PROTOCOL:

Title:

Prevalence and Patterns of Antimicrobial Resistance of Respiratory Pathogens isolated from Patients attending the Cystic Fibrosis Clinic at Charlotte Maxeke Johannesburg Academic Hospital, 2006-2010

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1 Introduction

Cystic Fibrosis is a genetic disorder which occurs due to mutations in the CF transmembrane conductance regulator gene (*cftr*). The gene has two key functions in chloride ion transport. The first is to mediate chloride ion conduction across the cell membrane. The second is to activate chloride ion channel activity (Dorwart et al 2004). Mutations in this gene result in reduced chloride secretion into the airways and increased absorption of sodium from the airways. This, in turn, results in a viscous airway mucous which adversely affects mucociliary clearance. The outcome is persistent mucin secretion and the formation of mucous plugs. The mucous plugs are a focus for persistent bacterial infections. (Boucher 2002)

Patients with cystic fibrosis may be infected with respiratory commensals or opportunistic pathogens. Among the organisms commonly isolated are *Haemophilus influenzae, Staphylococcus aureus, Pseudomonas aeruginosa, Burkholderia cepacia* complex, *Stenotrophomonas maltophilia, Alcaligenes xylosoxidans, Aspergillus fumigatus* and non-tuberculous mycobacteria. Historically, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were the organisms most prevalent in this population. Recently, some of the other pathogens mentioned have emerged globally. A number of reports across the globe have described the prevalence of each organism mentioned above. However, the prevalence differs from country to country and sometimes even from one centre to another (de Vrankrijker et al 2010). Thus management guidelines differ based on the findings of prevalence studies in each centre or country. A brief description of each of the aforementioned pathogens follows, with particular attention to prevalence, both overall and age-specific, changes in prevalence over time and antimicrobial resistance.

Haemophilus influenzae

Haemophilus influenzae is a pleomorphic gram negative bacillus usually implicated in respiratory infections, bacteraemia and meningitis in infants and young children. In cystic fibrosis, it is a common commensal of the respiratory tract of young children. The prevalence is at its peak in the 2- 5 year age group and decreases until adulthood (LiPuma 2010). It has been found that non-encapsulated *Haemophilus influenzae* is associated with chronic infections of the lung in cystic fibrosis patients. Exposure to multiple courses of antibiotics during the first few years of life leads to the development of multi-drug resistance in this pathogen (Cardines et al 2012).

Staphylococcus aureus

Staphylococcus aureus is a gram positive coccus implicated in a wide spectrum of infections including those of the skin and soft tissue, bone and joints, prosthetic devices, lung, bloodstream and central nervous system. In cystic fibrosis, *Staphylococcus aureus* is often the first pathogen recovered from respiratory secretions. Studies conducted in the USA through the Cystic Fibrosis Foundation have demonstrated the highest prevalence of *Staphylococcus aureus* in children between 6-10 years of age (LiPuma 2010). In more recent years, the problem of methicillin resistant *Staphylococcus aureus* (MRSA) has emerged as a worldwide problem, both in the healthcare setting, as well as in the community setting. The prevalence of MRSA in the cystic fibrosis population has mirrored this and is well described internationally (LiPuma 2010). Studies in the USA have noted an increased prevalence in children between 6 and 17 years of age (Razvi 2009). However, studies in the Netherlands have shown low rates of MRSA in their cystic fibrosis population as a result of strict MRSA infection control policies (de Vrankrijker et al 2010).

Pseudomonas aeruginosa

Pseudomonas aeruginosa is a non-fermentative gram negative bacillus which is ubiquitous in the environment. It is regarded as the most important pathogen in cystic fibrosis. Colonisation is initially intermittent, but gradually becomes persistent and chronic (Li et al 2005). With the establishment of chronic infection, the phenotype of the organism converts to a mucoid variety. This mucoid phenotype confers resistance to multiple antibiotics, making eradication difficult (Vrankrijker et al 2010). The prevalence of the organism increases with age, although the initial infection usually occurs soon after birth. Recent data from international studies have demonstrated conflicting results regarding changes in the prevalence of *Pseudomonas aeruginosa.* Some areas have described decreases in prevalence, whereas others have noted no change in prevalence (Razvi et al 2009; Millar et al 2009).

Burkholderia cepacia complex

This gram negative bacillus is also ubiquitous in the environment. The complex consists of 17 closely related species. It emerged as a pathogen in the 1980's and is today a well recognized pathogen in cystic fibrosis patients. The clinical course is variable and ranges from asymptomatic; to prolonged progressive deterioration; to rapid fulminant and often fatal deterioration. As with the aforementioned pathogens, the prevalence of *Burkholderia cepacia* differs from country to country, with the USA reporting a decrease in

prevalence during the late 1990's, the UK reporting an increase during the 1990's but a decline during the early 2000's and very low and consistent rates described in the Netherlands (Razvi et al 2009; Millar et al 2008; de Vrankrijker et al 2010). Once again, age specific prevalence has been noted, with higher prevalence in older patients (LiPuma 2010).

Stenotrophomonas maltophilia

Stenotrophomonas maltophilia is a gram negative opportunistic pathogen with intrinsic resistance to commonly used broad-spectrum antimicrobials, including carbapenems. It is a well known cause of a variety of opportunistic infections in immune-compromised patients, including pneumonia, bacteraemia, urinary tract infections, endocarditis, eye infections, bone and soft tissue infections and peritonitis. Recent reports indicate that infections due to this pathogen have increased. As with the increase in MRSA, the prevalence of *Stenotrophomonas maltophilia* has also increased among cystic fibrosis patients in recent years (Ballestero et al 1995; Talmaciu et al 2000). The recent emergence of this pathogen has been associated with the use of anti-pseudomonal antibiotics in cystic fibrosis (de Vrankrijker et al 2010). In particular, the use of quinolones has been found to be a risk factor for infection with *Stenotrophomonas maltophilia* in the cystic fibrosis population (Talmaciu et al. 2000). Unlike the persistent infection with *Pseudomonas aeruginosa* and *Burkholderia cepacia*, colonization with *Stenotrophomonas maltophilia* is usually intermittent (Demko et al 1998). Clinical development of resistance to the few antibiotics active against this pathogen has not been documented, and an in vitro study which examined a series of pre and post-treatment isolates of *Stenotrophomonas maltophilia* from cystic fibrosis patients demonstrated no increase in resistance development (San Gabriel et al 2004).

Alcaligenes xylosoxidans

This gram negative bacillus is similar to *Stenotrophomonas maltophilia* in that it is an opportunistic pathogen which causes nosocomial infections. In cystic fibrosis patients, the true prevalence is difficult to determine, since studies have shown broadly differing rates of infection (LiPuma 2010). *Alcaligenes xylosoxidans* is often a transient, although chronic infection may occur (Moissenet D A et al 1997).

Aspergillus fumigatus

Aspergillus fumigatus is the most common filamentous fungus involved in Cystic Fibrosis. Infection with this mould results in a chronic allergic inflammatory response known as allergic bronchopulmonary aspergillosis (LiPuma 2010). Patients usually present with a worsening of lung function. Many global cystic fibrosis centres have reported the prevalence of *Aspergillus fumigatus* and the range reported has increased over the last 2 decades, possibly as a result of improvements in culture methods and frequency of culturing. (Bakare N et al 2003; Valenza G et al 2008). Studies which have looked at prevalence by age stratification have noted increased prevalence with increased age (LiPuma 2010).

Non-tuberculous Mycobacteria

These mycobacteria are widely distributed in the environment. The most common species are *Mycobacterium avium* and *Mycobacterium abscessus complex* (de Vrankrijker et al 2010). These organisms were first isolated among cystic fibrosis patients in the 1970's (LiPuma 2010). The reported prevalence in cystic fibrosis patients ranges from <5% to almost 30% (de Vrankrijker et al 2010; LiPuma 2010). Age specific prevalence studies have reported lower rates in children less than 15 years of age (LiPuma 2010). A recent study reported that *Mycobacterium avium* and *Mycobacterium abscessus* complex target different cystic fibrosis patient subgroups. *Mycobacterium avium* was found in older patients as opposed to *Mycobacterium abscessus* complex, and *Mycobacterium avium* infected patients were usually diagnosed later in life. Patients infected with *Mycobacterium abscessus* complex received intravenous antibiotics prior to isolation of the pathogen and were often co-colonised with Aspergillus spp. (Catherinot et al 2012).

Much information is available globally regarding pathogens which chronically or intermittently infect patients with cystic fibrosis. However, no such data seems to be available from South Africa. Therefore, the aim of this study is to report on the range of respiratory pathogens infecting the cystic fibrosis population, to describe race and age-specific trends as well as to document prevalence and changes in antimicrobial resistance amongst these pathogens. This information may assist with development of empiric treatment policies in the unit. It may also better guide infection control policies in the unit to prevent transmission of multi-drug resistant pathogens among this population of immune-compromised patients.

2 Significance of the Study

No data regarding the pathogens infecting the cystic fibrosis population is currently available in South Africa. Knowledge of infecting pathogens and antimicrobial resistance among these pathogens will likely assist with development of future management guidelines as well as better direct infection prevention and control efforts against these pathogens.

3 Definitions

- Colonisation: 1 culture positive for an organism in a patient who has had >1 specimen submitted to the NHLS laboratory
- Chronic colonization: ≥ 2 cultures positive for the same organism during a 6 month period
- Intermittent colonization: > 1 culture positive for the same organism which does not meet the criteria for chronic colonization
- Age Category: The patient's age on 31 December of each year will determine which age category he/she will fall into for analysis of age- specific prevalence
- National Health Laboratory Service (NHLS): As South Africa's largest diagnostic pathology service the NHLS is responsible for providing laboratory services to > 80% of South Africa's population through a national system of laboratories.
- Disa System: The NHLS laboratory information system (LIS) onto which all patient results are loaded.

4 Study Aim and Objectives

4.1 Aim

The aim of this study is to describe the respiratory microbiology of patients attending the CMJAH cystic fibrosis clinic.

4.2 Objectives

4.2.1 Primary Objectives

- To describe the prevalence of the above- mentioned organisms among the cystic fibrosis population at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) during the study period.

- To describe the prevalence of antimicrobial resistance among the above-mentioned organisms during the study period.
- To determine whether there has been any significant change in prevalence to the above-mentioned organisms over the course of the study period.
- To determine whether there has been any significant change in prevalence of resistance to the above- mentioned organisms over the course of the study period

4.2.2 Secondary Objectives

- To describe the age-specific prevalence of the above-mentioned organisms among the cystic fibrosis population at Charlotte Maxeke Johannesburg Academic Hospital during the study period.
- To describe the race-specific prevalence of the above-mentioned organisms among the cystic fibrosis population at Charlotte Maxeke Johannesburg Academic Hospital during the study period. If the number of non-caucasian patients is suboptimal for statistical analysis, this objective may not be fulfilled.
- To describe the age-specific prevalence of antimicrobial resistance among the above-mentioned organisms during the study period.
- To describe the race-specific prevalence of antimicrobial resistance among the above-mentioned organisms during the study period. If the number of non-caucasian patients is suboptimal for statistical analysis, this objective may not be fulfilled.

4.2.3 Tertiary Objectives

- To describe co-infection with more than one pathogen.
- To report on the number of cultures with no growth of micro-organisms and growth only of normal commensals.
- to describe the pattern of colonization of each micro-organism; whether intermittent or chronic (refer to definitions)

5 Methods

5.1 Study Site

The study will be conducted at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH).

5.2 Study Population

The study will be conducted at the cystic fibrosis clinics at the Charlotte Maxeke Hospital.

5.3 Study Sample

The study sample will be the total number of patients from the cystic fibrosis clinics at CMJAH who meet the inclusion criteria during the period under study.

5.4 Sample Size

As this study is a retrospective review, a sample size cannot be calculated, however, an estimate of 100 patient files will be reviewed. If the number of files investigated is suboptimal for statistical analysis, the study period may be extended include file reviews of patients attending the adult cystic fibrosis clinic at CMJAH. The study may also be extended to include periods before 2006 and/or after 2010.

5.5 Inclusion Criteria

The following inclusion criteria will be used:

- Every clinic attendee during the period 1 January 2006 to 31 December 2010, who fulfils any one of the following requirements:
    - At least one respiratory specimen submitted to the NHLS laboratory for bacterial microscopy, culture and sensitivity testing.
    - At least one respiratory specimen submitted to the NHLS laboratory for fungal microscopy, culture and sensitivity testing.
    - At least one specimen submitted to the NHLS laboratory for mycobacterial microscopy, culture and sensitivity testing.

5.6 Exclusion criteria

- Any patient attended to at the cystic fibrosis clinics at Charlotte Maxeke Hospital during the period 1 January 2006 to 31 December 2010 with no respiratory specimens submitted to the NHLS laboratory for bacterial, fungal or mycobacterial microscopy, culture and sensitivity testing.
- Any patient whose laboratory results cannot be traced.
- Any patient whose clinical details cannot be traced.

5.7 Study Design

The study design is a cross-sectional, descriptive, retrospective review of respiratory pathogens from patients attending the cystic fibrosis clinic. The study will describe the prevalence of specific pathogens and the prevalence of resistance to antimicrobials among these pathogens.

5.8 Data Collection

Data is to be collected on a data collection sheet. The data to be collected can be divided into patient specific and pathogen-specific information.

Patient- specific details will be obtained from patient files at CMJAH cystic fibrosis clinic.

Pathogen- specific details include micro-organism identity and antimicrobial resistance. Every available laboratory result of respiratory samples from these patients submitted to the NHLS laboratory for bacterial, fungal and mycobacterial microscopy, culture and sensitivity testing during the period 1 January 2006- 31 December 2010 will be included in the study. These results will be obtained from patient files as well as from the NHLS Disa system.

For details of data to be collected, please refer to Appendix 1

6 Data Capture

Microsoft Excel spreadsheets will be used to capture the data collected

For details of data to be captured on Excel, please refer to Appendix 2

7 Statistical Analysis

Prevalence of organisms and prevalence of antibiotic resistance will be determined using the equations in Appendix 3.

Changes in prevalence and trend analyses will be calculated in consultation with a biostatistician. In addition, it is likely that further statistical analysis techniques may provide further insight. These will be evaluated and proposed in conjunction with a biostatistician.

8 Ethical Considerations

An Ethics Committee waiver will be obtained from the University of the Witwatersrand Ethics Committee. Permission to conduct the study will be obtained from the CMJAH hospital CEO and permission to access patient files will be obtained from the head of the CMJAH pediatric cystic fibrosis clinic (Dr. S. Klugman) as well as the head of the adult cystic fibrosis clinic (Prof. M. Mer).

The study will be conducted according to the World Medical Association's Declaration of Helsinki- Ethical Principles for Medical Research Involving Human Subjects.

9 Study Timeline

Protocol submission and Assessment: August- September 2012
Ethics Review: September 2012
Data Collection: October – December 2012
Data capture and statistical analysis: January – March 2012
Writing of report and writing of publication: April – June 2012

10 Funding

The costs involved in conducting this study total R1200.00 and will be funded by the Department of Clinical Microbiology and Infectious Diseases. The costs include:
Photocopying: R300.00
Transport: R700.00
Stationery: R200.00

No additional costs will be incurred by the University of the Witwatersrand or the Charlotte Maxeke Johannesburg Academic Hospital

11 Potential Limitations

- The study is contextual to the population being investigated. It may not be possible to generalize the findings of this study to other regions in South Africa. However, the cystic fibrosis clinics at

CMJAH are a large drainage site for the greater Gauteng region, and the findings of this study will provide important information regarding pathogens infecting this population of patients.

- The sample size is expected to be small and this may affect statistical analysis.
- The scope of the study is limited by time constraints.

12 Anticipated Problems

- There may be inadequate or incomplete data in patient files.
- Patient files may be misplaced in the filing system.
- Illegibility of clinician's handwriting may compromise the quality of data collected.
- Patient results may be inaccessible or misplaced.
- There may be inadequate numbers of non-causcasian patients for the race sub-group analyses

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14 Appendix

Appendix 1

<table>
<tr><td rowspan="3">Patient Details</td><td>Name</td><td colspan="2"></td></tr>
<tr><td>Age</td><td colspan="2"><u>Age Categories</u>:
1yr
5yrs
5- 10yrs
10- 15yrs
15- 20yrs
> 20 yrs</td></tr>
<tr><td>Race</td><td colspan="2">White
Black
Indian
Coloured
Other</td></tr>
<tr><td rowspan="4">Specimen Type</td><td colspan="3">Sputum</td></tr>
<tr><td colspan="3">Cough Swab</td></tr>
<tr><td colspan="3">Throat swab</td></tr>
<tr><td colspan="3">Broncho-alveolar lavage</td></tr>
<tr><td rowspan="20">Organism cultured and Antibiotics to be assessed for Resistance</td><td rowspan="3">Haemophilus influenzae</td><td>HI-b</td><td>Ampicillin</td></tr>
<tr><td>Non-type</td><td>Cefotaxime</td></tr>
<tr><td></td><td>Meropenem</td></tr>
<tr><td colspan="2">Staphylococcus aureus</td><td>Methicillin</td></tr>
<tr><td colspan="2" rowspan="10">Pseudomonas aeruginosa
• Non-mucoid
• Mucoid</td><td>Piperacillin- Tazobactam</td></tr>
<tr><td>Ceftazidime</td></tr>
<tr><td>Cefepime</td></tr>
<tr><td>Meropenem</td></tr>
<tr><td>Imipenem</td></tr>
<tr><td>Gentamicin</td></tr>
<tr><td>Amikacin</td></tr>
<tr><td>Tobramycin</td></tr>
<tr><td>Ciprofloxacin</td></tr>
<tr><td>Colistin</td></tr>
<tr><td colspan="2">Burkholderia cepacia complex</td><td>Trimethoprim-sulfamethoxazole</td></tr>
<tr><td colspan="2">Stenotrophomonas maltophilia</td><td>Trimethoprim-sulfamethoxazole</td></tr>
<tr><td colspan="2">Alcaligenes xylosoxidans</td><td></td></tr>
<tr><td colspan="2">Aspergillus fumigatus</td><td></td></tr>
<tr><td colspan="2">Non-tuberculous mycobacteria</td><td></td></tr>
</table>

Appendix 2

Study Number	Age category	Race	Organism cultured in 2006											
			HI	SA	PA	BCC	SM	AX	AF	NTM				Other
										MAC	MASC	MK	Other	

			Year:																																
Study Number	Age	Race	Lab Number	HI						SA		PA																		BCC			SM		
				AMP		CTX		MERO		MSSA	MRSA	CTZ		CPM		MERO		IMI		GENT		AMIK		TOB		CIPRO		COL		COTRI					
				S	R	S	R	S	R			S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	I	R	S	I	R

Appendix 3

Prevalence Calculations:

Prevalence of organism X =

Number of patients with 1st culture positive for organism X X 100

Total number of patients attending the clinic in that year

Prevalence of organism X in specific age category =

Number of patients in age category Z with first culture positive for organism X X100

Total number of patients in age category Z attending the clinic in that year

Prevalence of organism X in a specific race group =

Number of patients in Race Group A with first culture positive for organism X X100

Total number of patients in Race Group A attending the clinic in that year

Prevalence of Resistance of antimicrobial Y to organism X =

Total number of organism X isolates resistant to antimicrobial Y X100

Total number of organism X isolates cultured in that year

Prevalence of Resistance in age category Z of antimicrobial Y to organism X =

Total number of organism X isolates from age category Z which are resistant to antimicrobial Y X100

Total number of organism X isolates cultured from age category Z in that year

Prevalence of Resistance in Race Group A of antimicrobial Y to organism X =

Total number of organism X isolates in Race Group A which are resistant to antimicrobial Y X100

Total number of organism X isolates cultured from Race group A in that year