BACTERIA ISOLATED FROM THE AIRWAYS OF PAEDIATRIC PATIENTS WITH BRONCHIECTASIS ACCORDING TO HIV STATUS

Charl Verwey

Student Number: 0517054E

A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Masters of Medicine in the branch of Paediatrics

Johannesburg 2015

DECLARATION

I, Charl Verwey, declare that this research report is my own work. It is being submitted for the degree of Masters of Medicine in the branch of Paediatrics in the University of the Witwatersrand, Johannesburg. It has not been submitted for any degree or examination at this or any other university.

Signature: _____

_____ day of _____2015

Dedicated to Sanushka Naidoo, Akhil Carter Verwey and Kiran Luke Verwey

ABSTRACT

Background: It is routine to take airway samples from patients with bronchiectasis to determine the bacteria that colonize their airways. This guides the choice of antimicrobials to use when they have chest infection. It is not known whether there is a difference between the number, type and density of bacteria found in the airways of human immunodeficiency virus (HIV) positive and negative patients with bronchiectasis. **Objective:** To determine the bacteria isolated from the airways of children with bronchiectasis according to their HIV status. **Methods:** Records of children under 16 years of age with the diagnosis of bronchiectasis who had been seen by division of paediatric pulmonology at CHBAH between April 2011 and March 2013 were reviewed. Data were collected on the patient demographics, HIV status and characteristics of the airway samples collected from all the patients. Data collected on the airway samples included number of samples collected per patient, type and quality of the samples collected, number and type of organisms cultured and density of individual organisms cultured. Comparisons between HIV negative and positive patients were made. **Results:** A total of 78 patients with bronchiectasis were seen by the division of pulmonology over this 2 year period. Their mean age was 9.7±3.3 years. The majority of patients (79.5%) were HIV positive. 175 samples were collected and of these 85.7% were expectorated sputum. Gram negative bacteria (71.6%) were more common than gram positive bacteria (28.4%). *H. influenzae* was the most common bacteria identified (36.0%) followed by *S. pneumoniae* (13.1%), S. *aureus* (11.3%) and *M. catarrhalis* (10.8%). When comparing HIV positive and negative patients with bronchiectasis there was no difference in the number, type and density of bacteria isolated. **Conclusion:** Number, type and density of bacteria isolated in the airways of children with bronchiectasis is not associated with the HIV status of the patient.

ACKNOWLEDGEMENTS

Professor Sithembiso Velaphi for being there as supervisor

Dr Sanushka Naidoo for proof reading the completed manuscript

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

NOMENCLATURE

CF	-	Cystic fibrosis
HIV	-	Human Immunodeficiency virus
NHLS	-	National Health Laboratory Service
CHBAH	-	Chris Hani Baragwanath Academic Hospital
CXR	-	Chest X-Ray
CT	-	Computed Tomography scan
ARVs	-	Anti-retroviral drugs
BAL	-	Broncho-alveolar lavage
CFU	-	Colony Forming Unit

CHAPTER 1

1.0 INTRODUCTION

1.1 Definition and Presentation of Bronchiectasis

Bronchiectasis is a chronic suppurative pulmonary disorder that can generally be defined as an irreversible dilatation of the central and peripheral airways. It is characterized clinically by a chronic cough of more than four weeks duration with purulent sputum production, coarse crepitations on chest auscultation and features of chronic suppurative or obstructive lung disease[1-9] such as digital clubbing, lung hyperinflation, increased antero-posterior diameter of the chest and a Harrison's sulcus. Patients with bronchiectasis often present with recurrent chest infections. There may also be non-specific features such as exertional dyspnoea and growth failure. Radiologically it can be defined as a reversal in the ratio between the diameter of the lumen of a bronchus and the diameter of the adjacent pulmonary artery on computed tomography scan (signet ring sign). Usually this ratio is < 1 but in bronchiectasis it is > 1.[10] There is also a loss of tapering of bronchi as they extend to the periphery of the lung and areas of hyperlucency suggestive of small airway obstruction with air-trapping. Pathologically it is defined as a permanent dilatation of the large and medium sized airways and a reduction of the generations of subdivisions of one or more bronchi due to destruction of the structural elements within the bronchial wal1[1, 11] from repeated episodes of lower airway infection and the accompanying inflammation.[12]

It is associated with periodic infectious exacerbations which are defined as an increase in the severity and the wet character of the cough.[13, 14] There is chronic bacterial colonization of the bronchi and an increase in inflammatory cells, T lymphocytes and neutrophils, and mediators, interleukin-8 and tumour necrosis factor alpha, in the bronchial mucosa and

bronchial walls.[15, 16] This leads to a vicious cycle of recurrent chest infections and chronic inflammatory changes leading to the eventual destruction of the structural elements within the bronchial wall.

1.2 Epidemiology of Bronchiectasis

The incidence of non-cystic fibrosis (CF) bronchiectasis in children has not been well described. Field in 1927 reported the incidence of bronchiectasis in children under the age of fifteen in England and Wales as 7 per million children.[1] With improvement in housing conditions, sanitation, nutrition and the availability of antibiotics and immunisations against many of the organisms responsible for the development of bronchiectasis the incidence of bronchiectasis in developed countries has decreased.[17-20] In Finland the annual incidence of bronchiectasis in children under the age of 15 years between 1983 and 1992 was only 0.5 per 100 000.[18] In the USA the overall prevalence was reported as 52 per 100 000 irrespective of age.[21] Eastham reported a prevalence of one in 5 800 in Newcastle on Tyne in the north west on England.[22]

A few studies have shown that bronchiectasis is more common in developing countries and communities[5, 23] and in indigenous socioeconomically disadvantaged communities of developed countries.[19, 24] This was illustrated by Twiss in 2005 who reported an overall incidence of 3.7 per 100 000 children under the age of fifteen years in New Zealand. When divided along socioeconomic and indigenous lines the results were 17.8 per 100 000 in children of Pacific Island heritage and 4.8 per 100 000 in children of Maori descent compared to only 1.5 per 100 000 in children of European descent who are of higher socioeconomic status. This was statistically significant.[19] The children of Pacific Island

Heritage or from Maori descent were from families who had twice the average unemployment rate and were seven times more likely to live in homes where there were more than two occupants per bedroom. In another study from New Zealand Edwards showed similar results where he reported an overall prevalence of 1 in 5 900 in children under the age of fifteen years of age in Auckland but the prevalence was high at 1 in 1 875 and 1 in 4 244 for children from Pacific Island and Maori descent (low socioeconomic status) respectively compared to 1 in 24 900 reported in those of European descent (high socioeconomic status).[6] Similarly Chang reported an overall prevalence of bronchiectasis of 4.9 per 1000 live births in children under the age of fifteen in Central Australia, but in the indigenous children the incidence was 14.7 per 1000.[4]

1.3 Pathogenesis of Bronchiectasis

Chronic airway inflammation leading to bronchiectasis may be as a result of primary lung abnormalities that predispose the patient to recurrent or severe infections or due to normal lungs with either an abnormal host immune response or after an overwhelming severe lower respiratory tract infection in a previously normal lung.[4-9, 14, 16, 17, 19, 22, 24-28] In a number of children with bronchiectasis no cause can be found.[4-9, 14, 16, 17, 19, 22, 25-27, 29]

Cystic fibrosis and primary ciliary dyskinesia cause bronchiectasis due to impairment of mucous clearance from the airways.[8, 17, 27, 29] Chronic aspiration syndromes in the neurologically impaired as well in children with anatomical abnormalities such as tracheo-oesophageal fistula or laryngo-tracheal clefts may lead to bronchiectasis.[4, 6, 7, 9, 16, 17, 27, 29] Bronchiectasis may also develop in children with congenital lung abnormalities[4, 17, 19, 22] or after foreign body aspiration.[5, 6, 8, 16, 24]

Abnormalities in the immune system of a patient can lead to more recurrent and severe infections in a patient with normal underlying lungs and this can lead to the development of bronchiectasis.[5-9, 16, 17, 22, 25-27, 29] Humoral immunodeficiencies leading to a shortage or absence of immunoglobulins is an important cause of recurrent chest infections that eventually can lead to bronchiectasis if not managed adequately. These include IgG (including subtypes), IgM and IgA deficiencies.[4, 30] Examples of acquired immunodeficiencies are HIV infection, prolonged steroid usage and malignancies.

1.4 Bronchiectasis and Infections

Bronchiectasis has classically been described to be associated with infections due to measles virus, adenovirus[31] and *Bordetella pertussis*.[1] *Staphylococcus aureus (S. aureus), Haemophilus influenzae (H. influenzae), Streptococcus pneumoniae (S. pneumoniae),* respiratory syncytial virus and influenza virus infections have also been associated with the development of bronchiectasis.[6] *Mycobacterium tuberculosis (M. tuberculosis)* infection can also be complicated by bronchiectasis either due to extra-luminal airway obstruction by tuberculous lymph nodes or by severe parenchymal tuberculous infection.[25, 32, 33] In developing countries infections remain the most common cause of bronchiectasis.[6, 8, 25]

In HIV positive patients the cause of bronchiectasis is usually due to recurrent and severe respiratory tract infections on the background of an abnormal immune system. A study done by Berman et al in 2007 in Florida USA looked at the risk factors for developing bronchiectasis in HIV positive patients.[34] It was found that HIV positive patients with bronchiectasis were more likely to have had recurrent pneumonia ($P = < 0.001$), more

episodes of pneumonia (P = < 0.001) and were more likely to have more profound immunosuppression than HIV positive patients without bronchiectasis. In this study HIV positive patients with bronchiectasis were also more likely to have had a diagnosis of lymphocytic interstitial pneumonitis (P = 0.005). Patients with HIV have a higher incidence of pneumonia and develop more severe pneumonia.[35] The incidence of TB is also much higher in patients with bronchiectasis.[35, 36] South Africa has a very high prevalence of HIV positive patients. The National HIV and syphilis prevalence survey released by the National Department of Health in 2008 estimated that the national HIV positive prevalence in South Africa stands at 28.0% (CI: 26.9 - 29.1%).[37]

In South Africa antiretroviral drugs (ARVs) were only accessible to the general population in 2004. This led to a large cohort of patients in whom the introduction of ARVs was delayed. Due to the delay in diagnosis and a lack of adequate treatment for their HIV these patients often develop features consistent with chronic lung disease. Jeena et al described the chronic lung diseases found in children mean (range) age 36 months (18–144) with HIV in Durban before anti-retroviral medication was available in South Africa[38]. They reported that 57% of the HIV infected patients investigated had lymphocytic interstitial pneumonitis and 19% of these patients also had bronchiectasis. 14% of the patients had another interstitial pneumonia and in this study 0% had bronchiectasis in isolation. A study from Zimbabwe by Ferrand et al describing chronic lung disease in adolescents with vertically transmitted HIV showed that 100 of 116 (86%) met their definition of chronic lung disease.[39] The most common high resolution chest CT abnormality found (55%) was that of decreased attenuation suggesting small airway disease. 43% of patients who had

symptoms of chronic lung disease also had high resolution chest CT changes in keeping with bronchiectasis.[39]

1.5 Bacterial Colonization of the Airway and Bronchiectasis

Irrespective of the cause of the bronchiectasis the structural damage to the airways results in bacterial colonisation of the airways and predisposes the patient to repeated lower respiratory tract infections that can cause further damage to the lungs.[12] In order to limit the damage to the airways and lung parenchyma, episodes of acute infective exacerbations should be treated early and aggressively. Acute infective exacerbations are defined as an increase in the cough frequency and the character of the sputum produced as well as a change in the volume of the sputum produced and occasionally also a change in the chest auscultatory findings.[13, 14] Knowing which bacteria are found in the airways of patients with bronchiectasis is important in defining empiric antibiotic guidelines for acute infective exacerbations.

The airway colonizing bacteria resulting in acute infective exacerbations in patients with CF-related bronchiectasis have been extensively described, with colonization from an early age with *S. aureus* and *H. influenza* being common, and followed closely by *Pseudomonas aeruginosa (Ps. aeruginosa).*

Studies in adult non-CF related bronchiectasis patients have shown that *H. influenzae, Ps. aeruginosa* and *S. pneumoniae* are the most common organisms isolated.[40] Adult patients with CF-related bronchiectasis are more likely to be colonised with *Ps. aeruginosa* than patients with non-CF related bronchiectasis (84% vs 29.7% (P = <0.05)).[41] They are also

more likely to have *S. aureus* colonisation than in non-CF related bronchiectasis (65% vs 5% (P = <0.05)).[41]

There have been a few studies that have described the organisms colonizing the airways in paediatric non-CF related bronchiectasis.[6, 19, 22, 29, 42] Two studies from Turkey reported that in children presenting with non-CF related bronchiectasis the most common bacteria found in the airways were *H. influenzae* (37%), *Ps. aeruginosa* (29%), *S. aureus* (13%), *S. pneumoniae* (8%), *Klebsiella* (8%) and *Enterobacter* (4%).[8, 42] Eastham et al reported that *H. influenzae* and *S. pneumoniae* were the most common organisms isolated from children with non-CF related bronchiectasis from Newcastle, England.[22] Studies from other developed countries reported *H. influenzae, S. pneumoniae, Moraxella catarrhalis (M. catarrhalis), S. aureus* and *Ps. aeruginosa* as being the most prevalent organisms.[6, 14, 16, 19, 26, 29]

Bacterial carriage may change in individuals over time with the carriage of *H. influenzae* decreasing in children with bronchiectasis from both Alaska and Australia, that of *S. pneumoniae* remaining stable and that of *S. aureus* increasing over a 4 – 6 year period.[43]

The bacteria in the airways of children with HIV associated chronic lung disease have recently been described.[39, 44] Ferrand et al described the organisms found in the airways of 54 adolescents with vertically transmitted HIV in Zimbabwe.[39] A positive bacterial sputum culture was reported in 17 (31%) of these patients with *H. influenzae, S. aureus, M. catarrhalis, Ps. aeruginosa, S. pneumoniae, Klebsiella pneumoniae (K. pneumoniae)* and *Listeria monocytogenes* being the organisms reported. Masekela et al looked at a cohort of

HIV infected children in South Africa and found that the most common organisms were *H. influenzae* and *H. parainfluenzae* which accounted for approximately 49% of the organisms cultured.[44]

It is not clear if there is a difference in organisms colonizing the airways between HIV infected and HIV uninfected patients.

1.6 Management of Children with Bronchiectasis in Relation to Airway Colonization

Patients with bronchiectasis who are asymptomatic are not placed on antibiotics when airway samples return a positive culture result. When there is an acute deterioration in their clinical condition as judged by an increase in the volume of sputum production, change in sputum colour, fever, increase in respiratory rate or work of breathing, radiological changes or deterioration in pulmonary function a course of antibiotics will be started guided by the microscopy, culture and sensitivity results of the previous sputum cultures of the individual patient.

Having the information regarding the bacteria found in the airways of HIV infected and HIV uninfected patients with bronchiectasis will be vital in making the choice of which antibiotics to use in those patients with bronchiectasis who suffer an acute exacerbation. The majority of patients presenting with bronchiectasis to the paediatric pulmonology service at Chris Hani Baragwanath Academic Hospital (CHBAH) are HIV positive and whether to treat the HIV infected and HIV uninfected patients with bronchiectasis differently or with the same antibiotics is a question that needs answering.

CHAPTER 2

2.0 MATERIALS AND METHODS

2.1 Aim

To determine the bacteria found in airway samples of paediatric patients with bronchiectasis seen at Chris Hani Baragwanath Academic Hospital according to their HIV status.

2.2 Objectives

1. To describe the bacteria found in the airway samples of paediatric patients with bronchiectasis at Chris Hani Baragwanath Academic Hospital.
2. To compare the type and density of bacteria found in the airway samples of patients with bronchiectasis who are HIV infected to those who are HIV uninfected.

2.3 Study Design

A retrospective observational study of children with bronchiectasis who had been admitted to the paediatric wards or were attending the paediatric pulmonology clinic at Chris Hani Baragwanath Academic Hospital (CHBAH) from 01 April 2011 to 31 March 2013.

2.4 Study Setting

CHBAH is a tertiary hospital in the public sector in Soweto, Gauteng. It is a referral centre for patients from the surrounding primary and secondary hospitals that serve the southern part of Gauteng, as well as from numerous secondary hospitals in the surrounding provinces. The majority of patients who attend CHBAH paediatric pulmonology clinic with bronchiectasis are from the local Soweto community. Referrals to the paediatric

pulmonology clinic at CHBAH are received either from the in-house service or from the primary and secondary hospitals in the hospital drainage area. In-house referrals are received from one of four acute general paediatric wards or from other sub-specialty services, for example the haematology-oncology unit or the infectious diseases unit. The infectious diseases unit runs the hospital's paediatric HIV clinic.

2.5 Definition of Bronchiectasis

The diagnosis of bronchiectasis was suspected on history and clinical examination and confirmed by radiological findings with the aid of a chest x-ray (CXR) or a computed tomography scan (CT) of the lungs. Clinical features that were suggestive of bronchiectasis included a chronic cough productive of purulent sputum, signs of chronic suppurative lung disease such as digital clubbing, increased chest antero-posterior diameter, a Harrison's sulcus and coarse crackles on lung auscultation. CXR changes included dilated peripheral airways, cystic changes in the lung fields and features of air-trapping and hyperinflation such as air anterior to the cardiac shadow, narrowing of the mediastinal shadow and flattening of the diaphragm. In patients who had a chest CT done, diagnosis was made on features such as tram-tracking, signet ring sign and gross dilatation and cystic changes of the large airways. All patients involved in this study were assessed for bronchiectasis by the investigator, CV.

2.6 Study Population

All children under the age of 16 years with a diagnosis of bronchiectasis who were either admitted to CHBAH and referred to the paediatric pulmonology service or who attended the paediatric pulmonology clinic at this hospital from 01 April 2011 to 31 March 2013.

2.6.1 Inclusion criteria

1. Children under 16 years of age with bronchiectasis.

2. Children admitted in CHBAH or who attend the paediatric pulmonology clinic at CHBAH and who have had at least one airway sample collected for microscopy and culture.

3. Children whose airway samples were processed at the National Health Laboratory Services (NHLS) based at CHBAH.

2.6.2 Exclusion criteria

1. Children with the diagnosis of bronchiectasis who had airway samples collected but processed and analysed at a laboratory other than NHLS at CHBAH.

2. Children with bronchiectasis in whom CF had been confirmed.

2.7 Sampling Technique and Materials

As part of hospital protocol, airway samples were taken from all patients who had been diagnosed with bronchiectasis. The first sample was taken when the patient initially presented to the general paediatric wards for an acute lower respiratory tract infection and is subsequently diagnosed with bronchiectasis or when the patient arrived as a referral to the paediatric pulmonology clinic for the investigation and management of their bronchiectasis. Samples were then taken on a regular basis, approximately every three months, and during every acute exacerbation or admission. This was done for airway bacterial surveillance.

Airway samples were collected by one of the following methods:

- Spontaneous expectoration: the patient spontaneously coughs and the expectorate is captured in a sample bottle.
- Induced sputum: the patient is given a short-acting beta-agonist (salbutamol 200µg via metered dose inhaler) followed 10-15 minutes later by 5ml 5% hypertonic saline nebulisation. The patient is then asked to spontaneously cough and the expectorate is collected into a specimen bottle.
- Tracheal aspirate: airway samples are collected after instilling 1-2 ml 0.9% saline through an endotracheal tube in an intubated patient and then suctioning the sample into a sputum trap.
- Broncho-alveolar lavage during bronchoscopy: 0.9% saline is instilled into the airways of a patient during bronchoscopy. 10-20 ml 0.9% saline is instilled depending on the patient's weight. Negative suction is applied via the bronchoscope and the samples are collected in a sputum trap.

All samples were sent to the NHLS laboratory at CHBAH.

Procedure for processing Samples at the NHLS laboratory:

The most purulent portion of the sample is placed on a microscope slide with the aid of a disposable loop or a sterile swab. The sample is gently rolled on the slide and is allowed to air dry. The slide is stained with the Gram's stain method. Gram's crystal violet solution is added onto the slide and applied for one minute. It is then washed off with water. Gram's iodine solution is applied to the slide and allowed to remain for one minute before being washed off. The slide is then flooded with Gram's decolouriser for ten seconds or less until no more Gram's crystal violet washes off. The Gram's decolouriser is washed off with

water. Gram's safranin solution is applied to the slide and removed by washing off with tap water after thirty seconds. After this staining procedure the slide is ready for interpretation.

The samples are examined under a light microscope. The number of polymorphonuclear and squamous epithelium cells per low power field (10x magnification) is counted. A total of ten fields are counted per sample. The Bartlett's scoring system is then used to determine the likelihood of the sample being of upper or lower respiratory tract origin. The number of neutrophils per low power field are counted and a score of 0, +1 or +2 assigned depending on whether there are <10, 10-25 or >25 neutrophils per low power field. The number of squamous epithelial cells per low power field is then counted and a score of 0, -1 or -2 assigned depending whether there are <10, 10-25 or >25 squamous epithelial cells per low power field. The scores from the polymorphonuclear cells and the squamous epithelial cells are then added together. A score of 0 or less is indicative of a sample originating from the upper respiratory tract and represents a sample with a lack of active inflammation or one that is contaminated with saliva. A score of 1 or more is indicative of a lower respiratory tract sample and of a sample with features of active inflammation.

The Gram's stains are further examined to determine the bacterial and cellular density of the sample. The number of neutrophils per low power field are counted and a score of 1+, 2+ or 3+ assigned depending on whether there are <10, 10-25 or >25 neutrophils per low power field. The bacterial density is reported by assigning a symbol +, ++ or +++ for the following scenarios: + for when bacteria are present but not on every field observed under oil immersion 100x objective, ++ for when bacteria are present in every field but not in large amounts or +++ for when bacteria are present in all fields in large amounts.

Furthermore if the organisms on the slide have stained blue-purple they are gram positive organisms and if the organisms stain red-pink they are gram negative organisms.

With all samples the following media was inoculated by selecting the most purulent portion of the sample and transferring it with a disposable loop onto the media: 5% horse blood agar which is incubated in CO_2 at 35^{o}C, MacConkey agar which is incubated aerobically at 35^{o}C and Bacitracin heated blood agar which is incubated in CO_2 at 35^{o}C. Broncho-alveolar lavage samples were centrifuged at 1 500 rpm for five minutes before plating on the abovementioned media. All inoculated media were read after 24 and 48 hours. Identification of the bacterial organism was done if any of the plates yielded positive growth. Positive cultures were further quantified to establish the density of the bacterial growth in the culture. Culture density is reported as +, ++ and +++. + is when less than 10 colony forming units (CFUs) are counted on the initial inoculum, ++ is when 10 – 99 CFUs are counted on the initial inoculum and +++ is when ≥100 CFUs are counted on the initial inoculum. Bacterial sensitivity testing was done on samples when requested or in the following cases: when *S. pneumoniae, H. influenzae or M. catarrhalis* had been identified, when gram negative organisms were identified in a sample with a positive Bartlett score, or when *S. aureus* was identified without the presence of concomitant normal oral flora.

Broncho-alveolar lavage samples were additionally plated on a Sabouraud dextrose agar for fungal culture. These media were incubated in CO_2 at 35^{o}C. Fungal cultures on samples other than broncho-alveolar lavage samples were not done routinely but were done when

requested. Specific anaerobic cultures were not routinely performed due to the technical difficulties and low yield of this test.

HIV testing was routinely done on all patients who were admitted to the paediatric wards. HIV testing was performed as per National Department of Health Guidelines. HIV serology with the ELISA method was done for children older than eighteen months of age with a confirmatory serology test (HIV combination Ab/ P24 Ag) performed if an ELISA was reactive. HIV PCR (qualitative) testing was done for children under eighteen months.

2.8 Data Collection Procedures

Data was collected from the patient's paediatric pulmonology file and from the computerized NHLS results database. The paediatric pulmonology patient files are kept in the paediatric pulmonology department. Each patient has a paediatric pulmonology file opened on first presentation to the paediatric pulmonology service whether as an inpatient in the wards or as a referral to the paediatric pulmonology clinic. The results from the computerised NHLS results database were accessed via computers in each hospital ward as well as in the outpatient and paediatric clinics area.

The following data were collected from the patients' paediatric pulmonology file and from the computerised NHLS results database: patient demographic characteristics, radiological features, including chest x-ray and chest computed tomography scans, data on previous and current tuberculosis treatment and laboratory findings including HIV status, CD4 count, viral load, number and type of sputum samples collected, sputum microscopy and sputum culture results. All samples collected from patients were included in the analysis. This

included multiple samples from the same patient. Comparisons were made between all individual samples as well as between multiple samples from an individual patient. The initial CD4 count and viral load on presentation to the paediatric pulmonology team was used for analysis.

2.9 Data Analysis

All data were entered into an Excel spreadsheet and all patient identifiers were removed. The individual patient identifiers were only available to the principal investigator and were held in a secure database. Patient details were removed from the database prior to the data analysis. The data capture sheet for this study can be viewed in Appendix 1.

Statistical analysis was performed using Statistica Version 12. Description of the data was summarized using the mean and standard deviations for continuous variables with normal distribution; median, interquartile range and range for continuous data without normal distribution and proportions and percentages was used for categorical variables.

Comparisons between HIV positive and negative patients with bronchiectasis were performed using the Student t-test for continuous variables with normal distribution and Mann-Whitney U test for those without normal distribution. The Chi-square or Fisher's exact test was for categorical variables. The differences were considered to be statistically significant when the p-value was less than 0.05.

2.10 Ethics Considerations

2.10.1 Permission

In preparation for conducting of the study the investigator, attended a Good Clinical Practice course in 2012 (Appendix 2). Permission to perform the study was obtained from the Hospital Protocol Review Committee (Appendix 3) and the Human Research Ethics Committee of the University of the Witwatersrand (Clearance certificate number: M130415, Attached as Appendix 4).

2.10.2 Confidentiality

A list, which contained the names and hospital numbers of the patients, was kept confidential by the author. This list was used only during retrieving of the medical records.

CHAPTER 3

3.0 RESULTS

3.1 Number of Patients Enrolled and their Demographic Characteristics

Seventy-eight patients with non-CF bronchiectasis had airway samples analysed at CHBAH from 01 April 2011 to 31 March 2013 and were included in the study. Thirty-seven (47.4%) were from admission in the paediatric wards and 40 (51.4%) were being seen at the paediatric pulmonology clinic. Thirty-seven (47.4%) had bronchiectasis confirmed by means of a CT chest. The male-to-female ratio was 0.8:1. The mean age of the patients was 9.7±3.3 years. Seventy-five (96.2%) were of African descent and 3 (3.8%) were of coloured descent (Table 3.1).

3.2 HIV Status of Patients with Bronchiectasis

All 78 patients were tested for HIV. Sixty-two (79.5%) of them were HIV positive and 16 (20.5%) were HIV negative (Table 3.1). Fifty-five (88.7%) and 50 (80.6%) HIV positive patients had CD4 count percentage and HIV viral load recorded respectively. The mean CD4 percentage in these patients was 15.8±12.1% and the median viral load was 17 974.5 copies per millilitre (IQR 183 395). In 35 (56.5%) of the HIV positive patients the duration of ARV treatment was known. The mean duration of ARV treatment in these patients was 52.1±39.1 months.

There was no significant statistical differences between the HIV positive and HIV negative patients with regards to sex (p=0.353). There was a trend for the HIV positive patients to be older at presentation than the HIV negative patients, with a mean age in HIV positive patients of 10.0±3.2 years compared to 8.3±3.4 years in HIV negative patients (p=0.051).

Table 3.1 Demographic Characteristics and HIV Results of Study Patients (n=78)

Characteristics	**Total Number (%)**			**P - value**
	All (N = 78)	**HIV +ve (N = 62)**	**HIV –ve (N = 16)**	**HIV +ve vs. HIV -ve**
Age in years (mean)	9.7±3.3*	10.0±3.2*	8.3±3.4*	P = 0.051
- <1 year	0	0	0	
- 1-5 years	6 (7.7)	4 (6.5)	2 (12.5)	
- > 5 years	72 (92.3)	58 (93.5)	14 (87.5)	
Sex:				
- Male	35 (44.9)	29 (46.8)	6 (37.5)	P = 0.353
- Female	43 (55.1)	33 (53.2)	10 (62.5)	
Race:				
- African	75 (96.2)	59 (95.2)	16 (100)	P = 0.497
- Coloured	3 (3.8)	3 (4.8)	0	

* Mean ± Standard deviation

3.3 Underlying Conditions Associated with Bronchiectasis in this Cohort

Sixty-two (79.5%) patients were HIV positive. Forty-seven (60.3%) patients had previously been treated for TB and 7 (9.0%) patients were on TB treatment when they presented to paediatric pulmonology. Among the 47 patients who had previously been treated for TB, 35 (44.9%) had received a single course of TB treatment, 7 (9.0%) had received two courses, 2 (2.6%) had received three courses of TB treatment and data regarding the number of previous TB treatment courses was missing in 3 cases. HIV positive patients were more likely than HIV negative patients to have had previous TB treatment (p=0.016), but there was no statistical difference regarding the number of previous TB treatments received (p=0.567) or the number of patients currently on TB treatment (p=0.949).

Three (3.8%) patients had post-infectious bronchiectasis: 1 developed bronchiectasis post-measles infection and 2 with respiratory papillomatosis, 3 (3.8%) patients had a primary

immunodeficiency: 2 with agammaglobulinaemia and 1 with a natural killer cell deficiency, 1 (1.3%) had an acquired immunodeficiency post-chemotherapy for a Wilms tumour and 1 (1.3%) had an aspiration syndrome. In one patient no cause could be found. There were no patients with post-foreign body bronchiectasis, cystic fibrosis, primary ciliary dyskinesia or congenital airway abnormalities.

3.4 Sample Collection

3.4.1 Number of Samples Collected

A total of 175 samples were collected from the 78 patients. The median number of samples collected per patient was two. Thirty-four patients (43.6%) had one sample collected, 22 (28.2%) had 2 samples collected, 8 (10.3%) had three, and 14 (18.0%) had more than three samples collected (Table 3.2). There was no statistical difference between the number of samples collected from HIV positive and HIV negative patients (p=0.701).

3.4.2 Type of Specimen

One hundred and fifty (85.7%) were expectorated or induced sputum specimens and 25 (14.3%) were broncho-alveolar lavage (BAL) specimens (Table 3.2). There were more HIV –ve patients who had specimens collected using BAL, compared to expectorated or induced specimens, than HIV +ve patients (p=<0.05).

3.4.3 Quality of Specimens: Bartlett Score

Of the 150 samples on which quality assessment was performed 11 (7.3%) had a Bartlett score of -1 or -2 indicating a sample representative of the upper airways. Twenty-nine (19.3%) had a Bartlett score of 0 in which it is difficult to assess the representation of

upper or lower airways. One hundred and nine (72.7%) had a Bartlett score of +1 or +2 indicating a sample representative of the lower airways. Information on Bartlett score was missing on one patient. There was no statistical significant difference in the Bartlett score between HIV positive and HIV negative patients' samples (p=0.280).

There was no statistical difference between the number of bacteria cultured per sample based on the Bartlett score (p=0.620) or between the type of bacteria cultured based on the Bartlett score of the sample (p=0.812).

All analyses were done with all the samples included, irrespective of the Bartlett score. All analyses were also repeated dividing the samples into their different Bartlett score groups (-1 and -2 vs. 0 vs. +1 and +2). There was no difference between the results obtained when comparing the different groups amongst themselves or against the group with all the samples included.

3.4.4 Number of Bacteria Cultured per Sample

Forty-five (25.7%) samples did not culture any bacteria. Sixty-two (35.4%) cultured 1 bacteria, 47 (26.9%) 2 bacteria, 18 (10.3%) 3 bacteria and 3 (1.7%) samples cultured 4 bacteria (Table 3.2). There was no statistical difference between HIV positive and HIV negative patients when comparing positive and negative cultures (p=0.417), and comparing the number of bacteria cultured per sample. (p=0.488).

3.4.5 Density of Individual Bacterial Colonies

The density of the individual bacterial colonies was as follows: no colonies were described as scanty, 5 (2.3%) had 1+ growth, 46 (20.7%) had 2+ growth and 169 (76.1%) had 3+ growth. Data was missing on 2 samples (Table 3.2). There was no statistical significance between the density of the colonies in HIV + and HIV – patients' samples (p=0.387).

Table 3.2 Characteristics of all Samples Collected

Specimens	**Number (%)**			**P - value**
	Total	**HIV +ve**	**HIV -ve**	**HIV +ve vs. HIV –ve**
Number of samples per patient	n = 78	n = 62	n = 16	p = 0.701
1	34 (43.6)	29 (46.8)	5 (31.3)	
2	22 (28.2)	17 (27.4)	5 (31.3)	
3	8 (10.3)	6 (9.7)	2 (12.5)	
>3	14 (17.9)	10 (16.1)	4 (25.0)	
Types of samples	n = 175	n = 133	n = 42	p = <0.05
- Expectorated and induced sputum	150 (85.7)	123 (92.5)	27 (64.3)	
- Broncho-alveolar lavage	25 (14.3)	10 (7.5)	15 (35.7)	
Quality of expectorated and induced sputum	n = 150*	n = 122	n = 27	p = 0.291
- Sample representative of upper airways	11 (7.3)	10 (8.2)	1 (4.2)	
- Unsure regarding upper or lower airway representation	29 (19.3)	21 (17.2)	8 (29.6)	
- Sample representative of lower airways	109 (72.7)	91 (74.6)	18 (66.7)	
- Missing data	1 (0.7)			
Number of bacteria cultured per sample	n = 175**	n = 133	n = 42	p = 0.488
- 0	45 (25.7)	36 (27.1)	9 (21.4)	
- 1	62 (35.4)	48 (36.1)	14 (33.3)	
- 2	47 (26.9)	32 (24.1)	15 (35.7)	
- 3	18 (10.3)	14 (10.5)	4 (9.5)	
- 4	3 (1.7)	3 (2.3)	0 (0.0)	
Density of all individual bacterial colonies	n = 220	n = 164	n = 56	p = 0.387
- Scanty	0	0	0	
- <10 CFUs*** on initial inoculum	5 (2.3)	5 (3.1)	0	
- 10-99 CFUs on initial inoculum	46 (20.7)	33 (20.1)	13 (23.2)	
- ≥100 CFUs on initial inoculum	169 (76.1)	126 (76.8)	43 (76.8)	
- Missing data	2 (0.9)	0	0	

* Excludes BAL samples ** Includes BAL samples ***Colony-forming units

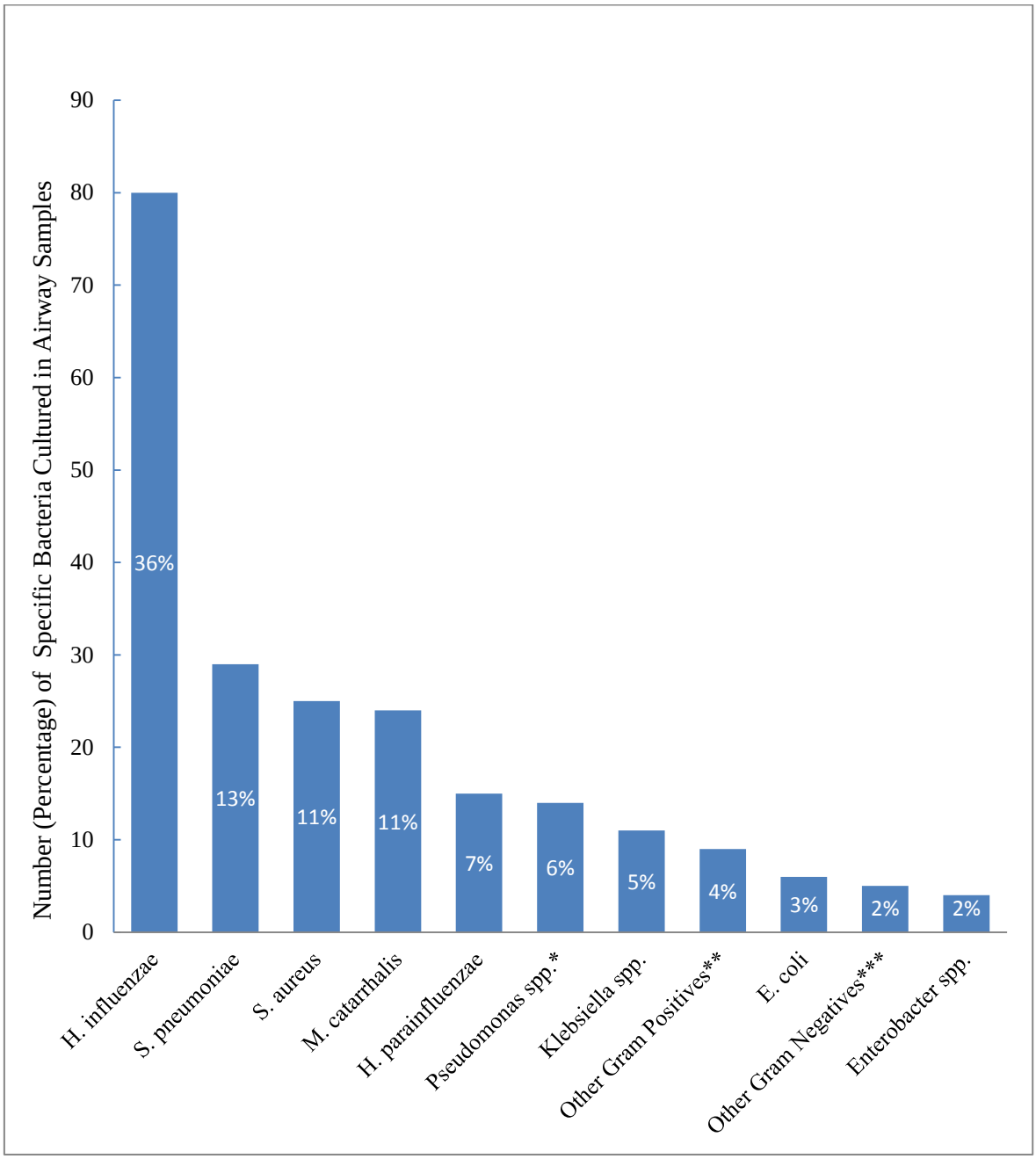
3.5 Names and Types of Bacteria Cultured

The total number of bacterial colonies grown from the 175 samples of the 78 patients was 222. The bacteria cultured were *H. Influenza* (n= 80, 36%), *S. pneumonia* (n=28, 13.1%), *S. aureus* (n=25, 11.3%), *M. catarrhalis* (n=25, n=10.8%), *H. parainfluenzae* (n=15, 6.8%), *Pseudomonas species* (n=14, 6.3%), *Klebsiella species* (all producing extended spectrum beta-lactamase) (n=11, 5.0%), *E. coli* (n=6, 2.7%), *Enterobacter species* (n=4, 1.8%), and others (n=17, 6.4%) (Figure 3.1). The majority of bacteria (n=159, 71.6%) cultured were gram negative bacteria and the rest were gram positive bacteria (n=63, 28.4%) (Table 3.3).

The common gram positive bacteria cultured were *Streptococci*, 58.7% and *Staphylococci*, 41.3%. The common *Streptococci* grown were *S. pneumoniae* (78.4%). Other streptococci grown were *S. viridans* (n = 1), *S. pyogenes* (n = 5) and others (n = 2). The common *Staphylococci* were Methicillin sensitive *S. aureus*, 69.2%. Other *Staphylococci* were Methicillin resistant *S. aureus* and only 1 (3.8%) was a coagulase-negative *staphylococcus.*

The common gram negative bacteria were *H. influenzae* (n=80, 50.3%), *M. catarrhalis* (n = 24, 15.1%), *H. parainfluenzae* (n=15, 9.4%), *Pseudomonas species* (n=14, 8.8%) and *Klebsiella species* (all extended spectrum beta-lactamase producing organisms) (n=11, 6.9%). The other gram-negative bacteria were *E. coli* (all extended spectrum beta-lactamase producing organisms) (n=6, 3.8%), *Enterobacter species* (n = 4, 2.5%) and a few of *M. morgagni*, *Proteus mirabilis*, *Pantoea species* and *Neisseria species* (n=5, all combined).

Figure 3.1: Type, Number and Percentage of Bacteria Cultured in all Airway Samples



**Ps. aeruginosa* and *Ps. flourescens*, ** Coagulase negative staphylococcus, *S. viridans*, *S. pyogenes* and Group C + G Streptococcus, ** *M. morgagni*, *P. mirabilis*, Pantoea species and Neisseria spp.

Table 3.3 Characteristics and Number of Bacteria Cultured in all Samples (n=222)

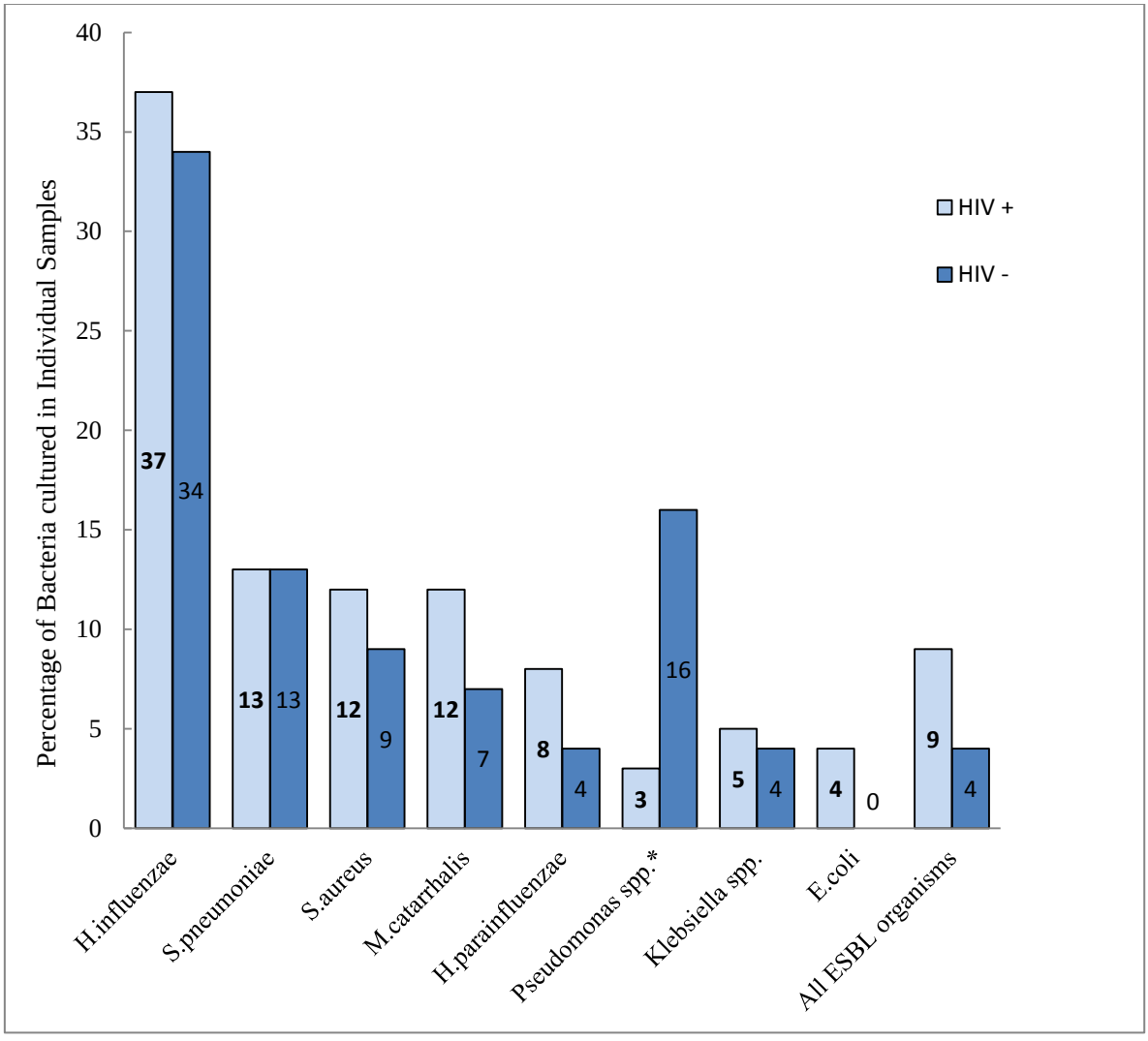
Bacteria	**Numbers (%)**			**P - value**
	All	**HIV +ve**	**HIV -ve**	**HIV +ve vs. HIV –ve**
Gram positive bacteria:	**63/222 (28.4)**	**46/63 (73.0)**	**17/63 (27.0)**	**p = 0.413**
Streptococci (n=37)	**37/63 (58.7)**	26/37 (70.3)	11/37 (29.7)	p = 0.308
- S.pneumoniae	29/37 (78.4)	22/29 (75.9)	7/29 (24.1)	p = 0.545
- Other streptococci[1]	8/37 (21.6)	4/8 (50.0)	4/8 (50.0)	p = 0.113
Staphylococci (n=26)	**26/63 (41.3)**	20/26 (76.9)	6/26 (23.1)	p = 0.501
- Methicillin sensitive S.aureus	18/26 (69.2)	15/18 (83.3)	3/18 (16.7)	p = 0.288
- Methicillin resistant S.aureus	7/26 (26.9)	5/7 (71.4)	2/7 (28.6)	p = 0.564
- CNS[2]	1/26 (3.8)	0/1 (0.0)	1/1 (100)	
Gram negative bacteria:	**159/222 (71.6)**	**120/159 (75.5)**	**39/159 (24.5)**	**p = 0.413**
H.influenzae	80/159 (50.3)	61/80 (76.3)	19/80 (23.8)	p = 0.405
M.catarrhalis	24/159 (15.1)	20/24 (83.3)	4/24 (16.7)	p = 0.224
H.parainfluenzae	15/159 (9.4)	13/15 (86.7)	2/15 (13.3)	p = 0.221
Pseudomonas[3]	14/159 (8.8)	5/14 (35.7)	9/14 (64.3)	p = 0.002
Klebsiella species (All ESBL)	11/159 (6.9)	9/11 (81.8)	2/11 (18.2)	p = 0.445
E.coli (All ESBL)	6/159 (3.8)	6/6 (100)	0/6 (0.0)	p = 0.171
Enterobacter species	4/159 (2.5)			
Other Gram negative bacteria[4]	5/159 (3.1)			
All ESBL organisms	17/159 (10.7)	15/17 (88.2)	2/17 (11.8)	p = 0.148

[1]*S. viridans, S. pyogenes* and Group C + G streptococci, [2]Coagulase negative staphylococcus, [3]*Ps. aeruginosa + Ps. flourescens,* [4]*M. morgagni, P. mirabilis*, Pantoea and Neisseria species

3.6 Comparisons of the Types of Bacteria Cultured in HIV Positive and Negative Patients

When comparing HIV positive and negative patients with regards to bacteria cultured in individual samples there was no statistical difference with regards to the presence of gram positive and gram negative bacteria (p = 0.844) (Figure 3.2).

Figure 3.2: Comparison of Bacteria Cultured in Individual Samples between HIV Positive and Negative Patients



* P=0.002 for Pseudomonas **

There was also no statistical difference for streptococci (p=0.308), *S. pneumoniae* (p=0.545) and other streptococci (p=0.113), staphylococci (p=0.501), MSSA (p=0.288) and MRSA (p=0.564).

There was no statistical difference with most gram negative bacteria including *H. influenzae* (p=0.416), *M. catarrhalis* (p=0.224), *H. parainfluenzae* (p=0.221), ESBL Klebsiella (p=0.445) and ESBL *E. coli* (p=0.171).

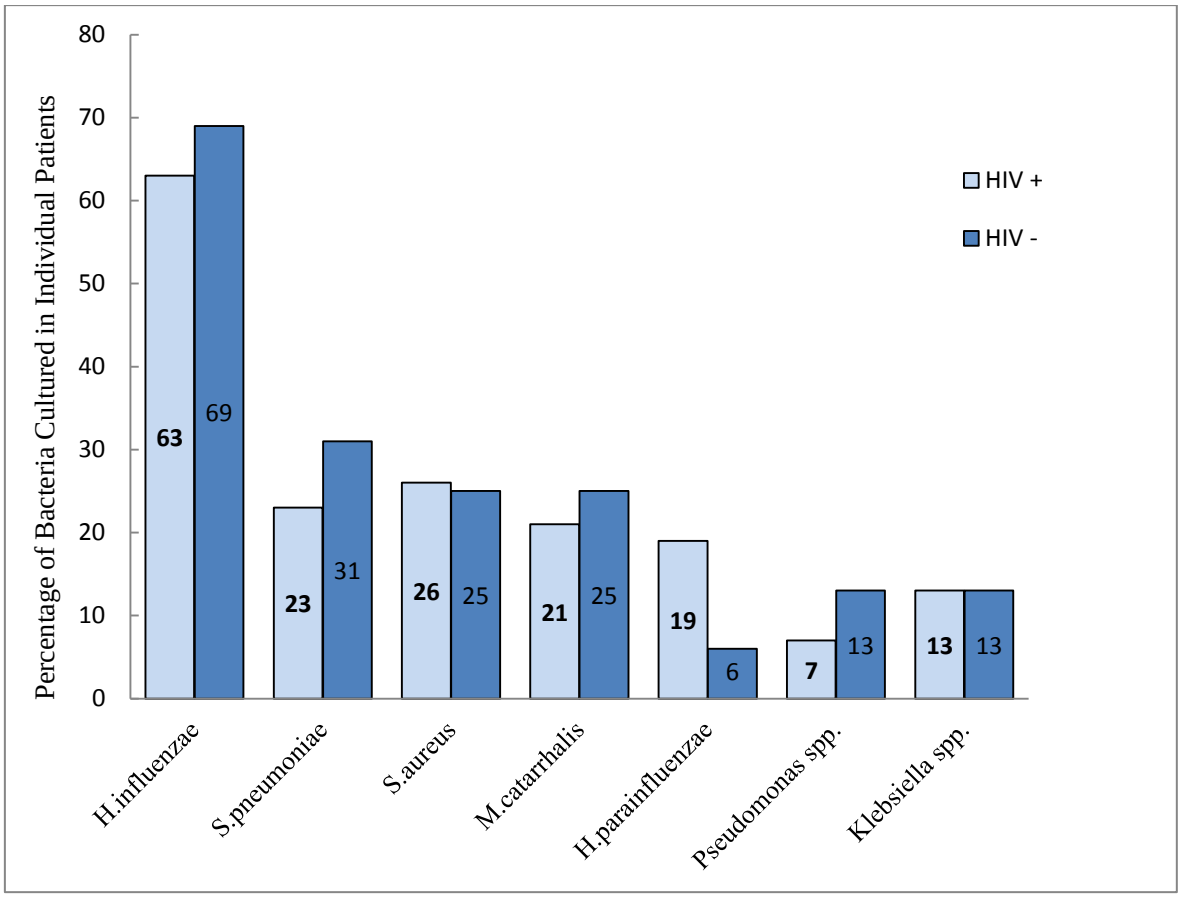
Similarly there was no statistical difference between groups when the ten most common bacteria were combined for analysis (p=0.357), when all gram positive bacteria were combined for analysis (p=0.413), when all gram negative bacteria were combined for analysis (p=0.413) and when all ESBL organisms were combined for analysis (p=0.148).

When comparing the presence of Pseudomonas in the individual samples of HIV positive and HIV negative patients statistical significance was reached (p=0.002) with HIV negative patient samples more likely to culture Pseudomonas. Similarly when the presence of the five most common bacteria per individual sample was compared in HIV positive and HIV negative patients statistical significance was reached (p=0.024) with HIV positive patients more likely to have one of the five most common bacteria in an individual sample.

When looking at the number of patients who ever cultured a specific bacteria and then comparing HIV positive and HIV negative patients no statistical difference was found for *H. influenzae* (p=0.450), *S. pneumoniae* (p=0.337), *S. aureus* (p=0.612), *M. catarrhalis*

(p=0.481), *H. parainfluenzae* (p=0.194), ESBL Klebsiella species (p=0.666) or Pseudomonas species (p=0.148) (Figure 3.3).

Figure 3.3: Comparison of Bacteria Cultured in Individual HIV Positive and HIV Negative Individuals

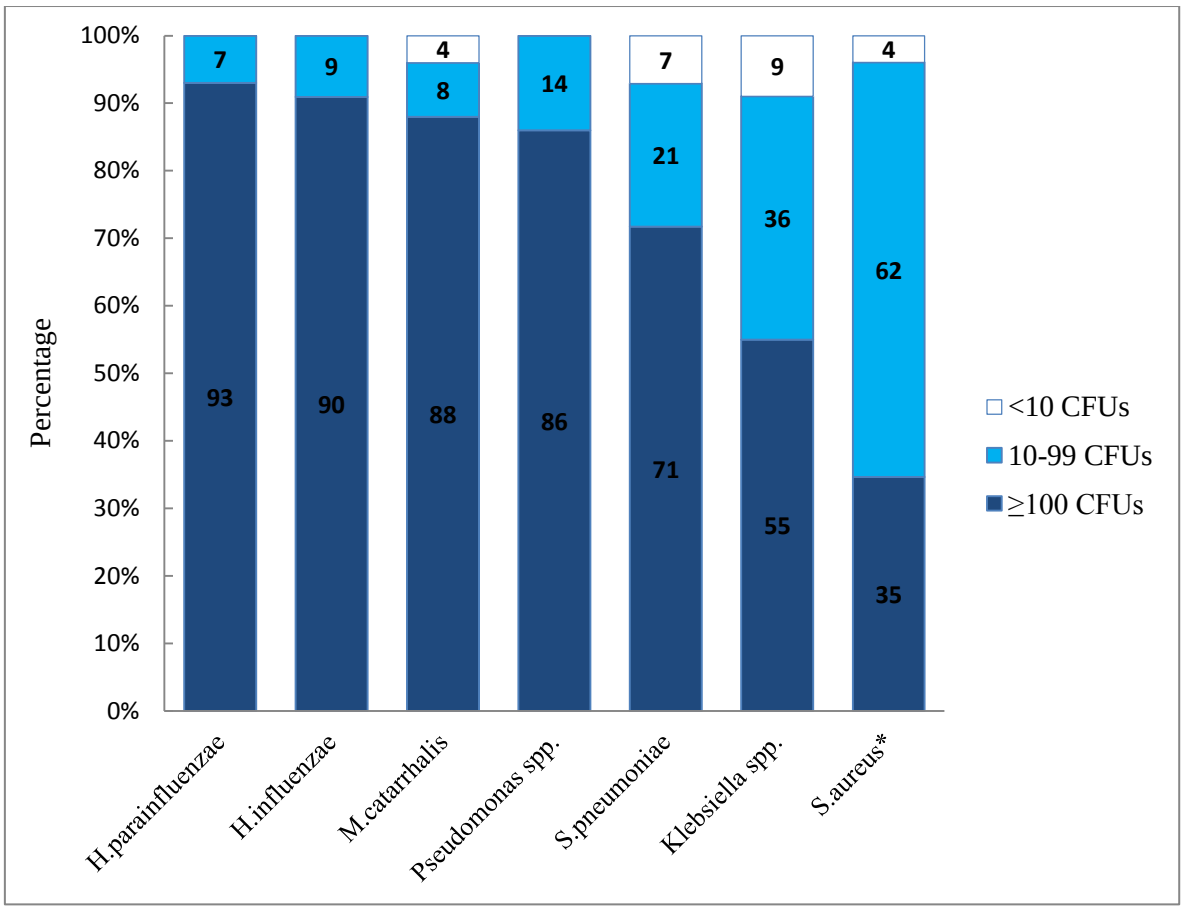


3.7 Density of Individual Bacterial Colonies

The colony densities of the individual bacteria are shown in Figure 3.4. *S. aureus* was statistically more likely to be present with 10 – 99 colony-forming units per inoculum when compared to all the other bacteria cultured who were more likely to be present with >100 colony-forming units per inoculum.

When looking at the densities of the individual bacterial colonies and comparing these between HIV + and HIV – patients there was no statistical significance for *H. influenzae* (p=0.296), *S. pneumoniae* (p=0.155), *S. aureus* (p=0.443), *M. catarrhalis* (p=0.710), *H. parainfluenzae* (p=0.921), Klebsiella species (p=0.118) and Pseudomonas species (p=0.346).

Figure 3.4: Density of Colony Forming Units on Bacterial Culture.



* P<0.05 for S.aureus

When grouping the density categories into the following two groups: scanty to 99 CFUs and >100 CFUs on initial inoculum, there was no statistical difference between the HIV + and HIV – groups when looking at individual bacterial densities.

There was no statistical difference between HIV + and HIV – patients when comparing the types and percentage of bacteria with more than 100 CFUs.

CHAPTER 4

4.0 DISCUSSION AND CONCLUSION

4.1 Discussion

A number of studies have reported on bacteria colonizing the airways of children with bronchiectasis, with most of them reporting from countries with a low incidence of HIV infection in children.[6, 8, 14, 16, 19, 22, 26, 29, 40, 42] Very few of these studies have reported on colonization of children who are HIV positive.[39, 44] It is not known whether there is a difference between the number, type and density of bacteria cultured in the airways of HIV positive and HIV negative children with bronchiectasis. The aim of this study was to determine which bacteria are isolated from the airways of children with bronchiectasis and whether there are differences in the type of bacteria cultured in the airways between HIV positive and HIV negative children.

The main findings in this study were that majority of patients with bronchiectasis were HIV positive with active disease as shown by low CD4 count and high viral loads. The bronchiectasis was most likely secondary to previous severe and recurrent lung infections. Most of the HIV positive patients also had a previous history of being treated for TB.

The most common bacteria cultured from the airways of children with bronchiectasis were *H. influenzae, S. pneumoniae, S. aureus, M. catarrhalis* and *H. parainfluenzae*. When comparing the bacteria cultured from the airways of HIV positive and HIV negative children with bronchiectasis there was no difference between the numbers of bacteria cultured per airway sample, the type of bacteria cultured per sample or per individual patient and the density of the bacterial colonies cultured in the children. The number, type

and density of bacteria cultured in children with bronchiectasis were not associated with their HIV status.

In this study about three quarters (74.3%) of specimens had at least one bacteria cultured, with 38.9% of them culturing two or more types of bacteria. This is a high rate of positive cultures compared to that reported in other studies.[8, 39] Karadag et al, isolated bacteria in only 46.9% of patients with bronchiectasis, and cultured one bacteria in 66.1% of patients. The reason for this difference is not clear, but it could be related to the age at presentation of the patients and that the majority of our patients were HIV positive, though we did not find differences in bacteria density between HIV negative and positive patients. The mean age of the patients in the Turkish cohort was 7.4 ± 3.7 years at presentation while the mean age of our patients was 9.7 ± 3.3 years. This would allow more time for the untreated patients to acquire more acute infective exacerbations and possibly to be colonised by a larger number of organisms. Ferrand reported on a cohort of adolescents with HIV associated lung disease and reported positive bacterial cultures on only 33% of their patients.[39] This low rate of positive cultures in this cohort is most likely due to the fact that this study looked at children with all HIV associated chronic lung disease where only 43% patient of the patients had bronchiectasis, compared to this study where all patients had bronchiectasis.

The high rate of positive samples is similar to some studies reporting from countries with low HIV incidence in children.[6, 7, 14, 16, 19] In a study by Twiss et al they cultured bacteria in 75% of samples[19], while Kapur cultured bacteria in 62% and 68% of their samples respectively in two different studies[14, 16] and Edward cultured bacteria in 66.7% of their

samples.[6] Even though all these studies were performed in settings with a very low HIV prevalence these studies' results compare favourably with this study's results. Masekela, in a study from South Africa had reported a positive culture rate of 65% when cumulative samples over a one year period were included.[44]

Gram negative bacteria were cultured more commonly than gram positive bacteria in this cohort (71.6% vs. 28.4%). This is in keeping with all the paediatric studies looking at bacteria in the airway of children with bronchiectasis.[6, 8, 14, 16, 19, 22, 26, 29, 39, 42, 44] The bacteria most commonly cultured in our study were: *H. influenzae* (36%), *S. pneumoniae* (13.1%), *S. aureus* (11.3%), *M. catarrhalis* (10.8%) and *H. parainfluenzae* (6.8%). These results are very similar to the results reported from different countries where similar studies have been done.[6, 8, 14, 19, 22, 26, 29, 42]

H. influenzae is the most common bacteria reported in most studies with a frequency ranging between 31% and 68%, and the frequency in this study was 36%. The majority of studies have reported similar results in terms of the top 5 bacteria cultured namely *H. influenzae* followed by *S. pneumoniae, M. catarrhalis, H. parainfluenzae* and *S. aureus*.[14, 19, 22, 26] Other studies have reported common bacteria that vary slightly from this study, with Dagli, Karadag and Li all reporting, in order of declining frequency, *H. influenzae, Ps. aeruginosa, S. aureus,* and *S. pneumoniae*.[8, 29, 42] The bacteria from these studies are similar to this study except for a higher frequency of *Ps. aeruginosa* found in their cohorts. The reason for this finding is not clear but it was reported by Kapur in one of their studies that the presence of *Ps. aeruginosa* colonisation of the airways of the non-CF child with bronchiectasis could signal the presence of a serious underlying co-morbidity.[16] Indeed in

the only case of *Ps. aeruginosa* in the airways of children without CF found by Edwards was in a patient with a tracheostomy.[7]

Masekela found that in samples from children who are HIV infected and have bronchiectasis the most commonly cultured bacteria were *H. influenzae* (30%) and *H. parainfleunzae* (21%) with *M. catarrhalis* following at 4% and none of S. *pneumoniae*, *S. aureus* or *Ps. aeruginosa* reaching a frequency of more than 2%.[44] These findings are very different to the ones in this study with a much higher frequency of *H. parainfluenzae* and much lower frequency of all the other bacteria. The reason for this difference is unclear. In the study by Ferrand the findings were similar to this study, but with a lower frequency of *S. pneumonia* and a higher frequency of *S. aureus*.[39] The numbers in the study were very small and therefore difficult to interpret.

There was no difference in this study between the HIV positive and HIV negative patients with regards to the presence of gram positive or gram negative bacteria and there was no difference between HIV positive and HIV negative patients with regards to the presence of *H. influenzae, S. pneumoniae, S. aureus, M. catarrhalis* or *H. parainfluenzae* in individual airway samples or in all samples collected per patient.

There was a higher frequency of *Ps. aeruginosa* in samples of HIV negative children than in those who were HIV positive ($P < 0.05$). When reanalysing the data on an individual patient basis, and not on a total airway sample basis, it was found that one patient had multiple samples positive for *Ps. aeruginosa* and there was no statistical difference between HIV positive and HIV negative patients in relation to the frequency of *Ps.*

aeruginosa in their airway samples (P = 0.148). There was also no difference in the densities of the individual bacterial colonies between HIV positive and HIV negative patients.

There are a number of limitations to this study. Firstly, it was a retrospective observational study and therefore it is possible that some of the information reviewed was not complete for some patients. Secondly, CT scans of the chest were not obtained on all patients to confirm the presence of bronchiectasis. Lastly, the number of HIV negative patients in this study was quite small. Though the number of HIV negative patients was much smaller than the HIV positive patients the strengths of this study is that a direct comparison in number, types of bacteria grown in the samples from airways of HIV positive and HIV negative patients was made, which has not been reported in previous studies.

4.2 Conclusion and Recommendations

The most common bacteria cultured in the airway samples of paediatric patients with bronchiectasis were: *H. influenzae* (36%), *S. pneumoniae* (13%), *S. aureus* (11%), *M. catarrhalis* (11%) and *H. parainfluenzae* (7%).

There is no difference in the type of bacteria nor the density of the bacterial colonies cultured in the airways of children with bronchiectasis with regards to their HIV status.

Antibiotics used during an acute exacerbation in patients with bronchiectasis should be based on the results of an individual's own sputum culture if available or else an antibiotic with both gram positive and gram negative cover, including cover for both *S. pneumoniae* and *H. influenzae* should be used.

Antibiotics used in the treatment of an acute exacerbation in patients with bronchiectasis should not differ depending on the HIV status of the patient.

APPENDIX 1: DATA CAPTURE SHEET

Bacteria found in the airway samples of a cohort of paediatric patients with bronchiectasis: Data Capture sheet

Dr Charl Verwey 082 411 8656

Patient Study Nr: ☐☐1

Date enrolled: (dd/mm/ccyy): ___/___/_____ ☐☐☐☐☐☐☐☐2

Patient age (months): _____ ☐☐☐3

Inclusion / exclusion criteria

Age < 16 years: Y / N
Bronchiectasis confirmed: Y / N
≥ 1 airway sample collected (CHBAH): Y / N
Known diagnosis of cystic fibrosis Y / N

Basics

Date of presentation to Resp. team: ___/___/_____ ☐☐☐☐☐☐☐☐4

First presentation: Ward[1] / Clinic[2] ☐5

Bronchiectasis diagnosis:

- Clinical dx: Y[1] / N[2] ☐6
- Radiological dx: Y[1] / N[2] ☐7

History **Study Nr:**

Aetiology of bronchiectasis: ☐☐8

1. Post-infectious (excl. TB):
2. Post-Tuberculosis:
3. Post-Foreign body (intraluminal obstruction)
4. Primary immunodeficiency
5. HIV acquired immunodeficiency
6. Acquired immunodeficiency (excl. HIV)
7. Mucociliary abnormalities (CF / PCD)
8. Congenital airway abnormalities
9. Cough abnormalities
10. Aspiration syndromes
11. Unknown
12. Other

Duration of symptoms (months): _____ ☐☐☐9

Radiology

Chest X-ray available: Y^1 / N^2 ☐10

- If yes: Bronchiectasis confirmed: Y^1 / N^2 ☐11
- If yes: Unilateral[1] / Bilateral[2] ☐12

  Number of lobes involved: _____ ☐13

Chest CT done: Y^1 / N^2 ☐14

- If yes: Bronchiectasis confirmed: Y^1 / N^2 ☐15
- If yes: Unilateral[1] / Bilateral[2] ☐16

  Number of lobes involved_____ ☐17

  Type of bronchiectasis:Tub[1]/Vari[2]/Cys[3]/Combo[4] ☐18

  Other findings: _____

Laboratory **Study Nr:**

HIV test done: Y[1] / N[2] ☐19

- If yes: PCR[1] / Elisa[2] ☐20
- Result conclusion: HIV positive[1] / HIV negative[2] ☐21

HIV positive:CD4 count(x10^6/*l*/%):_____/_____ ☐☐☐☐/☐☐,☐22

Viral load(cps/ml):_____ ☐☐☐☐☐☐☐23

ARVs: Y[1] / N[2] ☐24

- If yes: Duration (months) _____ ☐☐☐25

Previous TB treatment: Y[1] / N[2] ☐26

If yes: Number of treatments: _____ ☐27

Currently on TB treatment: Y[1] / N[2] ☐28

If yes: duration of treatment (months) _____ ☐29

Currently on Bactrim prophylaxis: Y[1] / N[2] ☐30

If yes: duration of treatment (months) _____ ☐☐☐31

Nr of airway samples collected: _____ ☐☐32

Type of samples collected: ☐33

1. Spontaneous expectoration: Nr: ☐34
2. Induced expectoration: Nr: ☐35
3. Tracheal aspirates: Nr: ☐36
4. Broncho-alveolar lavage: Nr: ☐37

Sample Nr: **Date:** **Study Nr:**

Type of sample: ES[1] / IS[2] / TA[3] / BAL[4] ☐38

Adequacy of sample: Bartlett score -2[1] / -1[2] / 0[3] / 1[4] / 2[5] ☐39

Gram stain: Positive[1] / Negative[2] ☐40

If positive: Neutrophils: 1+[1] / 2+[2] / 3+[3] ☐41

Epithelial cell: 1+[1] / 2+[2] / 3+[3] ☐42

Bacteria: +[1] / ++[2] / +++[3] ☐43

Organism: Gram +[1] / Gram –[2] / Combo[3]: _____ ☐44

Culture: Positive[1] / Negative[2] ☐45

- If positive: Normal flora[1] / Pathological[2] ☐46
- If pathological: No of Organisms: 1[1] / 2[2] / 3[3] ☐47
  - Organism 1: _____ ☐4
  - No of colonies: scanty[1] / 1+[2] / 2+[3] / 3+ ☐49
  - Organism 2: _____ ☐50
  - No of colonies: scanty[1] / 1+[2] / 2+[3] / ☐51
  - Organism 3: _____ ☐52
  - No of colonies: scanty[1] / 1+[2] / 2+[3] / 3+[4] ☐53
- Fungal culture: positive[1] / negative[2] ☐54
  - If positive: organism: _____ ☐55
- TB identified (smear or TBC) Y[1] / N [2] ☐56

Notes:

APPENDIX 2: GOOD CLINICAL PRACTICE CERTIFICATE

WITS HEALTH CONSORTIUM

(A wholly owned subsidiary of the University of the Witwatersrand, Johannesburg)

Clinical Research Department

Certificate of Attendance

Dr C Verwey

MP 0520586

has attended the course:

GOOD CLINICAL PRACTICE – Basic Course

22 & 23 August 2012

(Presented by: Lesley Burgess – course details overleaf – certificate is valid for 3 years)

Wits Health Consortium, 8 Blackwood Ave, PARKTOWN

The Health Professions Council of South Africa approved CPD reference is as follows:

Accreditation No: MDB08/106/01/2012 Activity No: 29953 Category: 1B Points: 11

Accreditation No: MDB08/109/01/2012 Activity No: 29957 Category: 2L Points: 4 Ethics

Lesley Burgess

HEAD OF DEPARTMENT

Date of Issue: 28 August 2012

COORDINATOR

APPENDIX 3: HOSPITAL PROTOCOL REVIEW COMMITTEE

CERTIFICATE

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

MEDICAL ADVISORY COMMITTEE
CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

PERMISSION TO CONDUCT RESEARCH

Date: 12 April 2013

TITLE OF PROJECT: Organisms colonizing the airways of children with non-cystic fibrosis related bronchiectasis at Chris Hani Baragwanath Academic Hospital

UNIVERSITY: Witwatersrand

Principal Investigator: Dr C Verwey

Department: Paediatrics

Supervisor (If relevant): Prof S Velaphi

Permission Head Department (where research conducted): Yes

Date of start of proposed study: 1 May 2013
Date of completion of data collection: 31 December 2013

The Medical Advisory Committee recommends that the said research be conducted at Chris Hani Baragwanath Hospital. The CEO /management of Chris Hani Baragwanath Hospital is accordingly informed and the study is subject to:-

- Permission having been granted by the Committee for Research on Human Subjects of the University of the Witwatersrand.
- the Hospital will not incur extra costs as a result of the research being conducted on its patients within the hospital
- the MAC will be informed of any serious adverse events as soon as they occur
- permission is granted for the duration of the Ethics Committee approval.

.....
Recommended
(On behalf of the MAC)
Date: 12 April 2013

.....
Approved/Not Approved
Hospital Management
Date: 12/04/13

APPENDIX 4: HUMAN RESEARCH ETHICS COMMITTEE

R14/49 Dr Charl Verwey

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M130415

NAME: (Principal Investigator)	Dr Charl Verwey
DEPARTMENT:	Department of Paediatrics Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	Organisms Colonizing the airways of Children with Non-Cystic Fribrosis Related Bronchietctasis at Chris Hani Baragwanath Hospital
DATE CONSIDERED:	26/04/2013
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof S Velaphi
APPROVED BY:	Professor PE Cleaton-Jones, Chairperson, HREC (Medical)

DATE OF APPROVAL: 26/04/2013

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**.

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX 5: PLAGIARISM REPORT

Turnitin Originality Report

CharlVerweyMMed.docx by Charl Verwey

From BACTERIA FOUND IN THE AIRWAY SAMPLES OF A COHORT OF PAEDIATRIC PATIENTS (poE53v9Z7a18A56L0Z71i16w84e66QYke09BAZ3G7sfq87pVOWrOKVsjqVPdAtiiDvFHWxy47881KC0halxkrPlb

- Processed on 23-Dec-2014 9:36 AM SAST
- ID: 492600324
- Word Count: 10440

Similarity Index
12%
Similarity by Source

Internet Sources:
7%
Publications:
9%
Student Papers:
2%

sources:

1 1% match (Internet from 08-Oct-2013)
http://wiredspace.wits.ac.za/bitstream/handle/10539/7545/dramowski_final%20report.pdf?se

2 1% match (publications)
Rossato Silva, Denise; Pozzebon da Silva, Larissa and de Tarso Roth Dalcin, Paulo, "Tuberculosis in hospitalized patients: clinical characteristics of patients receiving treatment within the first 24 h after admission", Jornal Brasileiro de Pneumologia, 2014.

3 1% match (publications)
Muldoon, E., and F. Mulcahy. "Syphilis resurgence in Dublin, Ireland", International Journal of STD & AIDS, 2011.

4 < 1% match (publications)
Grimwood, K., "Airway microbiology and host defences in paediatric non-CF bronchiectasis", Paediatric Respiratory Reviews, 201006

5 < 1% match (Internet from 28-Oct-2014)
http://www.repository.up.ac.za/bitstream/handle/2263/24164/Complete.pdf?

6 < 1% match (publications)
TETYANA ZAKHARKINA, "Detection of microorganisms in exhaled breath condensate during acute exacerbations of COPD : EBC, microorganisms and COPD exacerbations", Respirology, 08/2011

7 < 1% match (Internet from 26-Jan-2014)
http://www.broadinstitute.org/mpr/publications/projects/Metastasis/DatasetC_Rosetta_breast_outcome.res

8 < 1% match (Internet from 20-May-2014)
http://www.sajs.org.za/index.php/sajs/article/download/1523/523

REFERENCES

1. Field CE. Bronchiectasis in childhood; clinical survey of 160 cases. Pediatrics. 1949;4(1):21-46.
2. Chang AB, Glomb WB. Guidelines for evaluating chronic cough in pediatrics: ACCP evidence-based clinical practice guidelines. Chest. 2006;129(1 Suppl):260s-83s.
3. Chang AB, Grimwood K, Mulholland EK, Torzillo PJ. Bronchiectasis in indigenous children in remote Australian communities. Med J Aust. 2002;177(4):200-4.
4. Chang AB, Masel JP, Boyce NC, Wheaton G, Torzillo PJ. Non-CF bronchiectasis: clinical and HRCT evaluation. Pediatr Pulmonol. 2003;35(6):477-83.
5. Dogru D, Nik-Ain A, Kiper N, Gocmen A, Ozcelik U, Yalcin E, et al. Bronchiectasis: the consequence of late diagnosis in chronic respiratory symptoms. J Trop Pediatr. 2005;51(6):362-5.
6. Edwards EA, Asher MI, Byrnes CA. Paediatric bronchiectasis in the twenty-first century: experience of a tertiary children's hospital in New Zealand. J Paediatr Child Health. 2003;39(2):111-7.
7. Edwards EA, Metcalfe R, Milne DG, Thompson J, Byrnes CA. Retrospective review of children presenting with non cystic fibrosis bronchiectasis: HRCT features and clinical relationships. Pediatr Pulmonol. 2003;36(2):87-93.
8. Karadag B, Karakoc F, Ersu R, Kut A, Bakac S, Dagli E. Non-cystic-fibrosis bronchiectasis in children: a persisting problem in developing countries. Respiration. 2005;72(3):233-8.
9. Lai SH, Wong KS, Liao SL. Clinical analysis of bronchiectasis in Taiwanese children. Chang Gung Med J. 2004;27(2):122-8.
10. Matsuoka S, Uchiyama K, Shima H, Ueno N, Oish S, Nojiri Y. Bronchoarterial ratio and bronchial wall thickness on high-resolution CT in asymptomatic subjects: correlation with age and smoking. AJR Am J Roentgenol. 2003;180(2):513-8.
11. Reid LM. Reduction in bronchial subdivision in bronchiectasis. Thorax. 1950;5(3):233-47.
12. Cole P. The damaging role of bacteria in chronic lung infection. J Antimicrob Chemother. 1997;40 Suppl A:5-10.
13. Kapur N, Masters IB, Morris PS, Galligan J, Ware R, Chang AB. Defining pulmonary exacerbation in children with non-cystic fibrosis bronchiectasis. Pediatr Pulmonol. 2012;47(1):68-75.
14. Kapur N, Masters IB, Chang AB. Exacerbations in noncystic fibrosis bronchiectasis: Clinical features and investigations. Respir Med. 2009;103(11):1681-7.
15. Gaga M, Bentley AM, Humbert M, Barkans J, O'Brien F, Wathen CG, et al. Increases in CD4+ T lymphocytes, macrophages, neutrophils and interleukin 8 positive cells in the airways of patients with bronchiectasis. Thorax. 1998;53(8):685-91.
16. Kapur N, Grimwood K, Masters IB, Morris PS, Chang AB. Lower airway microbiology and cellularity in children with newly diagnosed non-CF bronchiectasis. Pediatr Pulmonol. 2012;47(3):300-7.
17. Nikolaizik WH, Warner JO. Aetiology of chronic suppurative lung disease. Arch Dis Child. 1994;70(2):141-2.
18. Saynajakangas O, Keistinen T, Tuuponen T, Kivela SL. Evaluation of the incidence and age distribution of bronchiectasis from the Finnish hospital discharge register. Cent Eur J Public Health. 1998;6(3):235-7.
19. Twiss J, Metcalfe R, Edwards E, Byrnes C. New Zealand national incidence of bronchiectasis "too high" for a developed country. Arch Dis Child. 2005;90(7):737-40.
20. Kolbe J, Wells AU. Bronchiectasis: a neglected cause of respiratory morbidity and mortality. Respirology. 1996;1(4):221-5.

21. Weycker D, Edelsberg J, Oster G. Prevalence and economic costs of bronchiectasis. American Thoracic Society International Conference. Amer J Resp Crit Care Med. 2004;169(7 supplement):330.
22. Eastham KM, Fall AJ, Mitchell L, Spencer DA. The need to redefine non-cystic fibrosis bronchiectasis in childhood. Thorax. 2004;59(4):324-7.
23. Field CE. Bronchiectasis in childhood; prophylaxis, treatment and progress with a follow-up study of 202 cases of established bronchiectasis. Pediatrics. 1949;4(3):355-72.
24. Singleton R, Morris A, Redding G, Poll J, Holck P, Martinez P, et al. Bronchiectasis in Alaska Native children: causes and clinical courses. Pediatr Pulmonol. 2000;29(3):182-7.
25. Karakoc G. Bronchiectasis: still a problem. 2001;32(2):175-8.
26. Munro KA, Reed PW, Joyce H, Perry D, Twiss J, Byrnes CA, et al. Do New Zealand children with non-cystic fibrosis bronchiectasis show disease progression? Pediatr Pulmonol. 2011;46(2):131-8.
27. Pasteur MC, Helliwell SM, Houghton SJ, Webb SC, Foweraker JE, Coulden RA, et al. An investigation into causative factors in patients with bronchiectasis. Am J Respir Crit Care Med. 2000;162(4 Pt 1):1277-84.
28. Valery PC, Torzillo PJ, Mulholland K, Boyce NC, Purdie DM, Chang AB. Hospital-based case-control study of bronchiectasis in indigenous children in Central Australia. Pediatr Infect Dis J. 2004;23(10):902-8.
29. Li AM, Sonnappa S, Lex C, Wong E, Zacharasiewicz A, Bush A, et al. Non-CF bronchiectasis: does knowing the aetiology lead to changes in management? Eur Respir J. 2005;26(1):8-14.
30. Rosen FS, Cooper MD, Wedgwood RJ. The primary immunodeficiencies. N Engl J Med. 1995;333(7):431-40.
31. Becroft DM. Bronchiolitis obliterans, bronchiectasis, and other sequelae of adenovirus type 21 infection in young children. J Clin Pathol. 1971;24(1):72-82.
32. Field CE. Bronchiectasis in childhood; aetiology and pathogenesis, including a survey of 272 cases of doubtful irreversible bronchiectasis. Pediatrics. 1949;4(2):231-48.
33. Kapur N, Karadag B. Differences and similarities in non-cystic fibrosis bronchiectasis between developing and affluent countries. Paediatr Respir Rev. 2011;12(2):91-6.
34. Berman DM, Mafut D, Djokic B, Scott G, Mitchell C. Risk factors for the development of bronchiectasis in HIV-infected children. Pediatr Pulmonol. 2007;42(10):871-5.
35. Madhi SA, Petersen K, Madhi A, Khoosal M, Klugman KP. Increased Disease Burden and Antibiotic Resistance of Bacteria Causing Severe Community-Acquired Lower Respiratory Tract Infections in Human Immunodeficiency Virus Type 1-Infected Children. Clinical Infectious Diseases. 2000;31(1):170-6.
36. Barnes PF, Bloch AB, Davidson PT, Snider DE. Tuberculosis in Patients with Human Immunodeficiency Virus Infection. New England Journal of Medicine. 1991;324(23):1644-50.
37. The National HIV and Syphilis Prevalence Survey South Africa. Report. National Department of Health South Africa, Africa NDOHS; 2008.
38. Jeena PM, Coovadia HM, Thula SA, Blythe D, Buckels NJ, Chetty R. Persistent and chronic lung disease in HIV-1 infected and uninfected African children. Aids. 1998;12(10):1185-93.
39. Ferrand RA, Desai SR, Hopkins C, Elston CM, Copley SJ, Nathoo K, et al. Chronic lung disease in adolescents with delayed diagnosis of vertically acquired HIV infection. Clin Infect Dis. 2012;55(1):145-52.
40. Angrill J, Agusti C, de Celis R, Rano A, Gonzalez J, Sole T, et al. Bacterial colonisation in patients with bronchiectasis: microbiological pattern and risk factors. Thorax. 2002;57(1):15-9.
41. Athanazio RA, Rached SZ, Rohde C, Pinto RC, Fernandes FL, Stelmach R. Should the bronchiectasis treatment given to cystic fibrosis patients be extrapolated to those with bronchiectasis from other causes? J Bras Pneumol. 2010;36(4):425-31.
42. Dagli E. Non cystic fibrosis bronchiectasis. Paediatr Respir Rev. 2000;1(1):64-70.

43. Hare KM, Singleton RJ, Grimwood K, Valery PC, Cheng AC, Morris PS, et al. Longitudinal nasopharyngeal carriage and antibiotic resistance of respiratory bacteria in indigenous Australian and Alaska native children with bronchiectasis. PLoS One. 2013;8(8):e70478.
44. Masekela R, Anderson R, Moodley T, Kitchin OP, Risenga SM, Becker PJ, et al. HIV-related bronchiectasis in children: an emerging spectre in high tuberculosis burden areas. Int J Tuberc Lung Dis. 2012;16(1):114-9.