial, but include: malabsorption, low vitamin D levels, corticosteroid use, alteration in growth and sex hormone axis's and genotype (58). Approximately 23% of patients with CF have osteoporosis and 38% have osteopaenia (59). Monitoring bone density from a young age forms an important part of CF care, with screening first occurring in children over the age of 8 who meet certain criteria (60). This is important as early recognition and treatment with vitamin supplementation, weight gain and weight bearing exercise can improve bone density and significantly reduce the risk of fragility fractures (61). As with CFRD, the presence of CF-related bone disease is also linked to poor lung function.

Low levels of testosterone have been found in 1 in 4 males with CF (62). This can further contribute to CF-related bone disease. Hypogonadism is not routinely screened for in CF but should be considered in males who have low bone mineral density.

1.6.4 Gastrointestinal

CFTR is present in epithelial cells throughout the gastrointestinal and hepatobiliary systems (63) and altered ion transportation in these systems may manifest clinically as meconium ileus, distal

intestinal obstruction syndrome (DIOS), constipation and liver cirrhosis. Meconium ileus can be the first manifestation of CF and is considered pathognomonic of CF. It occurs in approximately 20% of newborn patients with CF. In meconium ileus, viscid meconium obstructs the intestines, typically at the terminal ileum, and neonates are unable to pass stool. Healthy babies will normally pass meconium within the first 24 hours. In cases of meconium ileus, proximal loops of bowel dilate creating the clinical picture of obstruction. This can be further complicated by intestinal atresia, volvulus formation or perforation (64). As with many of the complications of CF, genetics play an important role, with individuals with Class I-III mutations being at higher risk of having meconium ileus.

In later life, a similar complication of intestinal obstruction as a result of viscous bowel contents can occur, known as DIOS. In DIOS, viscid bowel contents build up typically at the terminal ileum or proximal colon and can cause a partial or complete obstruction (65). DIOS is defined as acute complete or incomplete faecal obstruction in the ileocaecum (66). Treatment with stool softening laxatives or lavage is advised.

The main differential diagnosis for DIOS is chronic constipation, which is also a complication of CF. The prevalence of constipation in patients with CF is high, estimated to be around 47% (65). This is significantly higher than rates in the general population which range from 2% to 27% (67). Constipation is defined as gradual faecal impaction of the total colon (66) helping to differentiate it from DIOS. Patients are screened for constipation and DIOS at clinic visits via history and abdominal examination.

CF also affects ion transportation within the hepatobiliary system and can lead to gallstone formation and CF related liver disease (CFLD). Estimates for prevalence of CFLD differ, with studies ranging from 4.2% to 38% (68). Risk factors include: severe mutations (class I to III), male gender and history of meconium ileus. CFTR is present in the apical membrane of cholangiocytes and epithelial gallbladder cells. The absence or altered function of CFTR results

in altered chloride and bicarbonate transport creating an increased volume of viscous bile with reduced alkalinity. The viscous bile causes plugging, inflammation and fibrosis within the liver as well as predisposing to other hepatobiliary pathology including cholelithiasis. The most common pattern of hepatobiliary damage is focal biliary cirrhosis (69). CFLD can result in significant morbidity as it can lead to liver cirrhosis, the formation of oesophageal varices and in severe cases requires liver transplantation (68).

1.6.5 Reproductive

CF is one of few conditions where male infertility is expected. Azoospermia in males with CF was first described in the 1960s (70) and the cause, bilateral absent vas deferens, was identified soon after. Although there is normal sperm production, there is an inability to transport sperm and consequently males with CF are infertile. As with many of the complications of CF, as life expectancy has increased, this complication has become of greater significance. Between 95-98% of men with CF have obstructive azoospermia (71), although fertility is preserved in those with the 3849+10kb C > T mutation. Despite the fact that the majority of men with CF are infertile, reproductive technologies are now available to allow for aspiration of sperm from the epididymis allowing men with CF to father biological children (72).

CF does not cause any anatomical reproductive abnormalities in females. However, it may still impact on fertility. The CFTR is present in the cervix (73) and the absence or dysfunction of the CFTR in CF patients leads to thickened cervical mucus, which can inhibit passage of sperm through the cervical canal and even plug the cervical canal (74). However in spite of these changes, in healthy women with CF who have been able to maintain good nutrition and achieve normal ovulation and menstruation, there is little evidence that fertility is significantly affected (73).

1.6.6 Summary of clinical manifestations of CF

The clinical manifestations of CF are highly varied, and each individual will have a unique set of CF related complications depending on their environmental and genetic risk factors. The common and severe complications of CF have been focussed on in this review, but it is far from inclusive. There are multiple other conditions associated with CF including: sinus pathology, nasal polyps, rectal prolapse and pseudo-Bartter syndrome. The clinical manifestations of CF are summarised in Figure 3.

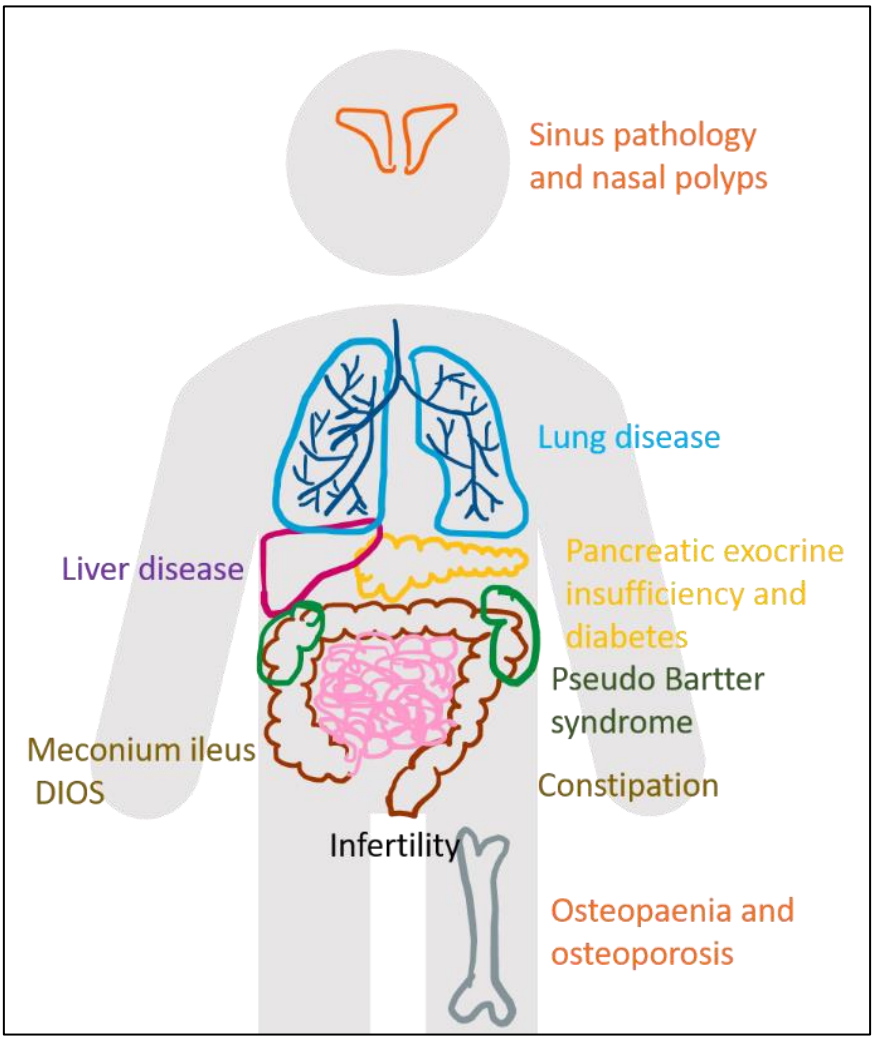

Figure 3 - A graphic to summarise the numerous, multisystem complications of CF.

1.7 Diagnosing CF

The introduction of a nationwide newborn screening programme to the UK in 2007 (Wales 1996, Scotland 2003) has revolutionised CF diagnosis. For example, in 2017, according to the

UK CF registry, of the 214 newly diagnoses of CF, 172 of these were identified by newborn screening (34). Newborn screening for CF forms part of the heel-prick test performed on neonates at 5 to 7 days old although the screening alone does not provide a definitive diagnosis of CF. If newborn screening is positive, further testing including sweat testing and genetic testing are then required to confirm the diagnosis of CF (75).

As with any screening test, CF screening has disadvantages. These include: false positives causing unnecessary anxiety for families and unnecessary tests; limited genetic testing to only the most common mutations; and CF screen positive patients with an inconclusive diagnosis (CF SPID). CF SPID patients are babies that initially screen positive upon newborn screening but on further investigation do not meet the criteria for CF. Either sweat testing is normal (<30 mmol/L) but they have two CTFR mutations, at least one of which must be of unclear phenotypical significance or they have an intermediate sweat test but one or zero CFTR mutations (76). The clinical significance of CF SPID is unclear. However, although testing undeniably has some pitfalls, newborn screening allows for early diagnosis and this has undisputable benefits such as improved nutritional status (77).

Although newborn screening is now responsible for identifying the majority of cases, testing based on symptomatology is still a key part in diagnosing those with CF who may have not been screened or may not have been picked up at screening. This may be for a variety of reasons including the specificity of the immunoreactive trypsinogen assay and the limited panel of mutations included in genetic screening (78). The National Institute for Health and Care Excellence (NICE) recommends sweat testing in children and young adults who present with clinical manifestations of CF or have a family history of CF, whilst genetic testing is recommended in in adults (75).

Antenatal diagnosis of CF is also possible. Until recently the main methods for diagnosis CF antenatally were chorionic villus sampling and amniocentesis. Both tests are invasive and are

associated with an increased risk of miscarriage (79). However new, less invasive and lower risk technologies are becoming rapidly available, such as non-invasive prenatal testing (NIPT), which simply involves taking a maternal blood test (80). In the UK, NIPT is currently available privately but not on the National Health Service (NHS) in all areas. These new technologies may enable parents to make informed decisions with regards to their pregnancy without the risks associated with amniocentesis or chorionic villus sampling.

1.8 Management of CF

The management of CF is complex and involves regular input from a multi-disciplinary team (MDT) of experts in CF care, including respiratory doctors, CF specialist nurses, dieticians, physiotherapists and psychologists.

1.8.1 Pulmonary management

Rigorous monitoring and management of CF lung disease is key in providing good outcomes. Pulmonary monitoring forms an integral part of management. In children monitoring occurs at least every 8 weeks and consists of: assessing lung function via spirometry (if the child is old enough), measuring oxygen saturations, clinical assessment and taking microbiological samples (81). Detailed annual review is required and includes blood tests, chest X-rays and further microbiological testing along with full involvement of the CF MDT.

Regular physiotherapy is an important cornerstone in maintaining good lung function in individuals with CF. A variety of techniques can be used depending on the age and needs of the individual but the essential purpose is to clear as many secretions as possible (82). In infants, chest percussion is performed by parents to help remove secretions, whereas in older children airway clearance devices are often used. Some airway clearance techniques do not require equipment and rely on different breathing techniques to help remove mucus, such as huffing and coughing. Other techniques require devices to aid mucus clearance, these include positive expiratory pressure (PEP) devices and oscillating PEP devices such as acapella. There is little

evidence to suggest either device is more effective than the other and as such choices in physiotherapy technique are a combination of expert physiotherapist input and individual needs and preferences (83).

Mucoactive agents are helpful in CF. The first line option is usually rhDNase. This can be used alone or in combination with hypertonic saline, which is the second line treatment. These agents are taken before physiotherapy to make secretions less viscous and aid their clearance (81).

Antibiotics are another key part of pulmonary management in CF. They have four main indications (84):

1. Treatment of a pulmonary exacerbation. This is typically achieved using with oral or intravenous antibiotics (85).
2. Oral prophylactic antibiotics, for example flucloxacillin to reduce infection with *S. aureus*.
3. Eradication of new organisms, such as *P. aeruginosa.* This usually requires courses of nebulised antibiotics with or without oral or intravenous antibiotics (86).
4. Nebulised antibiotics to suppress chronic infection such as *P. aeruginosa.*

There is no universally agreed definition of a pulmonary exacerbation in CF (85) meaning that identification and treatment of exacerbations can differ between CF centres and between clinicians at the same centre (87,88). The European Consensus Group defines a pulmonary exacerbation as the clinical need for additional treatment as indicated by a recent change in clinical parameters, although these parameters are not agreed upon (89). Although defining pulmonary exacerbations remains a challenge, there is consensus that exacerbations should be treated promptly and aggressively to improve quality of life, prevent a decline in lung function and ultimately reduce morbidity and mortality. A huge variety of organisms can be responsible for pulmonary exacerbations including viruses, bacteria, non-tuberculous mycobacteria and

fungi. The route, choice and duration of antibiotic will vary depending on the microorganism and sensitivities identified on culture, microorganisms previously grown by the patient and local practice (85).

In children, the current recommendation is for prophylactic antibiotics (typically flucloxacillin) to be used in order to reduce infection with *S. aureus*. However, although this has been shown to reduce the number of isolates of *S. aureus* in these patients, whether there is actually any clinical benefit in taking prophylactic antibiotics remains debatable (90). Further research is being conducted into the efficacy of anti-staphylococcal prophylaxis through the CF anti-staphylococcal antibiotic prophylaxis trial (CF START). There are concerns that the use of these prophylactic antibiotics may make children isolate *P. aeruginosa* earlier (17).

Eradication of new organisms also requires prompt and aggressive treatment particularly with organisms such as *P. aeruginosa.* As previously mentioned, inhaled antibiotics are typically used to achieve this, sometimes in combination with intravenous or oral antibiotics. There is no universal consensus on what the optimal management of early *P. aeruginosa* infection should be (91). Trials are also been undertaken into the best route of administration of antibiotics in early *P. aeruginosa* infection including the Trial of Optimal Therapy for Pseudomonas Eradication in CF (TORPEDO-CF) study (92). The current approach within the North West Midlands CF centre is a stepwise approach with nebulised colomycin for 3 months as the first line combined with either oral ciprofloxacin or intravenous antibiotics. Should this fail to eradicate the *P. aeruginosa,* this is followed by 4 weeks of tobramycin second line and 4 weeks of aztreonam third line (93).

Chronic infection with *P. aeruginosa* can be defined using the Leeds criteria (94) detailed in Table 1.2. As with early infection with *P. aeruginosa,* there is no universally agreed approach to management (95). The CF Trust recommends nebulised colistin as first line management (96). Anti-pseudomonal antibiotics have been shown to slow decline in lung function and reduce

morbidity including number of hospital admissions in patients with chronic *P. aeruginosa* infection (96).

Table 1.2 - The Leeds criteria, describing P. aeruginosa infections

Chronic infection	When more than 50% of months, when samples had been taken, were *P. aeruginosa* culture positive.
Intermittent infection	When 50% or less of months, when samples had been taken, were *P. aeruginosa* culture positive.
Free of infection	No growth of *P. aeruginosa* during the previous twelve months, having previously been *P. aeruginosa* culture positive.
Never	*P. aeruginosa* never cultured from sputum or cough swab.

1.8.2 Nutritional management

Nutritional management is key in CF care as poor growth and nutrition are associated with an increased morbidity and mortality (89,97). A high-fat, high-calorie diet is recommended alongside sufficient pancreatic enzyme replacement therapy in patients who are pancreatic insufficient. Growth should be monitored regularly and height or length, weight and, in infants, head circumference should be recorded at every clinic visit and regularly during inpatient stays (97). All CF patients should have access to a CF specialist dietician who can provide dietary advice and support (55).

Pancreatic insufficiency is objectively identified by a variety of tests, most commonly faecal elastase. In individuals who are pancreatic insufficient, replacement therapy is required. The amount of enzyme supplementation required depends on several factors including: weight, age, diet and clinical symptoms. Specialist dieticians can advise patients on the correct amount of enzyme supplements to be taken.

Alongside pancreatic enzyme replacement, fat soluble vitamins often require replacement in those who are pancreatic insufficient due to fat maldigestion and malabsorption. Vitamin levels are checked regularly to assess for insufficiency and many patients require supplements of

vitamins A, D, E and K. These vitamins play important roles in: vision, skin, immune systems, respiratory symptoms, bone strength, clotting and reduction of chronic inflammation (98). A new vitamin supplement, Paravit-CF, has recently been introduced as part of routine CF care. It contains vitamins A, D, E and K in one tablet, helping to reduce the treatment burden in patients with CF.

Patients with CF are also at increased risk of hyponatraemia due to excess sodium loss. Infants are at particular risk due to low dietary intake and increased surface area to volume ratio. There is no consensus on routine sodium supplementation (99), as such each case should be assessed on an individual basis. Factors that may wish to be considered include: infants' increased losses, illness increasing losses, hot temperatures and climate and any situations that may cause excessive losses such as exercise, foreign travel and occupational hazards.

Patients may also become calcium, zinc or magnesium deficient, and this is usually identified on blood tests. They may require supplementation in certain cases, and this again is judged on an individual basis and in accordance with local guidelines.

1.8.3 Screening for complications

As previously discussed, CF has multiple complications including diabetes and liver disease. A key part of managing CF is monitoring for complications to identify them at the earliest possible stage and optimise their management and prognosis. One way in which this is achieved is through annual review. Annual reviews are a yearly assessment for every patient with CF and include blood tests, clinical assessments, psychological assessment, assessments from physiotherapists, CF specialist nurses, social workers and dieticians. Blood tests include: liver function to look for early signs of CFRLD and testing for CFRD from the age of 10 onwards alongside other blood tests which help look at pulmonary and nutritional issues. Annual reviews have been associated with increased parent satisfaction and increased testing for potential complications of CF (100).

1.8.4 Psychosocial management

CF is an undeniable challenge to live with both for patients and their families as such a holistic approach to care is of vital importance. Clinical psychologists form an essential part of the CF MDT and their assessments and support are vital for families and patients struggling with CF(101).

CF management can also involve liaison with social workers and schools to ensure optimal care and social support for patients.

1.8.5 New treatments

Increased understanding of genetics presents an opportunity for targeted therapies in CF. CFTR modulators, drugs that work by improving the function of the CFTR channel, have shown the most promise in treating CF. Ivacaftor was the first CF treatment directly targeted at correcting or partially correcting the defect in the CFTR. Ivacaftor is a CFTR potentiator – meaning it works by improving the function of CFTR channels. It is thought to do this by increasing the open probability of CFTR channels enabling increased ion transportation can occur (102). Ivacaftor has been shown to be effective in patients with G551D mutation in terms of both lung function and quality of life scoring, but unfortunately was not shown to be effective in those with Phe508del mutations (103).

Lumacaftor has been the next advance in CF genetic medicine. Lumacaftor is a CFTR corrector and aims to correct the processing and trafficking of CFTR channels. In combination with ivacaftor it is effective in patients with Phe508del mutations and trials (104). However, combination therapy is currently not recommended by NICE. Lumacaftor and ivacaftor dual therapy is available in the US and Ireland. There is ongoing campaign around enabling access in the UK (105).

However, there are promising breakthrough's for individuals who are homozygous for Phe508del. Dual therapy with tezacaftor-ivacaftor has been shown to improve lung function

and reduce rates of pulmonary exacerbation (106). Triple therapy involves a combination of two CFTR correctors with the potentiator ivacaftor (105). Clinical trials of triple therapy VX-659–Tezacaftor–Ivacaftor (32) and VX-445–Tezacaftor–Ivacaftor (33) are even more promising, showing greater increases in lung function for individuals who have one or two Phe508del mutations.

Gene replacement therapies have also been explored as a potential treatment for CF. Unlike the gene specific therapies already discussed, gene replacement therapies aim to replace the faulty CF gene as opposed to improving the function of CFTR. Gene replacement therapies typically use viruses as a vector to try and incorporate healthy CF genes into the individual's cells to replace the faulty CF genes. Although there has been some promise in mice models (107), transferring these principals to humans remains challenging (108) and the results of gene replacement therapy remain underwhelming.

1.9 Prognosis

An increased understanding of CF, multidisciplinary care and new treatments have meant CF is now very rarely a cause of childhood mortality. It is predicted that should life expectancy continue to rise as it has done in recent years, children born with CF in 2010 can be expected to live to approximately 56 years old (109).

1.10 Summary

CF is a clinically important, life-shortening, non-curable genetic disorder affecting over 10,000 individuals in the UK. There are a wide variety of symptoms and complications associated with CF, but respiratory complications are the leading cause of morbidity and mortality. Respiratory complications occur as a result of repeated airway infections leading to inflammation and fibrosis. Surveillance for infection forms an important element of respiratory management in patients with CF and this will be discussed in chapter 2.

2.0 Respiratory Microbiology in CF

2.1 Respiratory pathogens in CF

One of the defining features of CF is recurrent respiratory infections which over time lead to progressive inflammation and destruction of lung parenchyma.

Evidence suggests that infants affected by CF rapidly acquire infections following birth (110) although they may be asymptomatic. Even in infants, who may only be a few months old, there can already be radiological evidence of structural lung disease. Although initial infection may not be present at birth, studies have shown that CF lungs are abnormal from birth, with mucus plugging and increased inflammatory markers present in bronchoalveolar lavage fluid even in absence of identifiable infection (111). Infection in infants is associated with poor nutritional outcomes (74).

The bacteria that cause infection in children with CF are distinct from those that cause infection in unaffected children. In children without chronic respiratory disease, bacteria are a rare cause of respiratory infection with viruses causing most infections. Respiratory syncytial virus and rhinovirus are the major causes of infection (112) with common bacteria including *Streptococcus pneumoniae* and *Haemophilus influenzae* (113). However, in children with CF, initial infection in infancy and childhood is often with *Staphylococcus aureus* and *Haemophilus influenzae* (114). Figure 4 (34) highlights the changes in bacterial infections with age in individuals with CF.

Older children and teenagers with CF are at increased risk of developing infection with *P. aeruginosa.* This is potentially the most clinically significant infection associated with CF as it has been shown to decrease lung function and increase morbidity and mortality (115). As such, early infection with *P. aeruginosa* is treated aggressively with antibiotics in an attempt to prevent infection becoming chronic. Furthermore, the subtype of *P. aeruginosa* affects a

patient's prognosis as mucoid strains cause a more significant deterioration in lung function as compared to non-mucoid strains (116).

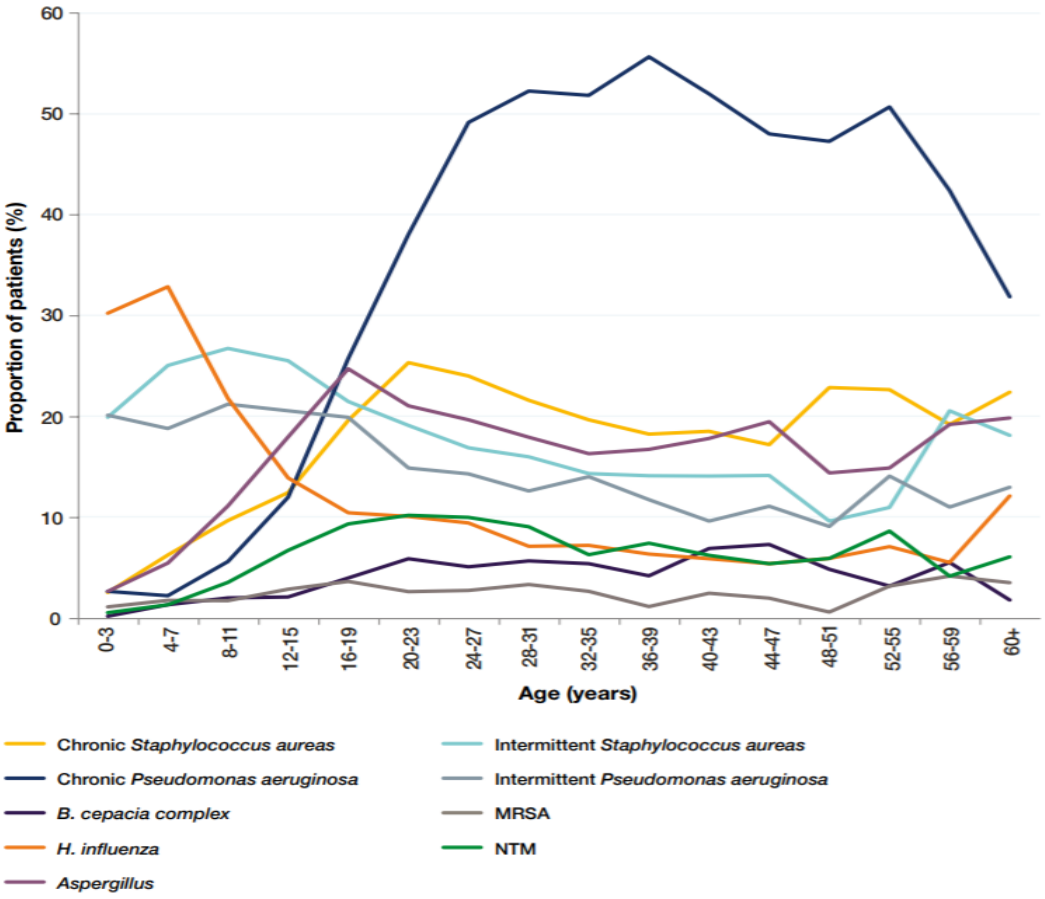

Figure 4 (26) - A graph to show prevalence of different respiratory pathogens in different age groups from all individuals on the CF registry in 2017. Reproduced with permission from the CF Trust.

P. aeruginosa is particularly challenging to treat partly due to its inherent resistance to some antibiotics and partly due to its lifecycle which involves the formation of biofilms (117). Biofilms are extracellular matrices which hold colonies of bacteria together, which makes antibiotic penetration and treatment significantly more challenging. Initially when *P. aeruginosa* infects and attaches to the lung surface the bacteria are in planktonic form, the bacteria then divide and go through several phenotypical changes finally resulting in large mucoid colonies surrounded by biofilms.

Other significant pathogens commonly seen in children with CF include *Burkholderia cepacia* complex. Infection with *Burkholderia* is also associated with a decrease in lung function and increased mortality (118) but infection is less prevalent than with *P. aeruginosa.*

Viral, fungal and nontuberculous mycobacterial infections are also common in children with CF (119).

2.2 The lung Microbiome in CF

Not all microbes in the lungs are pathogenic. The microbiome is defined as the "ecological community of commensal, symbiotic and pathogenic organisms that share our body space" (120). Extensive research into the gut microbiome and its relation to multiple diseases has been undertaken (121–123). However, research into the lung microbiome is much more recent and, until recently, it was taught by some that the lungs are a sterile environment (124). Understanding the lung microbiome has important implications, as the lung microbiome has been shown to be altered and less diverse in those with chronic lung disease (125). Determining whether changes in the lung microbiome are merely a marker of disease, or contribute to disease pathogenesis is important and may be a potential target for disease modifying treatments (124).

New technology is allowing researchers to gain a better insight into the CF lung microbiome with increasing numbers of bacteria, including those that have not previously been identified in CF lungs, now being researched (126–129). In one recent study, presence of anaerobes in the lung microbiome was positively associated with pancreatic sufficiency, better lung function and better nutrition (130) demonstrating that the lung microbiome may be a marker of disease progression in CF. However, some of these anaerobic bacteria are associated with increased antibiotic resistance and inflammation and so the lung microbiome may directly be contributing to disease pathogenesis (131).

There are now several different methods for analysing the lung microbiome (131) including DNA, RNA and culture based methods.

2.3 Symptoms caused by respiratory infection

One of the challenges in identifying and treating infections in children with CF is that they can present in a variety of ways. Children may be entirely asymptomatic; a recent study found that infants with CF are more likely to be asymptomatic with viral infections than infants without CF (132). Asymptomatic infections are challenging as firstly, unless routine samples are taken, they may not be identified and secondly as the child appears well acceptance and adherence to treatment may be poor. Preschool children are particularly likely to have infection in the absence of symptoms and signs (133).

Although some children may present asymptomatically, there are key symptoms that children with exacerbations of CF may present with. Notable symptoms include but are not confined to: increased cough, increased sputum production, new crackles and a decline in weight percentile. Early treatment with antibiotics can help reduce these symptoms and minimise further complications (134). Other factors that may indicate a pulmonary exacerbation of CF include: new or increased haemoptysis, increased dyspnoea, malaise, fatigue or lethargy, sinus pain or tenderness, change in sinus discharge, decrease in pulmonary function by 10% or more from previously recorded function and radiographic changes (26). These factors may indicate the need for antibiotic treatment and some studies have suggested that presence of 4 or more symptoms may indicate requirement for parenteral antibiotics (135).

2.4 Identifying Respiratory Infections in Patients with CF

Due to the lung damage caused by infections in children with CF, identifying and treating infections as promptly as possible is key and surveillance cultures should be performed on a regular basis. In children respiratory samples are taken at every outpatient appointment, these typically occur every 2 months although may be more frequent in infants or symptomatic

children. Cough swabs (CS), cough plates (CP), sputum samples and bronchoalveolar lavage (BAL) are the core methods used to identify infection. Table 1.3 summarises some of the methods used for respiratory sampling.

Table 1.3 - Methods used for respiratory sampling

Sample Type	Description
BAL	Fluid squirted into and collected from the lungs during bronchoscopy
Sputum	Spontaneously expectorated sputum sample
Induced sputum	Sputum obtained following administration of hypertonic saline and physiotherapy
CS	Swab is placed in the pharynx, without touching the mucosa, the child is asked to cough
CP	Agar plates held 5 to 6cm from the child's mouth. The child is asked to cough on each plate

2.4.1 BAL

A spontaneously expectorated sputum sample is the optimal lower airway microbiology sample in CF. Unfortunately, a lot of children and adults with CF are unwilling or unable to expectorate sputum. The gold standard for identifying lower respiratory infections in non-expectorating CF patients is BAL (136). BAL involves squirting fluid directly into the lungs and recollecting this fluid during bronchoscopy. As such it is one of the best methods for identifying infections in the lower respiratory tract. However, the number of lobes sampled can affect whether all pathogens are identified on BAL (137). An additional benefit of performing BAL is that during bronchoscopy the lungs can be visualised to look for evidence of airway damage and mucus plugging, and biopsies can also be taken to look for evidence of structural lung changes (138).

However, it would be impractical to perform bronchoscopy on a routine basis to identify all infections. Bronchoscopy requires administration of a general anaesthetic and such an invasive procedure is not warranted for routine surveillance cultures (139,140). Therefore current NICE and CF Trust guidelines recommend if a patient is unable to provide a sputum sample, they provide CS as their routine microbiology surveillance sample (101,141).

2.4.2 Sputum

A spontaneously expectorated sputum sample is the optimal lower airway microbiology sample for routine samples in CF and in adults and older children who are able to expectorate, monitoring for respiratory infections involves regular sputum samples. Sputum has a high yield for identifying pathogens and a high concordance with BAL samples (142) and is considered the gold standard for surveillance cultures in expectorating patients.

2.4.3 Induced Sputum

For children who are not able to expectorate, it is sometimes possible to induce sputum. The recent CF sputum induction trial (CF-SpIT) found that sputum induction, which involves giving hypertonic saline via nebuliser and physiotherapy to help the child produce sputum, was superior to CS at identifying pathogens (143). Induced sputum also identified 77% of the pathogens identified on two-lobe BAL. Consequently, one of the recommendations in this study was that induced sputum could be considered as an alternative to invasive bronchoscopy in symptomatic children.

Although induced sputum is an important method for identifying infection in non-expectorating children, it is costly and time consuming in comparison to both CS and CP. Therefore, it would not be an appropriate method for sampling for routine surveillance at every outpatient appointment. Another disadvantage is that it is not always successful – 84% of procedures were successful in the CF-SpIT trial (143). However, the CF-SpIT trial has highlighted that sputum induction is a useful method for identifying infections in symptomatic children who are unable to expectorate.

2.4.4 CS

CS are the method of choice in almost all CF centres in the UK for children who are unable to expectorate. CS involve placing a swab in the pharynx, which is then cultured onto agar plates. They are quick, cheap and can be performed on children of any age.

However, CS have a significantly lower yield for identifying pathogens compared to sputum samples (144,145). As such, research into improving microbiological surveillance in young children with CF who are unable to expectorate is important. Positive CS are highly indicative of positive sputum cultures. However a negative CS, especially in the presence of symptoms, does not rule out infection (145).

2.4.5 CP

CP were first used as an alternative to CS in a study by Maiya *et al.* (2004) at Birmingham Children's Hospital (146). As with CS, they are comparatively cheap and quick to perform in comparison to other sampling methodologies. They cannot be performed on young children as they require the ability to cough on demand. However, one advantage of CP is that they are preferred to CS by the majority of patients (146,147).

CP have not been introduced at most CF centres as the evidence supporting their benefit in comparison to CS is conflicting (146–149). The evidence basis behind using CS and CP will be critically evaluated in the next section.

2.5 CS versus CP

There is debate as to whether CP are more effective at identifying infection compared to CS. A study into the relative efficacies of CS versus CP was performed by Maiya *et al.* (2004) and found the yield of pathogens identified on CP was significantly higher than from CS. In this study, 31 patients participated and had sputum, CP, moist and dry CS taken. Twenty positive sputum cultures identified pathogens, with sixteen positive CP and seven positive moist and dry CS (146).

However, as this study was performed in children who were able to expectorate, sputum would be the microbiological method of choice in these children. This brings into question the clinical relevance of this study and whether these results are applicable to non-expectorating children. A follow-on abstract was published by the team at Birmingham Children's Hospital, 130

children, only 17 of whom could expectorate were included and similar results were found (150).The number of samples taken in the original Maiya *et al.* study is also small and so these findings may not be generalisable but having similar findings in the larger population in the follow on abstract increases the generalisability of these results.

Increased identification of pathogens by CP has not been replicated in similar studies. A study of 95 subjects by Byrne *et al.* in 2013 found that pathogens were isolated on 18.2% of CS but only 8% of CP (147). This study did acknowledge there were benefits to CP as firstly, older children tended to isolate more specimens on CP – potentially due to a stronger cough mechanism and secondly, the majority of children preferred CP to CS.

Two other studies by Chavasse *et al.* (2007) and Orska *et al.* (2006) had similar findings (148,149) with CS identifying a higher number of pathogens in comparison to CP. The lack of clarity regarding the efficacy of CP means they are rarely used in clinical practice in most CF centres.

2.6 Non-culture-based techniques for identifying pathogens in CF

Regardless of which of the above methods for respiratory sampling is used, they are usually then cultured in the lab to grow pathogens. However, new non-cultured based techniques are revolutionising the identification of pathogens in children with CF. Polymerase chain reaction (PCR), has the potential to identify more pathogens, more accurately than culture-based techniques (151).

Use of molecular techniques such as PCR are especially relevant for clinically important pathogens such as *P. aeruginosa*, where PCR assays have been shown to have high sensitivity and specificity for identification (152). PCR is also beneficial for identifying bacteria that are not routinely identified by culture (151) which may be the cause of unexplained symptoms and failure to respond to treatment in children who appear to be growing no pathogens using culture based techniques.

2.7 Summary

The gold standard for routine microbiological surveillance is sputum cultures. However, many children are unwilling or unable to expectorate sputum. CS have traditionally been used for surveillance cultures in non-expectorating children, but they have a low sensitivity. Alternative methods such as CP have been examined but the results of clinical studies are conflicting. Consequently, collection of routine microbiological samples from non-expectorating children with CF remains a challenge without a clear solution.

3.0 Aims and Objectives

3.1 Introduction

There is conflicting evidence regarding whether CS or CP are superior at identifying pathogens for routine surveillance cultures in non-expectorating children with CF. This indicates the need for further research in this area.

Children with CF at Shrewsbury and Telford Hospital NHS Trust (SaTH) routinely perform CS and CP at each outpatient appointment and have done so for many years. There is a wealth of previously unused data at SaTH, including paired CS and CP results from children aged 2 to 18: potentially one of the largest collections of data regarding CS and CP use in the UK. This has the potential to answer important questions regarding the use of CS and CP including: does using both CS and CP increase detection of pathogens and do CS or CP identify important pathogens such as *P. aeruginosa* earlier.

This study utilises 5 years of retrospective data to evaluate the performance of CP in comparison to CS.

3.2 Aims and Objectives

The principle aim of this thesis is to compare the performance of CS and CP at identifying respiratory pathogens in non-expectorating children with CF. The specific objectives are:

Objective 1

To collate paired microbiological results for CS and CP performed on children with CF at SaTH between November 2013 to November 2018.

Objective 2

Collect relevant data at the time of these samples including: age of child, gender, lung function (percentage forced expiratory volume in 1 second -%FEV_1) and weight, height and body mass index (BMI) centiles.

Objective 3

Attempt to detect a difference in pathogen yield between CS and CP.

Objective 4

To determine if age, weight, height, BMI centile or lung function affected performance of CS or CP.

Objective 5

To investigate if different organisms were more likely to be identified on CS or CP.

Objective 6

To investigate whether CS or CP were superior in aiding the early identification of *P. aeruginosa* in young children under the age of 7.

3.3 Summary

Further research into the comparative efficacy of CS and CP in non-expectorating children with CF is required due to divergence in previous studies. This study aims to compare these two methods used for surveillance cultures as well as answering other clinically important questions such as which method is superior at identifying early cases of *P. aeruginosa.*

4.0 Study Methodology

4.1 Introduction

This project comprised of three components: literature review, clinical experience of CF and microbiology, and data collection and analysis.

4.2 Literature Review

To gain an understanding of CF and respiratory microbiology a review of literature was undertaken. The review was non-systematic in its approach; however, I attended several lectures and workshops on systematic reviews at Keele University to gain an understanding of searching databases and critically appraising literature. Core medical databases including PubMed and Medline were searched for relevant literature. In addition, key articles were suggested by the project supervisor. Other important sources of information included Google Scholar, the Cystic Fibrosis Trust and the NICE for relevant clinical guidelines.

4.3 Clinical Background and Relevance

In order to appreciate the clinical relevance of this study, I attended the paediatric CF clinic at Royal Stoke University Hospital (RSUH) on a weekly basis for a year in order to gain a greater understanding of the disease and the multidisciplinary approach required to manage CF. One clinic was also attended at SaTH to observe paired cough swab and cough plate sampling being performed.

A week's observation was undertaken within the microbiology department at RSUH to gain an understanding of how respiratory samples are processed, pathogens are identified, and antibiotic sensitivities are determined. This was done at RSUH, as this was my base site and the site of both my supervisor and co-supervisor. However, as this experience did not happen at SaTH I did not have the opportunity to observe the processing of CP samples.

Time was spent in the containment level 3 laboratory to observe sputum samples and CS being inoculated onto agar plates. Plate reading and reporting was observed in the containment level 2 laboratory, to gain an understanding of how pathogens are identified on plates and which pathogens can be identified on different media. An important learning point from this was that the majority of samples from patients with CF will grow bacteria and to a novice it can be difficult to differentiate between normal respiratory flora and pathogens. Another important lesson was that in vitro results are not always representative of the microbiology and sensitivities in vivo.

Table 4.1 - The media used for CF respiratory samples at RSUH and the pathogens targeted by this media

Media	Pathogen targeted
Chocolate Agar	*H. influenzae, M. catarrhalis, S. aureus*
Blood Agar	S*. pneumoniae*
CLED	Enterobacteriaceae, Pseudomonads
SAID	*S. Aureus*
Sabouraud Agar	Fungi
B. cepacia selective agar	*B. cepacia* complex

During the week in microbiology time was also spent observing antibiotic sensitivity testing, helping to stain slides for mycobacterium and visiting the virology laboratories to gain an understanding of how viral samples are tested.

4.4 Methodology for collecting CS and CP at SaTH

CP and CS are performed routinely at every outpatient appointment for children with CF, which typically occur every 2 months although can be more or less frequent. CP and CS are taken by one of the CF team (specialist nurse, doctor or physiotherapist). CP involve a series of 4 CP

being held approximately 5cm from the child's mouth. The child is then asked to cough twice forcefully on each plate. The 4 plates used at SaTH are: blood agar, chocolate agar, salt agar and *Burkholderia* cepacia selective agar. The plates are then sent for culture and read at 24 and 48 hours.

CS are performed using the standard methodology used at all CF centres, a swab is held in the pharynx and the child is asked to cough. The swab is then sent to the lab where it is cultured onto agar plates. At SaTH CS are cultured onto 6 plates: blood agar, chocolate agar, salt agar, *Burkgolderia* cepacia selective agar, CLED agar and a SAB+CHLOR (SABC) plate. The plates used and their costs are summarised in Table 4.2.

Table 4.2 – The plates used to culture pathogens at SaTH and their costs.

Plate	**CS or CP**	**Cost of plate (pence per plate)**
Blood agar	Both	18
Chocolate agar	Both	28.4
Burkholderia cepacia Selective Medium	Both	54
Salt agar	Both	24
CLED agar	CS only	17
SAB + CHLOR (SABC) plate	CS only	20.4

The costs of performing CS alone are £1.62 per child with CP costing an additional £1.24, although this does not take into account the cost of clinics and analysis of samples. CS and CP are not performed in any set order in clinic and the order will often be the result of patient preference.

Although the addition of CP means patients are required to provide more samples and this can add to the length of clinic visits, the use of CS and CP appears to be well accepted by children, their families and staff at SaTH. Despite having to cough a total of 9 times for samples, the team at SaTH have had no complaints from families or children about the use of CP.

4.5 Study Design and Population

The study design was a retrospective review of paired CS and CP microbiological results for children with CF attending outpatient appointments at SaTH. Performing CS and CP on all non-expectorating children who are old enough to perform CP has been standard practice at SaTH for over a decade.

All paediatric CF patients who attended outpatient appointments with CF team at SaTH between November 1st 2013 to November 1st 2018 were included. They were identified by the SaTH Paediatric CF team providing a list of all their patients during this time. Only patients who were able to perform both CS and CP were included. This meant that children under the age of two were excluded. Patients capable of expectoration were not necessarily excluded. Although they may be productive at some appointments, this may not always be the case. Therefore, each appointment was checked to determine whether that patient had produced a sputum sample or whether paired CS and CP had been required.

4.6 Data Collection

4.6.1 Clinical Appointment Data

Dates for outpatient appointments were collected from the outpatient appointment diaries and cross-checked against patient notes or clinic letters on the SaTH online system "Clinical Portal". Occasionally, additional appointments were found in the notes or in clinical letters, which were not in the appointment's diary. Using the diary, patient notes and clinical letters reduced the chance of missing any outpatient appointments. However, as this study is retrospective and

relied on hand written or typed records, there is a possibility that a small number of appointments may have been missed.

4.6.2 Microbiology Results

Once appointment details were collected, the results system "Review" was checked for CS and CP results collected on that day. As occasionally samples were not processed immediately, samples labelled as coming from the Paediatric Outpatient Department within 2 days of the clinic appointment were included. Only data for finalised reports of paired CS and CP was collected. If the report was interim and not finalised it was excluded. If there was only CS, sputum or CP performed the data was excluded.

For a small number of children, two samples with different sample numbers were sent to the lab however, both samples were reported as CS or CP. Although it would appear that both a CS and CP had been taken in these children, both samples had to be excluded as it would be impossible to determine which result was from the CS and which result was from the CP.

A small number of samples were collected in patients over the age of 18 as they were transitioning from paediatric to adult services. These samples were excluded for this study as it focuses on the paediatric CF population. Inclusion and exclusion criteria for samples are summarised in Table 4.3.

Table 4.3 - Inclusion and exclusion criteria for microbiological samples

Inclusion Criteria for Samples	Exclusion Criteria for Samples
CS and CP from Paediatric Outpatient Department processed within 2 days of outpatient appointment Final microbiological report	Unpaired samples i.e. just cough swab, plate or sputum or sputum with only CS or CP Two samples both reported as CS or CP Interim report with no final report available Patient 18 years or older

Initially only one year of data was collected (November 1st 2017 to November 1st 2018) to assess the number of patients, paired samples and pathogens identified. However, following discussion with the statistics team at RSUH in the Research and Development (R&D) Department, it was recommended that five years' worth of data would be required to have enough samples which grew pathogens to be able to perform significant statistical analysis. Power calculations were not performed, however they were performed for Byrne *et al.*'s study, which stated a sample size of approximately 400 was needed to provide 80% power to detect an effect size of 20% for growth of pathogens at the 0.05 significance level, taking into account repeated measures (147).

4.6.3 Lung function and BMI data

Alongside collecting microbiological results, data on lung function (% FEV_1), height and weight were collected. These were initially collected from either the notes or from electronic clinic letters. Clinic letters were used for patients whose notes weren't readily available as for example they had died, moved out of the area or transitioned to adult services. For any missing spirometry data, the spirometry software at SaTH, "Spirotrac", was used to find missing results. For some of the younger patients, spirometry results were not available as although they were old enough to perform CS and CP samples, they were not old enough to perform spirometry. Whilst collecting the data on height, weight and lung function, the microbiology results were rechecked. This was done in order to minimise the chance of human error when collecting large volumes of data.

Following collection of all the results, height, weight and BMI centiles were calculated from the recorded measurements. To calculate the centiles, the "Growth Charts UK-WHO" application was used.

4.7 Information Governance

All data collected were recorded anonymously and stored on an encrypted memory stick. All patients were given a unique study identification number enabling data to be collected anonymously. Access to data was enabled by the team at SaTH who provided access to paper notes and information technology (IT).

4.8 Incomplete data

Due to the retrospective nature of this project, data were missing for some variables. Gender, age and microbiological results were collected for all 663 paired samples. However, data for BMI centile and lung function was not always available. 73 of the 663 samples (11%) did not have height and weight recorded to enable calculation of BMI centile. With regards to lung function, for 133 of the samples (20%) the child was too young to perform lung function. For the remaining 530 samples, where the child was old enough to perform lung function testing, 26 samples were missing lung function data. 2 lung function samples were also removed as outliers, leaving a total of 502 samples for lung function.

4.9 Cleaning Data

Once data was collected, all variables were checked for outlying results. Outliers were defined as any result that was significantly higher or lower than other results for the same variable in that patient and did not follow the general trend of that patients results. Only 2 outliers were identified, which were low percentage FEV_1s. These were both in young children who may have struggled with the techniques required to perform spirometry, it is possible these could be explained by exacerbations but is unlikely given how low these percentages were and the age of the children. These results were removed before data analysis.

Microbiological results positive for non-pathogenic organisms: yeast, *Candida albicans* and bacillus species were also removed from the data-set. This was done on advice from the project supervisor, an experienced CF clinician, and considering relevant literature which highlights that

in the majority of cases these organisms are non-pathogenic (153,154), although there is some controversy around the role of *C. albicans* in CF (155). *Bacillus* species was labelled as a likely contaminant on the microbiological results and hence was removed from data analysis. 14 paired samples identified non-pathogenic organisms alone, and 5 paired samples identified both non-pathogenic and pathogenic organisms. Of the 19 non-pathogenic organisms identified, 17 were cultured from CS.

4.10 Statistical analysis

In preparation for data analysis, I attended a module on statistics and epidemiology at Keele University to gain an understanding of commonly used study methodologies, statistical tests and their interpretation. Although not all of the statistical tests used in this study were covered, this module gave insight and competency in basic statistics.

Appropriate statistical tests to meet the study aims and objectives were identified with help from the project supervisor, co-supervisor and the R&D department at RSUH. These tests include: number needed to sample (NNS), McNemar's test, the generalised estimating equation and multivariable logistic regression. Simple tests including descriptive statistics and NNS were carried out using Microsoft Excel. Other statistical tests were carried out using STATA version 8 (STATA Corp, Texas, USA).

4.9.1 Number needed to sample

In most CF centres in the UK, CS alone are used as the routine method for respiratory sampling for non-expectorating children. Therefore, one important component of this study was to compare whether taking paired CS and CP samples could improve identification of pathogens. The NNS is one method for estimating how many more children would require paired CS and CP compared to CS alone to identify one additional pathogen.

NNS was determined by first calculating the absolute risk reduction (ARR) using the formula $\frac{a}{a+b} - \frac{c}{c+d}$ (see Table 3.4). The ARR was divided by 1 to calculate the NNS.

Table 4.4 - How NNS is calculated

	Positive	Negative
Test 1	a	b
Test 2	c	d

4.9.2 McNemar's Test

Previous studies that have compared the results of CS and CP cultures have used McNemar's test to determine whether there is a statistically significant difference between CS and CP performance (146,147).

McNemar's test is a non-parametric test and is used with paired measures dichotomous data (156). It is used to compare proportions, and specifically uses discordant pairs (in this case a negative CS and positive CP and vice versa) to determine whether there is a significant difference in proportions. To perform the McNemar test data is converted into a 2x2 contingency table as shown in Table 4.5, the formula $\chi^2 = \frac{(b-c)^2}{b+c}$ is then used to calculate chi squared test statistic and p value.

Table 4.5 - How the McNemar test is calculated

	Test 2 Positive	Test 2 Negative
Test 1 Positive	a	b
Test 1 Negative	c	d

This test is appropriate for this study as the groups are repeated, the same children had CS and CP performed, and the data is dichotomous, the CS and CP were either positive or negative. As this test was used in previous comparisons of CS and CP it also allows us to compare our results easily with previous studies. The null hypothesis in this study is the proportion of pathogens identified by CS is equal to the proportion of pathogens identified by CP.

However, application of McNemar's test on this data set provides complications. The most important reason McNemar's test is not optimal for analysis of the results in this study is the test does not adjust for the multiple paired measures we have from each patient. This is problematic as if one patient identifies more pathogens on CS or CP this could skew the results. This was not a concern in some previous studies, such as Maiya *et al.*(2004), as each patient only gave one set of samples (146). However, in Byrne *at al.'s* (2013) study, 4 repeated sets of paired samples were taken from patients (147). McNemar's test was performed separately on the first, second, third and fourth paired samples from each patient and an overall McNemar's test using all samples was performed. It is unclear how multiple paired samples were accounted for in the overall McNemar's test using all samples. For this reason, McNemar's test was also calculated taking just one sample from each patient from the first to tenth sample in this data set.

One potential solution is to use an adjustment for McNemar's test that accounts for repeated measures (157). The statistics required for this are complex and beyond the scope of this thesis. However, we have contacted a clinical statistician to advise on this methodology before considering this work for publication elsewhere.

4.9.3 Multivariable logistic regression

The second aim of the data analysis was to identify whether age, BMI centile, gender and percentage FEV_1 affected CS or CP positivity. In order to determine this, multivariable logistic regressions were performed for all variables for CS and CP separately. This analysis was suggested by the project supervisor, and basic information regarding logistic regressions was taught at the statistics and epidemiology course I attended.

Logistic regression is a predictive analysis and is used to explain the relationship between one or more independent variables and a dichotomous or binary dependent variable. Logistic regression was used on this data set as the dependent outcome is binary – the CS or CP was

either positive or negative for a pathogen. Multivariable analysis was performed as it enables multiple variables to be analysed simultaneously and minimises the effects of confounding (158). All 663 CS and CP samples were input with the corresponding data for BMI centile, age, gender and percentage FEV_1.

However, as with McNemar's test, repeated measures from patients are again problematic and mean results from the logistic regression should be interpreted cautiously. Logistic regression has been used in other studies, such as CF-SpIT, to assess the relationship between independent variables such as age and the likelihood of a positive sample (143).

4.9.4 Generalised Estimating Equation

Due to the issues surrounding multiple measures from patients, the generalised estimating equation was proposed as an alternative method for comparing CS and CP positivity and to overcome the limitations of logistic regression. This method does account for multiple measures but does not provide an easy comparison to previous studies. The generalised estimating equation was proposed as an alternative method of analysis by the project co-supervisor. I then researched generalised estimating equations to gain a basic understanding of their applications to the analysis of longitudinal data.

The generalised estimating equation is a statistical approach used in the analysis of longitudinal data and can be used where there are repeated measures from the same individual or from related individuals, for example family members or people in the same geographical area (159).

The generalised estimating equation was used in analysis of the results from the CF-SpIT trial to overcome issues with repeated measures (143). Although this study did not include CP, the study was comparing the use of CS to induced sputum and induced sputum with BAL. Therefore, as the statistical questions being asked were similar to the questions I was aiming to answer, a generalised estimating equation appeared to be the most appropriate method for addressing

the issues surrounding repeated measures in both the McNemar test and multivariable logistic regressions.

Further work with statistician support will also be performed to identify whether the generalised estimating equation is the most appropriate method for analysis or whether another method is available which can account for the repeated measures from patients, before this research is published elsewhere.

4.9.5 Organisms identified on CS and CP

An additional aim of statistical analysis was to compare the types of pathogen identified on CS and CP. The number of times each different pathogen was isolated on CS and CP samples were collated and compared.

For these more rarely isolated organisms, it was important to determine whether they are pathogenic or commensal as non-pathogenic organisms were excluded from analysis. There were 8 organisms only cultured a maximum of 4 times. These were: *Streptococcus pneumoniae,* coliform organisms*, Enterobacter cloacae, Enterobacter asburiae, Moraxella catarrhalis, Acinetobacter pittii, Streptococcus pyogenes* and *Streptococcus dysgalactiae*

S. pneumoniae, M. catarrhalis and S. pyogenes are common documented causes of respiratory tract infection (160–162) and *E. cloacae* and *E. asburiae* are causes of nosocomial respiratory infection (163). Coliform organisms have been documented to cause pneumonia (164).

A. pittii has been reported as a cause of nosocomial respiratory infection (165) and *Acinetobacter* species have been cultured from CF patients (166). However in vitro studies have shown that *A. pittii* does not adhere to respiratory epithelium (167). Despite this as it has been shown to cause clinically relevant respiratory infections *A. pittii* was included.

S. dysgalactiae has several subspecies and the specific subspecies is not known for these samples. *S. dysgalactiae* is a documented cause of respiratory infections and has similar

infective patterns to *S. pyogenes* (168). Therefore, all results were included in analysis, although it is possible some of these organisms may be non-pathogenic.

4.9.6 Early detection of *P. aeruginosa*

As discussed in Chapter 2, *P.aeruginosa* is one of the most clinically important pathogens in children with CF. It is a major cause of morbidity and mortality in children with CF, and early identification and treatment are one way to minimise the adverse outcomes associated with *P. aeruginosa* infection (169,170). *P. aeruginosa* is also being detected at increasingly younger ages, possibly as the result of advances in diagnostic techniques (171). As such it is important to see if the addition of CP aids in the identification of *P.aeruginosa,* particularly in young children. *P.aeruginosa* infections in children under the age of 7 in this cohort will be identified and will be analysed to see whether *P.aeruginosa* was identified by CS or CP and whether *P.aeruginosa* was identified again during the time period included in the study and by which sampling method.

4.10 Ethical Considerations

For this study, no official ethical approval was required, as according to the National Institute of Health Research (NIHR) this study is classified as service evaluation. Service evaluation defines or measures current practice within a service. As collection of both CS and CP samples in children with CF has been standard practice at SaTH for more than a decade, this project can be categorised as assessing this service. This was confirmed by the author and project supervisor completing the Health Research Authority (HRA) tool questionnaire (172), which established this project does not classify as research (see Appendix A). Ethical approval was therefore not sought.

5.0 Results

5.1 Introduction

The results will be detailed in this section. The aims of the statistical analysis are to address the following three objectives:

1. To determine whether there was a difference in pathogen yield in CS compared to CP.
2. To investigate if age, weight, height, BMI centile or lung function affected the likelihood of CS or CP being positive.
3. To investigate which organisms were more likely to be identified on CS or CP.
4. To identify whether CS or CP were superior at identifying *P. aeruginosa* in children under the age of 7.

5.2 Study population

663 paired CS and CP samples were identified from 38 patients from November 2013 to November 2018. 21 were males and 17 were females. The characteristics of the patient sample are outlined in Table 5.1, any outliers were omitted.

Table 5.1 Characteristics of study population

Characteristic	Range	Median	Mean	Standard Deviation
Age (years)	2.2 to 18.0	8.8	9.9	4.7
BMI centile	1st to 98th	52	51.7	25.9
Lung function (percentage FEV_1) *	38% to 115%	80	79.1	15.0
Number of paired samples per patient	1 to 34	20	17.4	10.0
*Normally distributed variables				

5.3 Overview of microbiological results

Of the 663 paired samples, the CS and/or the CP was positive for a respiratory pathogen on 118 (18%) of the paired samples. 14 samples only grew non-pathogenic organisms: yeast, *Candida albicans* and *Bacillus* species. These samples were not counted as positive as the organisms are not clinically significant.

There were 66 (10%) positive CS and 87 (13%) positive CP samples, some of which grew multiple pathogens. For 35 of the paired samples both the CS and CP were positive from the same appointment, there were 31 occasions where the CS was positive, and the CP was negative and there were 52 occasions where the CP was positive and the CS was negative. Hence there only being 118 positive paired samples in spite of 66 positive CS and 87 positive CP.

Only 27 (23%) of the paired samples with a positive culture identified the same pathogen(s) on both CS and CP.

Although there were only 66 positive CS, as some samples grew multiple pathogens CS identified 75 pathogens. The 87 positive CP identified 92 pathogens.

5.4 Number needed to sample

In this sample, 13 children needed both CS and CP samples to identify one additional respiratory pathogen compared to CS alone, this was calculated using the values in Table 5.2.

Table 5.2 - The NNS in this population when comparing CS alone to using both CS and CP, demonstrating that 13 children would require both CS and CP as compared to CS alone to identify one additional pathogen.

	Positive	**Negative**
CP and CS	118	545
CS	66	597

ARR = 0.078

NNS = 13

5.6 Determining whether there is a significant difference between CS and CP yield

Two statistical tests were used to evaluate the yield of CS compared to CP. These were: McNemar's test and the generalised estimating equation.

5.6.1 McNemar's Test

McNemar's test, shown in Table 5.3, demonstrates that there was a significant difference in the proportion of pathogens identified on CP compared to CS (p=0.028).

Table 5.3 - Contingency table used in the calculation of McNemar's test using all 663 paired samples.

The p value of 0.028 demonstrates there is a statistically significant difference in the number of pathogens identified by CS and CP.

	Cough Plate	
Cough Swab	Positive	Negative
Positive	35	31
Negative	52	545

Chi-Square= 5.31

McNemar's Significance Probability= 0.028

One method to account for the multiple paired measures in this study was to take just the first sample from every patient and perform McNemar's test on those samples, the results for this are detailed in Table 5.4. When only taking one sample from each patient, there was no significant difference between CS and CP positivity (p=1.00). This was repeated for the 2nd to 10th samples from each patient and no significant difference between CS and CP positivity was identified (see Appendix B). However, this method significantly reduces the power of this test, as the sample size is decreased from 663 to 38 for the first sample. Sample size continues to decrease with each subsequent test due to patients providing different numbers of samples over the study period.

Table 5.4 - Contingency table used in the calculation of McNemar's test taking only the first sample from each patient.

The p value of 1.000 demonstrates that when taking just one sample from each patient the difference between identification of pathogens by CS and CP is not statistically significant.

	Cough Plate	
Cough Swab	0 (negative)	1 (positive)
0 (negative)	3	4
1 (positive)	5	26

Chi-Square= 0.11

McNemar's Significance Probability= 1.000

5.6.2 Generalised Estimating Equation

To calculate the generalised estimating equation, all 663 CS were input and labelled 0 and all 663 CP samples were labelled 1 for the variable CS or CP. All other variables (age, BMI centile, gender and percentage FEV_1) were included. The patient identification number was used to account for the repeated samples from each patient. The results of this analysis are shown in Table 5.5.

Table 5.5 - Generalised estimating equation for the variables: CS or CP, age, gender, BMI centile and percentage FEV_1 in predicting pathogen growth.

CS were input as 0, CP were input as 1, the positive coefficient therefore indicates that CP were superior at identifying pathogens (p=0.020). The only other statistically significant variable was percentage FEV_1 (p=0.005).

Variable	Coefficient with 95% confidence interval	Standard error	P value
CS or CP	0.046 (0.007 to 0.084)	0.020	0.020
Age	-0.006 (-0.016 to 0.004)	0.005	0.244
BMI centile	0.000 (-0.002 to 0.001)	0.001	0.612
Gender	0.022 (-0.067 to 0.111)	0.045	0.630
Percentage FEV_1	-0.003 (-0.005 -0.001)	0.001	0.005
Unique ID	0.005 (0.000 to 0.010)	0.003	0.051

Wald Chi (6) = 19.23

The generalising estimating equation showed that only 2 variables were statistically significant, i.e. had a p value of <0.05 and a 95% confidence interval that does not cross zero. These 2 variables were CS or CP (p=0.020) and percentage FEV_1 (p=0.005).

As CS were labelled as 0, and CP labelled as 1, the positive coefficient demonstrates that, according to this model, CP was associated with positive culture growth. The low coefficient of 0.046 indicates that although the relationship is statistically significant, the magnitude of this relationship is small as CP are only associated with a 0.046 times increase in the likelihood of identifying a pathogen compared to CS. The coefficient of -0.003 for percentage FEV_1, means that according to this model, a 1% increase in FEV_1 is associated with a 0.003% decrease in the likelihood of a positive culture growth.

5.7 Determining whether age, BMI centile, gender and percentage FEV_1 predict CS and CP positivity.

The results of multivariable analysis for CS can be found in Table 5.6 and the results of analysis for CP can be found in Table 5.7.

Table 5.6 - Multivariable analysis with odds ratio (OR) for variables: age, gender, BMI centile and percentage FEV_1 predicting CS positivity.

The only significant predictor of CS positivity was percentage FEV_1 (p=0.017).

	N=	OR * Adjusted with 95% CI	P value*
Age	663	1.022 (0.936, 1.116)	0.627
Gender	663	0.783 (0 .398, 1.538)	0.477
BMI centile	590	1.002 (0.986, 1.018)	0.826
Percentage FEV_1	502	0.969	0.017

Table 5.7 - Multivariable analysis with odds ratio (OR) for variables: age, gender, BMI centile and percentage FEV_1 predicting CP positivity.

BMI centile (p=0.031) and percentage FEV_1 (p=0.002) were the only statistically significant predictors of CP positivity.

	N=	OR * Adjusted with 95% CI	P value*
Age	663	1.066 (0.989, 1.148)	0.093
Gender	663	1.192 (0.672, 2.113)	0.549
BMI centile	590	1.015 (1.001, 1.030)	0.031
Percentage FEV_1	502	0.964 (0.941, 0.987)	0.002

*Adjusted for: age, gender, BMI centile and percentage FEV_1

For CS, the only statistically significant predictor of CS positivity ($p<0.05$) was percentage FEV_1 (p=0.017). FEV_1 was associated with positive culture growth using both the generalised estimating equation and multivariable analysis.

For CP, percentage FEV_1 was also a statistically significant predictor of CP positivity in multivariable analysis (p=0.002). BMI centile also contributed to CP positivity (p=0.031).

5.8 Identification of different organisms on CS and CP

The final aim of data analysis was to compare the different types of organisms identified on CS and CP.

14 different pathogens were isolated from the CS and CP. Although CP identified a greater number of pathogens, a greater variety of pathogens were identified on CS. CS identified 6 pathogens that were not identified on CP: *Enterobacter. cloacae, Enterobacter. asburiae, Moraxella. catarrhalis, Acinetobacter. pittii, Streptococcus. pyogenes* and *Streptococcus. dysgalactiae*. Some of these pathogens were cultured on multiple occasions resulting in 10 growths that were not identified by CP. As one CS or CP could identify multiple pathogens, the total number of pathogens identified by each sampling method is greater than the total number of positive CS and CP. In total CP identified x pathogens and CS identified x pathogens. Figure 5 summarises the pathogens identified by CS and CP and the number of times they were cultured.

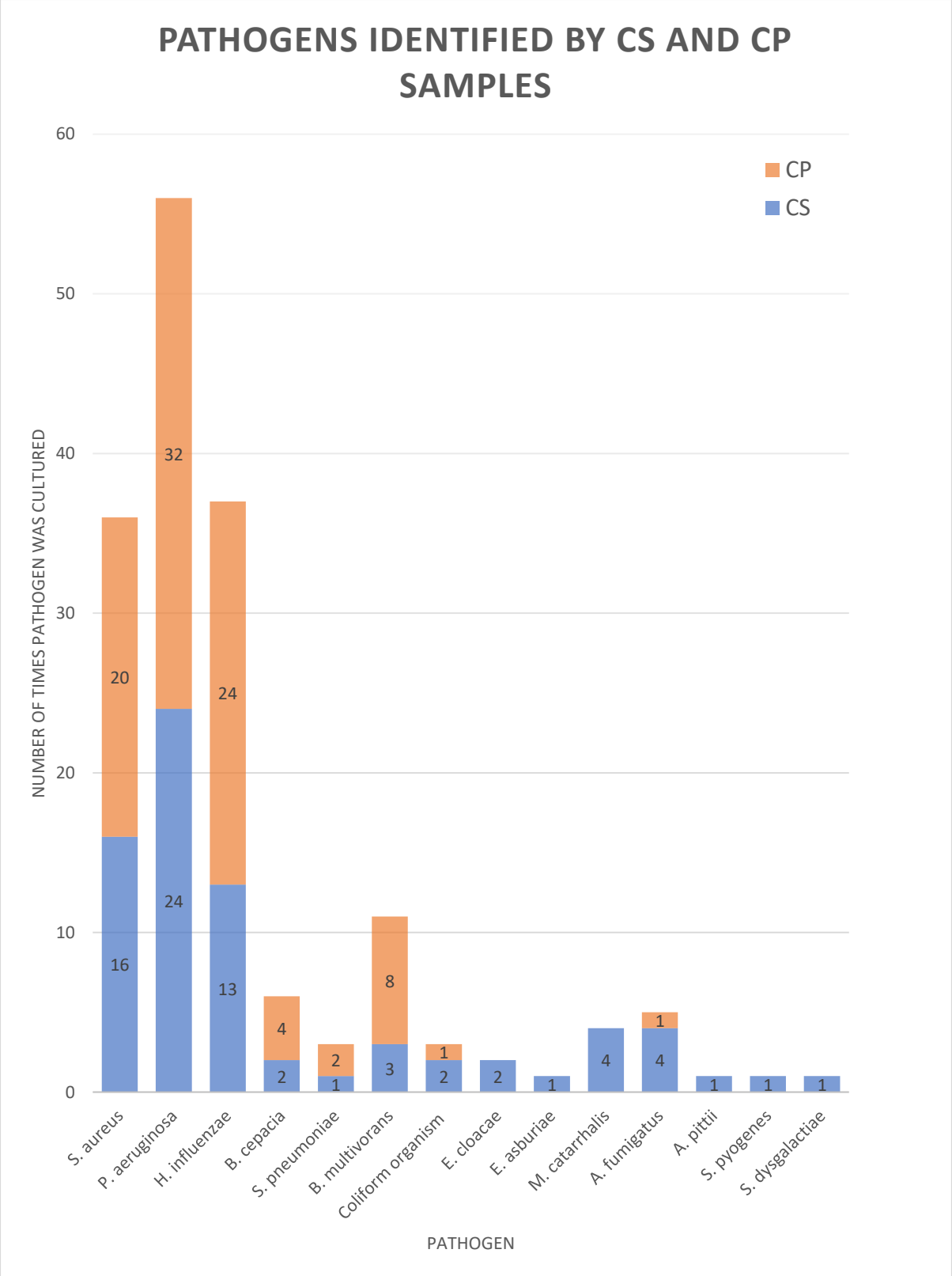

Figure 5 – A graph to show pathogens identified by CS and CP samples. There were only 66 positive CS, as some samples grew multiple pathogens CS identified 75 pathogens of which there were 14 different types of pathogen. The 87 positive CP identified 92 pathogens. CP identified 8 types of pathogen.

5.9 Growth of *P. aeruginosa* in children under the age of 7

Of the 38 children included in this study, only 4 children under the age of 7 grew *P. aeruginosa* during the study period on either CS or CP. In these 4 children the first growth of *P. aeruginosa* during the study period was from CP 2 times, CS 1 time and both CS and CP 1 time. In two cases, *P. aeruginosa* was grown again during the study period and in both these cases the second time *P.aeruginosa* was cultured it was from CS. Table 5.8 summarises these findings.

Table 5.8 – Growth of P. aeruginosa in children under the age of 7.

P. aeruginosa was isolated from four children during the study period, 2 times from CP once from CS and once from both. Two children grew P. aeruginosa again during the study period, both times from CS.

Patient	Age *P. aeruginosa* first isolated in study period (years)	Method *P. aeruginosa* identified by	Did *P. aeruginosa* grow again during study?	Method second growth identified by	Age at second growth (years)
1	4.7	CP	No	-	-
2	5.8	CS	Yes, once	CS	6.5
3	6.3	CP	No	-	-
4	5.0	CS and CP	Yes, once	CS	6.3

5.10 Summary

663 paired samples were identified in data collection. CP were positive more frequently than CS (87 times compared to 66) but whether there is a significant difference between CS and CP performance is challenging to ascertain due to repeated measures. McNemar's test and the generalised estimating equation would suggest that CP were statistically significantly more likely to identify pathogens. Although the comparison of CS and CP is important, a key finding from these results is that performing both CS and CP increased the yield of positive samples to 18% compared to 10% when using CS alone.

Percentage FEV_1 was the only consistent predictor of CS and CP positivity when looking at the variables age, BMI centile, gender and percentage FEV_1 in multivariable analysis. BMI was a significant predictor of CP positivity in multivariable analysis.

14 different organisms were isolated on CS and CP samples. CS identified a greater range of organisms including 6 organisms not identified on CP.

6.0 Discussion

6.1 Introduction

This chapter focuses on discussing the results of this service evaluation, in relation to current literature and the study aims, and considers the strengths and limitations of this study as well as recommendations for future research and clinical practice.

6.2 Comparing CS and CP yield.

The principal aim of this project was to compare the performance of CS and CP at identifying respiratory pathogens. 663 paired samples were analysed, and overall CP identified a greater yield of pathogens compared to CS (87 positive CP compared to 66 positive CS). 10% of CS were positive, this is lower than rates found in previous studies where between 13 and 26% of CS positively identified pathogens (145–147). 13% of CP were positive, this is consistent with findings in previous studies which have ranged between 8 and 52% (146,147).

Past studies looking at the use of CS and CP have focussed on comparing the two sampling methodologies (146,147). However, one of the key findings is that the yield of CS was 10% compared to 18% when using both CS and CP. This demonstrates that the addition of CP could benefit many patients and clinicians by identifying a greater yield of pathogens.

6.2.1 Comparison of results to previous literature

6.2.1.1 CF-SpIT

The CF-SpIT is one of the most recent trials to look at methods of identification of respiratory pathogens in non-expectorating children with CF (143). As one arm of this trial 167 paired CS and induced sputum results were collected. 14% of CS were positive for pathogens in this trial, which is higher than the yield found in this study.

There are numerous differences between the study population included in the CF-SpIT compared to this service evaluation. Firstly, the age range of children included was larger in the

CF-SpIT as children could be recruited from 6 months to 18 years. Secondly, children would be recruited not just from outpatient appointments, as in this study, but children who were inpatients for exacerbations, having routine surgery under general anaesthetic or attending for clinically indicated bronchoscopy were also invited to participate. As inpatients who were acutely unwell were also included in the CF-SpIT, this may partially explain the higher rates of isolation on CS in this study as children with exacerbations are more likely to have infections and therefore positive samples. Finally, children who were on antibiotic treatment at the time of sampling were excluded from CF-SpIT and again this may partially explain the higher yield of positive CS.

The key findings of CF-SpIT were that sputum induction was positive for pathogens significantly more often than CS (38% compared to 14%) and that sputum induction was positive a similar number of times compared to two-lobe BAL (69% of pathogens identified by sputum induction compared to 72% on two-lobe BAL). The key recommendations of this research were that performance of sputum induction in symptomatic children is beneficial and could reduce the need for BAL. These finding are very important but do not address the low sensitivity of CS as a routine sampling method at every outpatient appointment. Sputum induction could not be performed on every non-expectorating child at every clinic visit as it is time consuming, with the procedure taking up to half an hour in the CF-SpIT. Therefore although sputum induction is a useful method for pathogen identification in symptomatic children, it does not negate the need for research into alternative methods for routine surveillance cultures such as CP.

6.2.1.2 CP versus CS in patients with CF; a pilot study (Maiya *et al.*)

Maiya *et al.* reported CS were positive 23% of the time and CP 52% of the time in their study on 31 children (146). These yields are both higher than those found in this service evaluation and there are several differences between the study populations which may account for this. The Maiya *et al.* study recruited older children aged 8 to 16 who were able to expectorate so

that the results could be compared to sputum. Older children are more likely to have chronic infections and reduced lung function and as such it is unsurprising that a higher yield of pathogens was identified.

CS were also only inoculated onto 4 plates in this study. This does not explain why the yield is higher than in our service evaluation but may partially explain why there was such a marked difference between CS and CP yield.

In spite of the differences in methodology and study population, the key findings of the Maiya *et al.* study do mirror the findings in this service evaluation as CP identified more pathogens than CS.

6.2.1.3 The efficacy of CP in the identification of bacterial pathogens in children with CF (Byrne *et al.*)

The study into the efficacy of CP by Byrne *et al.* is the study most discordant with our findings. Byrne *et al.* reported CS to have an 18.2% positivity and CP to have an 8% positivity with concordance in just 5.5% of samples (147). There are some differences in the study population and methodology which may account for this.

Firstly only 3 CP were used in this study, which could partially explain the lower yield of positive CP. However, CS were also only inoculated onto 3 agar plates, so this does not fully explain the higher yield of CS.

Children over the age of 3 were recruited for the Byrne study, and so the study population age is most similar to this service evaluation. Only non-expectorating children were included in the Byrne study and it is not clear whether this means the child was unable to expectorate at that appointment or was never productive of sputum. In this service evaluation, whether a child was able to expectorate was judged at every appointment so children who were sometimes able to expectorate were included. This may partially explain some of the observed differences

between this service evaluation and Byrne *et al.'s* study, but it is unclear why the difference in results is so marked.

6.2.2 Number needed to sample

Current clinical practice in the majority of CF centres is for non-expectorating children to perform CS alone at every outpatient appointment. By taking paired CS and CP samples, the NNS was 13, meaning that for every 13 children to have paired CS and CP samples taken, one additional pathogen is identified compared to CS alone. This further suggests that by taking duplicate samples identification of pathogens may be improved.

6.2.3 McNemar's Test

While difficult to ascertain, the difference between CS and CP yield is statistically significant. The McNemar test has been used in previous studies to compare CS and CP performance (146,147) but poses problems in this study due to the inclusion of repeated paired CS and CP samples from each patient. When McNemar's test was performed on all samples, it suggested there was a statistically significant difference between CS and CP ($p=0.028$). However, when one sample was taken from each patient, from the first paired sample provided during the study period to the tenth paired sample provided, none of the results demonstrated a statistically significant difference. This illustrates that an unadjusted McNemar's test may not be a reliable method for determining whether there is a statistically significant difference in CS and CP positivity when there are multiple paired measures from each patient and is the major reason why McNemar's test is not the optimal statistical test for analysis of these data.

Similar findings were seen in a study undertaken by Byrne *et al*. (2013) where patients had paired CS and CP samples taken at four different clinic visits. For three out of the four clinic visits there was no statistically significant difference between CS and CP (147).

The McNemar test provides an additional challenge as the different organisms identified by CS and CP cannot easily be incorporated into the analysis. For example, a CS and CP sample from

the same appointment could both be positive for different organisms. The McNemar test dictates that this result would be considered the same as a CS and CP that were both positive for the same organism as the test only compares the binary variable of whether the CS or CP are each positive or negative. This may be a theoretical criticism, as only two of the 663 paired samples in this study identified different pathogens on CS and CP in the same appointment, and therefore were unlikely to significantly alter results. However, in one of these discordant cases, CP identified *P. aeruginosa* whereas CS identified *H. influenzae.* Although both clinically important, *P. aeruginosa* is the most important pathogen to identify and so counting these samples as the same does risk missing important information about the types of pathogen identified by CS and CP.

Multiple pathogens growing from one CS or CP sample are also overlooked in this test. This makes interpretation more challenging. The McNemar test should therefore be interpreted cautiously both in this study but also in previous studies comparing CS and CP.

6.2.4 Generalised estimating equation

In addition to performing McNemar's test, a generalised estimating equation was performed as an alternative method to establish whether using CS or CP was predictive of positive pathogen results. This was done as the generalised estimating equation accounts for repeated measures and can also adjust for additional variables: age, gender, BMI centile and percentage FEV_1.

The generalised estimating equation demonstrated that CP was a statistically significant predictor of positive results (p=0.020). For the purposes of the generalising estimating equation CP was defined as 1 and CS was defined as 0. The positive coefficient (0.046) demonstrates therefore that CP were predictive of pathogen positivity.

Percentage FEV_1 was also statistically significant (p=0.005) and had a negative coefficient (-0.003). This demonstrates that the children in this study were more likely to have a positive result on CS or CP if they have reduced lung function.

The coefficient for both percentage FEV_1 and CS or CP was low and therefore, although statistically significant, the clinical significance of these results remains unknown. Further exploration with statistician support will be required to investigate the predictive capacity of CS and CP in identifying pathogens.

6.2.5 Sampling technique – is it fair to compare CS and CP?

Another factor to consider when comparing CS and CP yield is the technique required to perform CS and CP. CS involve coughing once, occasionally twice, onto a swab held in the pharynx. CP involve the child coughing forcefully onto a plate held 5-6cm from their mouth. At SaTH, four CP are collected from each child. The observed difference between CS and CP may be the result of CS only requiring one cough and CP requiring eight coughs. By coughing more times, there is an increased likelihood of producing an adequate respiratory sample to identify pathogens from.

It could be argued that the comparison between CS and CP is an unfair one to make as CP involve more coughs and therefore it is unsurprising more pathogens will be detected. Conversely, CS have the advantage of being cultured on to more agar plates and so perhaps it is unsurprising that in this study they identified a greater variety of pathogens. Ultimately if one method was intrinsically better than the other, it is unlikely that there would be the discordance of results seen in previous studies (146,147).

To make the comparison between CS and CP fairer, one suggestion would be to ask children to cough onto four CS as well as four CP. However, this is unlikely to be acceptable for many children and more time consuming for staff. Although, this method may be an option for future research, it is highly unlikely that CF centres would be willing to adopt this for routine clinical practice.

Another potential method for making the comparison between CS and CP fairer would be to have multiple types of agar on one plate. Research would need to be conducted to determine

whether multiple types of agar are still as effective at identifying pathogens, but this could potentially reduce the number of coughs required from children both for research purposes and in clinical practice.

6.3 Predictors of CS and CP pathogen identification

Lung function, indicated in this case by percentage FEV_1, was the only significant predictor of both CS and CP positivity in multivariable analysis and analysis with the generalised estimating equation. As both multivariable logistic regression and the generalised estimating equation showed similar results, this increases the confidence that the relationship between lung function and positive CS or CP is a relevant finding.

In multivariable regression, for both CS and CP the OR was less than 1, meaning an increased FEV_1 is associated with a decreased likelihood in CS or CP identifying a pathogen. For CS the OR was 0.969 (95% CI 0.944, 0.994), for CP the OR was 0.964 (95% CI 0.941, 0.987), demonstrating that for both CS and CP and increase in FEV_1 of 1% was associated with a 3-4% reduced likelihood of a positive CS or CP. This is perhaps an unsurprising finding as it is well established that respiratory infections are associated with reduced lung function even in infancy (84,114,173). Ultimately, children with poor lung function in CF are more likely to have an infection and therefore more likely to have a positive CS or CP.

BMI centile was also a significant predictor of CP positivity (p=0.031). Surprisingly the odds ratio for this was 1.015 (95% CI 1.001, 1.030) suggesting an increase in BMI by 1% is associated with 1.015 times greater odds of the CP being positive. This is surprising as it is well documented that reduced BMI is associated with poor respiratory outcomes (174,175). One potential explanation is that increased BMI may be associated with increased cough strength. Further research would be required to assess the relationship between BMI centile and CP and the relationship between BMI centile and cough strength. Why this relationship was observed in CP and not CS is unclear.

It is important to note that as with the McNemar test, there are issues surrounding repeated measures from the same patient with regards to the multivariable logistic regression. However, as similar findings were shown in the generalised estimating equation, which does account for repeated measures, this increases the confidence that there is a statistically significant relationship between percentage FEV_1 and positive CS or CP. The relationship between BMI centile and positive CP is less clear as BMI centile was not associated with a positive result when using the generalised estimating equation. It is also possible this may be a chance finding.

The only other study to consider predictors of CS and CP positivity is Byrne *et al*. (2013) (147). Due to the low number of positive CP identified in this study, statistical analysis was not performed on predictive variables. However, Byrne describes a trend of older patients isolating more pathogens on CP and postulates this may be the result of increased cough strength. Given the findings in this study, the increased positivity of CP with age may be a consequence of declining lung function as patients age increases. Cough strength was not explored in this study and could still be an important variable in predicting whether pathogens are identified on CS or CP.

Another variable not explored in this study or past studies is the technique required in performing CP. CS can be performed on all children regardless of age, as even if the child is unable to cough on demand, insertion of the swab into the pharynx often triggers a cough. CP are reliant on children being capable of generating a strong cough on command. Byrne's observation of older children isolating more pathogens on CP, may be the result of them being able to produce adequate CP samples which may be more challenging in younger children.

6.4 Types of pathogen identified on CS and CP.

Non-pathogenic organisms were identified in greater numbers using CS. This finding is similar to that of Maiya *et al*. (2004), who also demonstrated that CS identified more non-pathogenic organisms compared to other respiratory sampling methods (146). One potential explanation

for this trend is that CS are more likely to be contaminated by pathogens as they may touch the oropharyngeal tissues when they are performed when compared to CP.

The common and important organisms seen in patients with CF were identified more frequently by CP. Namely: *P. aeruginosa*, *S. aureus* and *H. influenzae*. A similar pattern was seen by Maiya *et al*. where CP identified more cases of *P. aeruginosa* than any other sampling method used. However, these findings have not been replicated in other studies and are discordant with findings from Byrne *et al*. (2013), Chavasse *et al*. (2007) and Orska *et al.* (2006) (147–149).

Although CP identified a greater number of pathogens, a greater variety of pathogens were identified on CS. This may be because CS can be inoculated on to a wide variety of media whereas CP are limited to the number of plates the child has coughed on. At SaTH it is standard practice for CP to be performed on 4 plates: blood agar, chocolate agar, salt agar and *cepacia* specific agar, whereas CS can be plated onto 6 plates.

It is also possible that some of the additional organisms identified on CS may be commensal organisms. Although there is evidence that some of the less frequently isolated organisms can be pathogenic, there is also evidence they may be commensal. When taking CS samples, there is a greater risk they may be contaminated as the swab is held in the pharynx. Although CS should not touch the oropharyngeal mucosa (176), the risk of this occurring is much greater than with CP as they are held approximately 5 centimetres from the child's mouth.

6.5 Identification of *P. aeruginosa* in children under the age of 7

P. aeruginosa is arguably the most clinically significant pathogen in children with CF but early identification and treatment has the potential to reduce some of the adverse outcomes associated with *P.aeruginosa* infection. In this cohort of patients only 4 children under the age of 7 isolated *P.aeruginosa.* 2 of these cases were identified on CP, one on CS and one on both. It is possible that *P.aeruginosa* may have been isolated in these children before the study period

began, before they were old enough to perform both CS and CP or by other methods during the study period such as induced sputum or BAL.

No strong conclusions about the benefit of the addition of CP for the early identification of *P. aeruginosa* can be drawn from just 4 patients. However, without CP 2 of these 4 cases could have been missed, this suggests that CP may aid with the early diagnosis of *P. aeruginosa*. CP also grew a greater number of *P. aeruginosa* isolates in the whole study population cohort compared to CS, CP grew *P. aeruginosa* 32 times, CS 24 times. If these findings could be replicated in a larger population of patients, this could have important ramifications for clinical practice as the early identification of *P. aeruginosa* is very important for children with CF.

6.6 Study Strengths and Limitations

Although this study addresses the aims and objectives set, it is not without limitations. Firstly, due to the retrospective nature of the study design it data were missing from records for some variables. Also, as a retrospective study there are limitations as to what variables we could explore in relation to CS and CP positivity such as cough strength. Important additional variables that would be useful to explore in future studies include: presence of bronchiectasis, whether the child was symptomatic or asymptomatic at the time the sample was taken, the genetics of the child and the antibiotic sensitivities of pathogens.

Secondly, although this study has a large number of paired samples, the samples come from a relatively small population of 38 patients. Therefore, the findings of this service evaluation may not be generalisable to all children with CF.

Another limitation of this study is that when comparing pathogen growth only a binary positive or negative growth was used. When microbiological results are reported they also describe the amount of pathogen grown: scanty, moderate or heavy growth. This detail was not included in our analysis and therefore details on the amount of growth when comparing CS and CP may have been missed.

Finally, these samples were only collected from an outpatient setting as inpatients with CF at SaTH do not routinely have CP performed as ward staff are not trained to take both CS and CP. Therefore, the results of this study may not be applicable to acutely unwell children who require admission. However, as discusses in chapter 2, induced sputum may be the optimal method for identifying pathogens in symptomatic non-expectorating children with CF (143).

However, this study does have strengths when comparing it to other studies that have explored the use of CS and CP. Firstly it has the largest number of paired CS and CP samples explored in any of the past studies.

Secondly this is the first study to explore with statistical analysis whether age, gender, BMI centile and percentage FEV_1 predict a positive CS or CP result and therefore provides novel insight into these variables.

6.6 Recommendations for further research

This service evaluation demonstrates that by taking multiple samples from each patient at every clinic visit, the yield of pathogens identified is increased. However, it is not currently clear if the increased identification is due to having taken both CS and CP samples or simply the result of having multiple samples. Therefore, further research is required to discern whether taking multiple CS or CP would also increase the identification of pathogens. Additionally, another avenue that could be researched is whether increasing the frequency of taking respiratory samples increases the identification of pathogens.

Cost benefit analysis of performing both CS and CP samples is another potential area of future research. At SaTH CS alone cost £1.62, whereas performing both CS and CP costs £2.86. Although analysing the cost of additional CP samples is straightforward, determining whether any savings are made, such as through reduced need for induced sputum or BAL would be more challenging.

Finally, this study suggests that CP are statistically significantly more likely to identify pathogens. Due to the small number of participants in this study, it is important to determine whether these findings are generalisable to all children with CF. Therefore, comparison of CS and CP in a significantly larger study population is recommended.

6.6.1 Future study proposal

This service evaluation identifies several options for further research. However, the most important findings from this service evaluation are that by performing both CS and CP a greater yield of pathogens are identified, and that the addition of CP may be beneficial in the early identification of *P. aeruginosa*. Demonstrating these findings in a larger study population to ensure generalisability is the most pressing need for future research.

Therefore, my ideal future study would be a study into the benefits of performing both CS and CP in a larger patient population. Ideally it would involve a larger CF centre or several CF centres from different geographical locations. Non-expectorating children old enough to perform CP would be invited to participate and CS and CP would be performed on these children at every clinic for a year. Although the comparison of CS and CP may not in itself be a fair comparison (as detailed in section 6.2.5) unless advances could be made in using multiple agars on one plate, it would be impractical and unfair to expect children to perform 4 CS and 4 CP, therefore I would use the same methodology for collection of CS and CP samples as outlined in this study.

At the time of taking samples, data on the child's lung function, age and BMI would be collected. It would also be important to have more background data on the child than was collected for this service evaluation including: whether the child was symptomatic or asymptomatic at that appointment, their genetics, whether they have any evidence of bronchiectasis and whether they have grown P. aeruginosa in the past or from BAL or induced sputum during the study period. This additional information would enable further analysis as to whether any of these variables predict CS or CP positivity. Importantly the information regarding *P. aeruginosa* would

enable stronger conclusions to be made about the benefits of CP in the identification of initial *P. aeruginosa* infection.

Collecting samples over a year would provide challenges statistically due to repeated measures. However, the longitudinal data enables further analysis that would not be possible if only one sample was taken from each patient. For example, you could follow children up who grew *P. aeruginosa* by any of the sampling methods to see if they grew it again during the study period and whether CS or CP identified it.

Instead of the focus of this study being a comparison between CS and CP, the aims of this study would be to identify whether by performing both CS and CP, more cases of *P.aeruginosa* are identified and whether the yield of respiratory pathogens as a whole increases compared to the current practice of CS alone. Additionally, it would be important to ask children, their families and staff whether they would be happy to perform CS and CP routinely to ensure that this research has the potential to be applied clinically.

There would be numerous barriers to conducting a study as outlined above. Cost implications would be important to consider, as although CP are relatively cheap, funding would be required to cover these costs. This study would also potentially require collaboration between not only CF centres but also microbiology departments and all departments would need to have access to the same agar plates which can vary between hospitals. Ethical approval would also be needed.

However, in spite of some of the challenges, performing both CS and CP has the potential to identify more pathogens than CS alone and may allow for earlier detection of *P. aeruginosa* which causes airway damage, increased morbidity and earlier mortality in children with CF. Hence, ascertaining whether the addition of CP in clinics is beneficial should be a research priority.

6.7 Recommendations for clinical practice

This service evaluation has highlighted key points to consider in clinical practice:

- CS and CP are both valid methods for routine identification of respiratory infections in non-expectorating children with CF.
- Taking duplicate samples from non-expectorating children may increase yield of pathogens.

There is insufficient evidence from this service evaluation to recommend the use of CP instead of CS and vice versa. This argument is reinforced by inconclusive past literature comparing CS and CP (146–149). However, this study indicates that performing multiple samples increases pathogen detection rates and as such a recommendation from this study could be to take both CS and CP from non-expectorating patients. Further work on whether taking duplicates CS or CP can improve identification of pathogens is required before recommendation for changing current clinical practice can be made.

6.8 Conclusions

Respiratory complications are the leading cause of morbidity and mortality in individuals with CF and identification and treatment of respiratory infection is vital in managing CF related lung disease. Routine samples should be taken at every outpatient appointment to identify pathogens, as some children may be asymptomatic, and presentations of pulmonary exacerbations and chronic infections differ with every child. In most children and adults, sputum cultures are the optimal method for identifying respiratory infections. However, this is not possible in all patients, particularly in young children who may be unable or unwilling to expectorate.

Nearly all CF centres in the UK use CS as the method of choice for routine surveillance cultures in non-expectorating children. However, CP have been explored as an alternative. In this service

evaluation **the addition of CP increased the yield of pathogens from 10% when using just CS to 18% when using both CS and CP.**

A greater number of pathogens were grown from CP compared to CS, but CS identified more different types of probable pathogens. Although statistically superior, it is not possible to confidently determine whether CP are superior to CS in indentifying pathogens clinically. However, it is evident from this study is that duplicate CS and CP samples improve identification of pathogens. Subsequent research is required to establish whether taking multiple CS or CP samples or increased sampling frequency has similar improvements for pathogen identification.

The addition of CP also has the potential to identify more early cases of *P. aeruginosa.* Although the numbers of young children identified in this study were small, cases of *P. aeruginosa* would have been missed without the addition of CP. Due to the small population included in this study, further work is required to determine whether these results translate to a larger population of patients and are generalisable.

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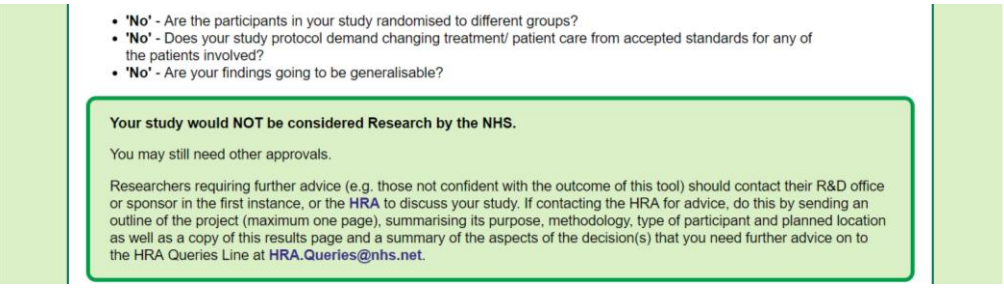
7.0 Appendix

Appendix A



Appendix B

Contingency tables used in the calculation of McNemar's test taking only one sample from each patient from the second to tenth sample provided from each patient.

Second sample

	Cough Plate	
Cough Swab	Negative	Positive
Negative	4	1
Positive	2	30

Chi-squared = 0.33

McNemar's significance probability = 1.000

Third sample

	Cough Plate	
Cough Swab	Negative	Positive
Negative	2	1
Positive	2	32

Chi-squared = 0.33

McNemar's significance probability = 1.000

Fourth sample

	Cough Plate	
Cough Swab	Negative	Positive
Negative	3	2
Positive	3	27

Chi-squared = 0.20

McNemar's significance probability = 1.000

Fifth sample

	Cough Plate	
Cough Swab	Negative	Positive
Negative	3	1
Positive	4	24

Chi-squared = 1.80

McNemar's significance probability = 0.375

Sixth sample

	Cough Plate	
Cough Swab	Negative	Positive
Negative	3	0
Positive	1	25

Chi-squared = 1.00

McNemar's significance probability = 1.000

Seventh sample

	Cough Plate	
Cough Swab	Negative	Positive
Negative	0	1
Positive	1	26

Chi-squared = 0.00

McNemar's significance probability = 1.000

Eighth sample

	Cough Plate	
Cough Swab	Negative	Positive
Negative	2	0
Positive	2	23

Chi-squared = 2.00

McNemar's significance probability = 1.000

Ninth sample

	Cough Plate	
Cough Swab	Negative	Positive
Negative	1	4
Positive	2	19

Chi-squared = 0.67

McNemar's significance probability = 0.688

Tenth sample

	Cough Plate	
Cough Swab	Negative	Positive
Negative	2	3
Positive	2	19

Chi-squared = 0.20

McNemar's significance probability = 1.000