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MICROBIAL AETIOLOGY OF COMMUNITY ACQUIRED PNEUMONIA AT A TERTIARY INSTITUTION IN JOHANNESBURG, SOUTH AFRICA

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfillment of the requirements for the degree of Master of Medicine in the branch of Clinical Microbiology and Infectious Diseases

Johannesburg, 2013

DECLARATION

I, Dr Parastu Meidany, declare that this dissertation is my own work. It is being submitted for the degree of Master of Medicine at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

_____

Dr Parastu Meidany

_____ day of _____2013

This is dedicated to

Shamim Afshani

&

Anisa Miri

PRESENTATION ARISING FROM THIS STUDY

FIDSSA Congress 2011; Oral Presentation, September 2011, Durban, South Africa

ABSTRACT

Introduction

To determine the spectrum of aetiological agents in adult Community Acquired Pneumonia (CAP) admitted to an academic hospital in Johannesburg using a novel transport medium (PrimeStore™ MTM), in addition to traditional specimen processing. PrimeStore™ MTM preserves released nucleic acids, including labile RNA.

Materials and Methods

Forty-eight adult patients with radiologically confirmed CAP were prospectively studied over three months. Microbiological investigation included culture from blood and sputum, with pulmonary tuberculosis being excluded by sputum microscopy and culture. Nasopharyngeal swabs (PrimeStore™ MTM) were analysed using two commercial multiplex PCR assays for the detection of 6 major bacteria and 12 major respiratory viruses. The BinaxNOW Legionella urinary antigen test was also used.

Results

A probable microbial aetiology of CAP was established for 62.5% (30 of the 48 patients) when the PCR platform was added to the conventional methods with the use of the PrimeStore™ MTM swabs. In contrast, the definite bacterial aetiology was 16.7% (8 of 48 patients) when conventional culture methods were used; none had more than one bacterial species identified. Five patients had no aetiological pathogens determined. The urine *Legionella pneumophila* antigen was negative in all patients. Of the eight patients (16.7%) with a definite bacterial aetiology; *Streptococcus pneumoniae* was isolated from blood cultures of all eight patients.

Blood and sputum cultures were negative in 5 patients, but the clinical and radiological reporting was indicative of *Pneumocystis jiroveci* pneumonia with a positive BDG>500. For this group of patients the term possible aetiology was introduced.

Conclusion

The five most prevalent pathogens identified were *Streptococcus pneumoniae*, *Haemophilus influenzae*, human rhinovirus, coronaviruses and metapneumovirus. The use of PrimeStore™ MTM significantly enhanced the diagnostic yield in determining the microbial aetiology of CAP in adult patients.

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ABBREVIATIONS

ATS- American Thoracic Society

BDG- Beta D-Glucan

CAP- Community Acquired Pneumonia

CDC- Center for Disease Control

ERS- European Respiratory Society

ESCMID- European Society for Clinical Microbiology and Infectious Diseases

HIV- Human immunodeficiency virus

ICU- Intensive Care Unit

IDSA- Infectious Disease Society of America

IF- Immunofluorescence Assay

PCR- Polymerase Chain Reaction

PJP- *Pneumocystis jiroveci* pneumonia

RT-PCR- Reverse Transcriptase Polymerase Chain Reaction

WHO- World Health Organisation

CHAPTER 1: INTRODUCTION

1.1 Background

Community-acquired pneumonia (CAP) is a common, potentially life-threatening infectious disease. Pneumonia affects about 12 individuals per 1000 annually in the United States (Guthrie, 2001), resulting in approximately 1 million patient hospitalisations annually (Arnold et al., 2003), with a mortality of 13.6% (Dean et al., 2001), and a cost of 10 billion US dollars annually (Gross et al., 2003).

CAP is a common infection in all age groups and is the most important lower respiratory tract infection. The demographics of the general patient population have changed over recent years. This includes an increase in the number of individuals over the age of 65 years, and the number of immune-compromised patients, especially in association with human immunodeficiency virus (HIV) infection (Luna et al., 2000). These factors have significantly increased the number of individuals at risk of pneumonia, the number of infections that occur, and the variety of pathogens found in association with the infection.

1.2 Epidemiology of CAP in South Africa

Despite the accessibility to effective vaccines and potent antimicrobials, in a 2008 Statistics South Africa report on mortality and causes of death, second to tuberculosis as the leading cause of death in the South Africa (12.6% of natural causes of death), the other most common causes of natural death were influenza and pneumonia (7.7%) (Statistics South Africa, 2008).

Despite the use of many microbiological techniques, in nearly 50% of cases of CAP the aetiology is unknown (Steinhoff et al., 1996). A study was undertaken to determine the aetiology of CAP in South Africa in 1987 where a cohort of 178 ICU admissions with severe CAP was reviewed. In that study, the incidence of Gram-negative bacteria causing infections was 15% (predominantly *Klebsiella pneumoniae*), the incidence of *Staphylococcus aureus* was 8% and *Legionella pneumophila* was 5% (Potgieter and Hammond, 1992).

A retrospective analysis of data of all patients admitted to the then Hillbrow Hospital ICU in Johannesburg, between 1982 and December 1992 was analysed to assess the aetiology of severe CAP (Feldman et al., 1995). The most common organism shown by Feldman et al. (1995) was *Streptococcus pneumoniae* (51.3%) and the second commonest organism was *Klebsiella pneumoniae* (31.9%).

A prospective study carried out on adult patients in Cape Town at Groot Schuur Hospital with pneumonia from July 1987 to July 1988 found 35.9% of patients to be infected with atypical bacteria namely; *Chlamydophila pneumoniae* (20,7%) and *Legionella pneumophila* (8,7%) (Maartens et al., 1994).

1.3 Aetiology of CAP

1.3.1 Bacterial aetiology

Numerous pathogens are listed as aetiological agents of CAP. In a review of 26 clinical trials in a cohort of adult patients who were hospitalised with CAP, it was found that a responsible organism was identified in only 3349 (33.7%) of 9933 patients (Echols et al., 2008). Among the patients for whom an aetiological pathogen was identified, in the atypical pathogen group there were 601 patients (17.9%) and in the bacterial pathogen group there were 2748 patients (82%). The bacterial pathogens were *Streptococcus pneumoniae, Haemophilus influenzae,* and *Staphylococcus aureus,* and the atypical pathogens were *Mycoplasma pneumoniae, Chlamydophila pneumoniae,* and *Legionella pneumophila*.

Most studies identify *Streptococcus pneumoniae* as the most common aetiological organism in CAP (Jokinen et al., 2001). Other bacterial agents including *Haemophilus* influenzae and atypical pathogens (*Mycoplasma pneumoniae, Chlamydophila pneumoniae, Coxiella burnetii and Legionella pneumophila*) are described in up to 35% of CAP episodes (Jokinen et al., 2001).

The Neufield Quellung typing method has been the “gold standard” for *Streptococcus pneumoniae* (Lalitha et al., 1996). The traditional method of serotyping *Streptococcus pneumoniae* uses pneumococcal typing anti-sera obtained from Statens Seruminstitut, Copenhagen, Denmark.

Pneumococcal conjugate vaccine (PCV) usage has reduced the burden of invasive disease due to vaccine types (i.e., serotypes 4, 6B, 9V, 14, 18C, 19F, 23F). Vaccine

efficacy has led to significant decreases in morbidity and mortality due to *Streptococcus pneumoniae* in the young with vaccine-serotype invasive pneumococcal disease being 83% in HIV-uninfected children compared to 65% in HIV-infected children (Madhi et al., 2012). Disease management has been complicated by the unexpected increase in resistant serotypes, such as 19A.

Serotype 1 is among the most prevalent invasive serotypes across the world (Garcia et al., 2006). Among vaccine serotypes, serotype 4 was significantly associated with invasive disease. Serotypes 1, 2, 7, 9, 14, and 16 were noted among the most invasive serotypes, and serotypes 3, 6, 15, 19, and 23 were among the least invasive serotypes (Smith et al., 1993).

1.3.2 Viral aetiology

The outbreaks of severe acute respiratory syndrome (SARS) and human cases of H5N1 influenza infection have shifted attention towards viral pneumonias. The identification of human metapneumovirus has led to numerous studies seeking to clarify the role of this virus in human respiratory tract infections (Crowe, 2004). Human bocavirus (Arnold et al., 2003) and human coronavirus NL6311 have been associated with CAP in children and adults (Van Der Hoek et al., 2006). As optimal replication of rhinovirus is restricted at higher core body temperature it was initially thought that its replication was prevented in the lower respiratory tract. However increasing evidence indicates that rhinovirus, may be associated with CAP in children and adults (Hayden, 2004) though its role in pneumonia still remains controversial.

Respiratory viruses are recognised as aetiological agents of CAP, with influenza virus being one of the leading viral pathogens (Ruiz et al., 1999). Current studies based on molecular diagnostics indicate that viruses acknowledged as the causative agent of CAP may have been underestimated because of a previous limited range of diagnostic options and methods (Murdoch, 2004).

It is unclear whether a virus on its own causes pneumonia or whether the virus acts in conjunction with other respiratory pathogens. Some studies have proven that some respiratory viral pathogens are capable of invading and multiplying in the lower respiratory tract mucosa (Papadopoulos et al., 2000). In addition, a few cases indicate that the incidence of mixed infections may be quite significant among patients admitted to hospital with CAP (Madhi et al., 2006).

The major causes of viral CAP in adults reported in literature are influenza A/B, parainfluenza virus (PIV) type 1(PIV1), PIV2, PIV3, respiratory syncytial virus (RSV), adenovirus, and rhinovirus (Domachowske and Rosenberg, 1999). Other viruses such as coronavirus (CoV), bocavirus, enterovirus, PIV4, the newly discovered parvovirus types 4 and 5, and mimivirus (Dare et al., 2008) have also been proven to infect the respiratory tract, but at a much lower frequency. The clinical significance of bocavirus, parvovirus types 4 and 5, and mimivirus is not known.

Rhinoviruses and CoVs were identified as human pathogens in the 1960s, but they have been largely ignored by the medical community because their clinical impact was uncertain (Tyrrell and Bynoe, 1965). It is now clear that rhinoviruses and CoVs may cause fatal CAP. All of the viruses mentioned above have similar clinical

presentations of CAP and attending clinicians cannot distinguish the aetiological agent without a proven laboratory diagnosis (Chiu et al., 2005).

Traditionally, rhinoviruses have been undiagnosed for several reasons. First, rhinovirus infections were not considered to be aetiological agents of CAP by clinicians. Second, the traditional culture of this virus is rather complex and tedious (Landry, 2007). Thirdly, rhinoviruses were neglected by clinicians, and no diagnosis by monoclonal antibodies has been developed. Lastly, rhinovirus serotypes make the possibility of broadly reacting antibodies unlikely since they lack a common group antigen (Arruda et al., 1996).

Since 2000, avian influenza viruses (H5N1, H7N7, and H7N3), human metapneumovirus (hMPV), severe acute respiratory syndrome (SARS) CoV, and human CoVs (HCoV) NL63 and HKU1 have emerged (Cheng et al., 2007).

Human MPV causes CAP and the signs and symptoms are very similar to those caused by RSV (Van den Hoogen et al., 2003). Human MPV outbreaks occur predominantly in the winter in temperate climates, often following the winter RSV outbreak (Kaida et al., 2006). Human MPV infections can be diagnosed by using serology, virus isolation, and antigen or nucleic acid detection. The major disadvantage of cell culture is the length of time required to identify a virus and its poor sensitivity (Boivin et al., 2002).

The human coronaviruses are the largest of all RNA viruses. There are five HCoVs, which include 229E, OC43, SARS-CoV, NL63, and HKU1 (Vallet et al., 2004).

HKU1 was discovered in January 2004 in a 71-year-old man returning to Hong Kong from Shenzhen, presenting with fever and a productive purulent cough with a chest radiograph showing patchy infiltrates (Woo et al., 2005). All attempts to grow a virus failed, however CoV RNA was detected by RT-PCR.

There have been numerous studies from over 12 countries reporting bocavirus prevalence ranging from 2 to 11% in respiratory tract specimens, indicating that the virus has a worldwide distribution (Kesebir et al., 2006).

Despite multiple studies identifying influenza virus as an identifiable aetiological pathogen of adult hospitalised patients with CAP, especially during a known influenza season, anti-influenza antiviral agents are uncommonly used empirically for CAP. Often clinicians wait for viral confirmation of influenza infection before prescribing antiviral treatment. However, in a routine clinical setting, diagnostic testing for influenza is rare, the more common rapid diagnostic methods have low sensitivity, and the more sensitive diagnostic methods such as RT-PCR are neither readily available nor affordable (Murdoch, 2004).

A primary viral pneumonia with a subsequent bacterial infection is also a recognised entity. In a cohort of patients admitted with (outside of influenza season) CAP, influenza was frequently found as a co-pathogen alongside other respiratory pathogens such as *Streptococcus pneumoniae* (Markos et al., 2006). Recently, radiological evidence of pneumonia was observed in 18-66% of patients hospitalised with confirmed H1N1 influenza (Mu et al., 2010).

The management of CAP in adults has traditionally focused little on potential viral causes. This situation is largely due to the lack of specific antiviral agents and the impression that viral pathogens play a relatively minor role in adult pneumonia (Hayden, 2006). Increasing awareness that adult CAP is commonly associated with viral infections, the potential impact of viral vaccines and the increasing availability of antiviral chemotherapeutic agents should direct researchers and clinicians to focus more on the role of respiratory viruses in adult CAP.

Furthermore, mixed infections involving bacterial and other viral pathogens are common and mixed rhinovirus/pneumococcal infection appears to be associated with severe pneumonia (Hayden, 2006). It is likely that the prevalence of both viral and mixed bacterial-viral infection is even higher than currently estimated. Further research should focus on the better characterisation of the viral burden in adult CAP and the interaction between bacterial and viral pathogens (Pevear et al., 2005). The use of empiric antiviral therapy, in addition to empiric antibiotic therapy should be considered in our current CAP guidelines.

1.3.3 Atypical aetiology

The atypical pneumonias could be classified as those that are of zoonotic origin which includes psittacosis, Q fever, and tularaemia, and the non-zoonotic agents which include *Chlamydophila pneumoniae, Mycoplasma pneumonia and Legionella pneumophila*. Both the zoonotic and non-zoonotic atypical pneumonias differ mainly from bacterial CAP with regards to the presence or absence of extra-pulmonary features.

"Atypical" pathogens are an important cause of CAP in a very specific subset of the population (elderly adults, tobacco smokers, immune-compromised and those with chronic illnesses such as chronic obstructive pulmonary disease) should be considered in aetiologic studies to capture epidemiological changes. An example is the prevalence of *Chlamydophila pneumoniae* in a study that showed cyclic variation (van der Eerden et al., 2005).

The clinical importance of the atypical pneumonias is not only closely linked to their clinical incidence, but rather on other clinical and public health aspects (Murray and Tuazon, 1980). The atypical pneumonias require antibiotics that inhibit or eradicate microorganisms (Sopena et al., 1998) such as macrolides and doxycyline. Studies confirm that atypical bacterial pathogens frequently co-infect patients with CAP, possibly in as much as one-third of cases of pneumococcal pneumonia (Ortqvist et al., 1990). Atypical pathogens are often under recognised unless specifically tested for by serology or molecular detection (Houck et al., 2001).

Mycoplasma pneumoniae is difficult to culture, and routine diagnosis is dependent on serology. Multiple primers for PCR-based detection of *Mycoplasma pneumoniae* have been developed for respiratory specimens but there may be a poor correlation between antibody response and PCR because the pathogen could be detected in patients without respiratory disease or remain undetected in patients with respiratory manifestations (Daxboeck et al., 2003).

Legionellosis clinically presents as two distinct entities: (i) Legionnaires' disease, a severe disease presenting as pneumonia and (ii) Pontiac fever, an influenza-like

illness (Fields et al., 2002). Legionnaires' disease manifests as a more acute and severe clinical picture than either chlamydial or mycoplasmal CAP. This is probably due to its ability to multiply intracellularly as well as its ability to avoid lysosomal destruction (Swanson and Hammer, 2000). A diagnostic tool is the urinary antigen test (Plouffe et al., 1995). The test is both highly sensitive and specific for *Legionella pneumophila* serogroup 1. A positive urinary antigen test on the day of admission indicates Legionnaires' disease. However, a negative test does not exclude the possibility of infection since this assay is limited to those who are infected with *Legionella pneumophila* serogroup 1. Those with mild disease due to other *Legionella* serogroups may also have a negative urinary antigen test.

During the period 11 November 1985 - 21 February 1986, 12 cases of Legionnaires' disease were identified at a Johannesburg teaching hospital. Only 2 patients were proven to have acquired the disease in hospital. An epidemiological investigation was undertaken which proved that the aetiological agent might have been acquired from a wide variety of water sources. *Legionella pneumophila* was cultured from the hospital hot-water system. Cases were clustered in the medical and surgical intensive care units. This was the first investigation of an outbreak of Legionnaires' disease in South Africa (Strebel et al., 1988).

1.3.4 Fungal aetiology

Even though CAP caused by fungi is not a common aetiology in the general population, pathology in a rapidly expanding immune-compromised population is encountered increasingly (Miyashita et al., 2001). Other directed epidemiological

studies have described the growing incidence of fungal pneumonia in high-risk groups such as those with AIDS (Bochud et al., 2001).

The diagnosis of *Pneumocystis jiroveci* pneumonia (PJP) has always been a challenge given the difficulty in culturing the responsible pathogen. Detection of *Pneumocystis jirovecii* (formerly known as *Pneumocystis carinii f. sp. hominis)* often relies on immune-fluorescent microscopy, and sensitivity is affected by sampling variability and experience of the technician. The results depend on the quality of the sample thus supporting the recommendation for routine induced sputum in these cases (Pitchenik et al., 1986). Molecular based diagnostics could improve PCP diagnosis by detection of DNA (Arcenas et al., 2006).

The Fungitell assay (Associates of Cape Cod, Inc.) intended for diagnosis of invasive fungal infections is a commercial test that detects (1→3)-β-D-glucan. (1→3)-β-D-glucan (BDG) is a component of the cell wall of fungi. Its presence in the bloodstream correlates with a possible aetiology of PJP (or other invasive fungal infections). Studies suggest that the Fungitel assay may be useful in the presumptive diagnosis of invasive fungal infections (Pickering et al., 2005). Staining with monoclonal antibodies for *Pneumocystis jirovecii* has gradually been replaced by BDG serum levels as a diagnostic assay with high negative predictive value and sensitivity and specificity above 80% for the diagnosis of PJP (Acosta et al., 2011).

1.4 Risk factors

Risk factors have been defined for a number of pathogens, including both typical bacteria and atypical organisms. A recent review of the aetiology of CAP listed

some of these factors (Apisarnthanarak and Mundy, 2005). Recognized risk factors for *Streptococcus pneumoniae,* the most common of the aetiological agents of CAP, include dementia associated with old age, seizure disorders, cardiac pathology, cerebrovascular disease, chronic obstructive pulmonary disease, HIV infection, African race, overcrowded living conditions, and smoking. Risk factors for *Staphylococcus aureus* include advanced age, chronic lung pathology, and previous antibiotic use, whereas the risk factors for *Haemophilus influenzae* are chronic obstructive pulmonary disease treated with repeated cycles of antibiotics or oral steroids.

1.5 Empiric treatment

Since an aetiological diagnosis of CAP is usually unknown, the initial empirical treatment is often guided by microbial patterns described with regards to the risk categories. The increased mortality in patients with severe CAP who do not receive empiric antibiotics that cover the infecting pathogens has been well studied (Leroy et al., 1995). Although conventional tests such as sputum and blood cultures have limited value in some cases of CAP (Waterer and Wunderink, 2001), an aetiological identification is relatively likely and aetiological-directed therapy is associated with a better outcome in patients with severe CAP (Van der Eerden et al., 2005).

Recommended antibiotic therapy differs between the various guidelines and is most likely due to differences of infections caused by CAP organisms, in antibiotic resistance, and in the interpretation of the clinical relevance as well as antibiotic licensing.

The challenge at the time that the clinician is providing treatment to a hospitalised patient with CAP is that he or she neither knows what the aetiological pathogen is nor knows its susceptibility patterns. Since the aetiological agent of CAP is often outdated and not determined locally, the initial empirical treatment is guided by microbial pathogens described for specific risk categories (Lim et al., 2003). For example, the currently available guidelines for CAP management advocate empirical antibiotic treatment based on the age of the patient, present co-morbidities and initial assessment of the severity of the illness (Lim et al., 2009).

Recommendations of empiric antimicrobial treatment according to the severity assessment are routinely based on the concept that the risk of adverse outcomes in patients receiving inadequate initial antimicrobial treatment increases with pneumonia severity (Roson et al., 2001). This includes excess mortality.

Guidelines for the treatment and prevention of CAP were first proposed by a European Respiratory Society (ERS) task force in collaboration with the European Society for Clinical Microbiology and Infectious Diseases (ESCMID) (Woodhead et al., 2005). Consensus guidelines from American Thoracic Society, Infectious Diseases Society of America, and Canadian Guidelines for the initial management of CAP advocate empiric therapy with macrolides, fluoroquinolones, or doxycycline (Mandell et al., 2001). The Therapeutic Working Group of the CDC discourages the use of fluoroquinolones due to potential resistance concerns.

Adherence to CAP guidelines also allows the screening of patients into those that need admission and those who don't and can be managed as outpatients (Marrie et al., 2000).

The main disagreement against a pathogen directed approach is the lack of sensitivity and specificity of the routine diagnostic methods currently employed (Niederman et al., 2001). For this reason, sputum examination by gram stain and culture were not recommended by the American Thoracic Society guidelines. Another argument against a pathogen directed approach is that atypical bacterial pathogens such as *Mycoplasma pneumoniae, Chlamydophila pneumoniae* and *Legionella pneumophila*, for which the prevalence ranges from 8% to 63%, cannot be identified by conventional microbiological methods during the first days of treatment (Fang et al., 1990).

The South African Guidelines for Community acquired pneumonia in adults was last updated by a working group of the South African Thoracic Society, which included members of the Critical Care Society of Southern Africa, and the Federation of Infectious Diseases Societies of Southern Africa in 2007. Serological testing for 'atypical pathogens', additional tests for microbial antigens or antibodies and polymerase chain reaction techniques are not recommended.

According to the Working Group of the South African Thoracic Society the treatment of choice for CAP in severely ill adults "is a combination of parenteral amoxycillin-clavulanate or a second or third generation cephalosporin or ertapenem in specific circumstances, together with an aminoglycoside (gentamicin or amikacin or

tobramycin) and a macrolide (erythromycin, clarithromycin or azithromycin)" (Feldman et al., 2007). The aminoglycoside is added initially because of the relatively high prevalence of CAP associated with aerobic Gram-negative bacilli documented previously in various intensive care unit studies in South Africa. Single daily dosing is recommended for the aminoglycosides, together with routine monitoring of trough serum drug levels. Aminoglycosides may be discontinued if organisms other than Gram-negative bacteria are isolated. Aminoglycosides are best avoided, or used with extreme caution, in the elderly and in patients with renal impairment (Feldman et al., 2007).

Empiric ertapenem should only be used in patients who have failed standard first-line antibiotic therapy for CAP, particularly as part of directed antibiotic therapy based on the results of microbiological testing (Feldman et al., 2007).

Considering the limitations of current diagnostic tests for viral confirmation, empiric therapy for viral causes CAP is an option as testing is more expensive (Mc Greer et al., 2007).

Seasonal and year-to-year variations in multiple studies indicate that, during numerous influenza seasons, an estimated 10%–15% of adult patients with pneumonia and/or symptomatic pulmonary pathology are likely to be caused by influenza virus (Jennings et al., 2008).

As the rates of viral and atypical pathogens are very similar in CAP, adequate treatment should be of consideration (Arnold et al., 2007).

1.6 Risk assessments

Clinicians estimate the severity of pneumonia and fail to recognise those at higher risk; therefore all guidelines recommend that clinical findings should be supplemented by objective severity scoring. Two main scoring systems are commonly in use: the pneumonia severity index (PSI) and CURB-65. PSI (Appendix 4) classifies patients in terms of their expected mortality based on the presence of co morbidity, vital signs and laboratory abnormalities. The CURB-65 (Appendix 3) score provides a guide to identify more severely ill patients on the basis of only five variables (Ewig et al., 2006).

The decision to provide treatment outpatient or inpatient treatment for CAP is extremely important, however admission rates for patients with CAP vary significantly (Lim et al., 2003). The two previously mentioned prediction rules are used to help in this selection process. The PSI assigns points to patients on the basis of 20 different variables and then assigns patients to 1 of 5 risk classes on the basis of the points calculated (Fine et al., 1997). Usually, patients that fall within class I–III receive treatment and clinical management as outpatients, whereas those that fall in classes IV and V are generally hospitalised.

CURB-65 is an acronym and represents a point scale based on confusion, urea level, respiratory rate, breaths/min, low systolic or diastolic blood pressure, and age. Patients with a score of 0 or 1 receive treatment as outpatients, those with a score of 2 receive treatment in a non- ICU hospital ward, and those with scores of >3 may require admission to the ICU.

However, few studies have assessed the independent effect of the microbial aetiology on PSI and CURB-65 severity scores (Roson et al., 2001). One notable study aimed to investigate the distribution of the aetiology according to a specific clinical setting and severity scores (PSI and CURB-65) and the relative mortality rates in a large series of patients with CAP (Cillóniz et al., 2011). The performance of CURB-65 in predicting mortality in community-acquired pneumonia (CAP) has been tested in two large observational studies in patients with sepsis and implementation of warning scores, for identification of high-risk patients in acute medical wards (Barlow et al., 2007).

In the United Kingdom, the BTS guidelines promote the use of CURB-65. This tool, proved to have 75% sensitivity and 75% specificity at predicting mortality at 30 days in CAP. This was a large prospective multicentre, multinational study (Lim et al., 2003).

In another study, PSI and CURB-65 assessments performed similarly in predicting the 28-day and in-hospital mortality; however; there was a significant mortality in patients with a CURB-65 <3 and PSI <5 whilst differences in the categorisation of severe CAP were noted (Richards et al., 2011).

A large amount of CAP research has been dedicated to comparing the various assessment systems to try and identify which is the most reliable system. In general, all of the previously mentioned systems have limitations, particularly in younger patients, and cannot replace a reliable clinical decision. The results depend on the use to which each system is being put (e.g. inpatient vs. outpatient

management, the requirement of an intensive care bed, or predicting morbidity or mortality, etc.), the particular institution to which it is applied and the severity of CAP of the cohort studied (Waterer et al., 2011).

1.7 Microbiological diagnostics

There is little evidence to suggest that microbial investigation affects mortality, but serves as a guide to antibiotic use as well as providing information for epidemiological purposes (Armitage and Woodhead, 2007). Controversy still exists over sputum Gram stain reporting. Some guidelines recommend that Gram stains of sputa prior to culture are useful to expand antimicrobial coverage (Lévy et al., 1988) but others recommended positive results as a guide to narrow the therapeutic antimicrobial spectrum (Gleckman et al., 1988). In addition, 5 to 38% of CAP cases are due to mixed organisms (Brown et al., 1986). Therefore, Gram staining results should be considered when interpreting the significance of sputum cultures (Gross et al., 2003).

All sputum investigations are dependent on the quality of the specimen (Bartlett 2011). The premises of the Gram stain according to the Bartlett score are that neutrophils indicate infection/inflammation and squamous epithelial cells suggest contamination. Gram stains of the specimen are read at low magnification (x100) and are scored 0, +1, or +2 according to the number of neutrophils seen per field and 0, -1, and -2 according to the number of squamous epithelial cells seen per field. Specimens with a zero or less score are considered inadequate (Bartlett 1974).

Those containing more than 25 neutrophils and fewer than 10 squamous epithelial cells per field are optimal specimens.

Except for the Japanese Respiratory Society (JRS), all societies recommend that two sets of blood cultures be taken from all patients admitted to hospital with pneumonia, preferably before the administration of antibiotics (Macfarlane and Boldy, 2004). The JRS only recommend this in cases of severe pneumonia (Miyashita et al., 2006).

Routine serological investigation for urinary antigen testing for *Legionella pneumophila* and *Streptococcus pneumoniae* are only recommended in severe cases (Woodhead and Macfarlane, 1987). Serological testing may be useful during outbreaks for epidemiological investigations, but with no impact on clinical management. Despite more than a century of microbiological tests, there is still uncertainty about the actual importance of the various organisms that cause pneumonia. The most important reason for this is the difficulty in obtaining samples directly from lung tissue. Lung biopsy and other invasive procedures can be used in very few selected patients that are hospitalised. Microbiological cultures of blood yield results for only 4%–24% of patients that are hospitalised (Sullivan et al., 1972) and <1% of patients that are being managed as outpatients (Woodhead and Macfarlane, 1987).

Numerous indirect microbiological methods may be better suited for patients with CAP. Currently, some clinicians rely mainly on microscopy (Gram stain) and culture of sputum with a good Bartlett score to determine bacterial aetiology (Mundy et al.,

1995). One could also exclude sputum findings for fear of interpreting a contaminated result and treating is as the aetiological agent (Bates et al., 1992). Others also look for bacterial antigen in sputum, serum, or urine (Ortqvist et al., 1990), a serum antibody response (Lieberman et al., 1996), or pneumococcal immune complexes in serum (Liebermann et al., 1996). Despite validation of these methods, none have predicted true microbial aetiology of CAP due to the lack of a well-defined reference standard.

In contrast, methods to determine viral aetiology, including serological methods, are more widely accepted. Conventional diagnostic tests of respiratory tract samples have low diagnostic yield.

1.8 Collection and detection methods

The collection and transport of clinical specimens to the microbiology laboratory is important as the first step in diagnostic testing. Apart from the nucleic acid test used or different RNA/DNA extraction protocol, specimen collection, specifically the inactivation of potentially infectious agents and the preservation and stability of pathogen RNA/DNA remains a critical gap in clinical diagnostics. It is important to note that the nasopharyngeal detection swabs are not of equal quality (Sautter and Wilson, l988).

The ability to recover organisms from swabs used for specimen collection is critical for the laboratory diagnosis of aetiological agents of CAP. It has to be considered that approximately 10-15% of the organisms collected on traditional spun fibre swabs can be recovered from cultures, as well as by nucleic acid amplification (Moore-Ness

and Sautter, 2004). Dacron or rayon flexible nasopharyngeal swab collected during the acute phase of illness is the single best test method for demonstrating current viral infection.

Nylon flocked swabs composed of open nylon fibres potentially release a much higher yield of viable organisms. The flocked swab traps bacteria by capillary action whereas traditional swabs absorb bacteria onto the surface of the cotton fibers thereby trapping them within the fiber matrix. Physical properties are commonly evaluated such as the ability to absorb clinical material from the site of infection, the potential to maintain viability of organisms during transport and the ability to release the material onto the agar surface prior to culturing.

A single nasopharyngeal swab performed moderately well in the detection of *Streptococcus pneumoniae* carriage, and the additional yield with two swabs was not sufficiently high upon culturing (Brugger et al., 2009). The low sensitivity of nasopharyngeal swab cultures needs to be considered into the interpretation of CAP studies.

The collection system, PrimeStore™ MTM, inactivates microbes from clinical specimens and prevents nucleic acid degradation during shipping and storage (Daum et al., 2008). It is temperature stable and suitable for outbreak surveillance. The pathogen specific detection system, PrimeMix, is an all inclusive, one-step PCR reagent mix for point of collection detection.

PrimeStore™ MTM formulation preserves and stabilises RNA/DNA in a wide range of sample matrices including nasal/throat swabs, nasal aspirates, culture and other biological specimens (Daum et al., 2007). PrimeStore™ MTM maintains RNA stability and preservation compared to other storage and transportation media. Using PrimeStore™ MTM, users can accurately quantify viral loads in samples stored under conditions that would significantly degrade RNA in alternative preservation solutions (Daum et al., 2008).

Specimens collected in PrimeStore™ MTM can be transported at ambient temperature without cold-chain shipping. Additionally, cells and microbes from collected specimens are lysed and killed (Daum et al., 2008). PrimeStore™ MTM is compatible with most commercial extraction systems(silica and bead-based) for nucleic acid isolation such as Qiagen and Life Technologies systems. PrimeStore™ MTM includes an internal positive control (IPC) that provides a built in measure of specimen integrity and serves as a carrier species for increasing the DNA and RNA extraction efficiency from samples with few copies. PrimeMix is one-step, real-time PCR blend formulated and optimised as a ready use solution that does not require additional 'mastermix' preparation. It is rapid (about 1 hr from collection to detection), and is stable from days to weeks if refrigerated, and months if frozen (Daum et al., 2007).

1.9 Molecular diagnostics

Nucleic acid detection with the use of real-time polymerase chain reaction (PCR) has been developed for many bacterial and viral pathogens causing respiratory tract infections (Welti et al., 2003).

Conventional microbiological assays for detection of bacterial and viral respiratory tract pathogens have proven to be slow, insensitive, unable to strongly distinguish between infection and colonization, and the yield is heavily decreased by introduction of antibiotic treatment prior to collection of relevant specimens. Molecular diagnostic assays for bacteria and atypical pathogens of community acquired pneumonia have the potential to significantly improve the diagnostic yield and decrease turnaround time required for accurate results. Due to various reasons, these tests often lack standardisation and are not widely available. Consideration should be given to the development and evaluation of companion molecular diagnostic tests for detection of respiratory pathogens in future clinical trials of antimicrobials intended to treat community-acquired pneumonia (Nolte, 2008).

One of the advantages of molecular diagnostics is the possibility of identifying pathogens in patients already receiving antibiotic treatment. Some of these new PCR methods have been evaluated concurrently in a prospective study of patients with CAP (Kais et al., 2006) as prior antimicrobial treatment is an important factor which decreases the diagnostic yield in traditional culture based methods. In a large series, no diagnosis was made in 45% of the cases (Fang et al., 1990).

Molecular diagnostics undertake detection of the common and atypical bacterial pathogens that cause CAP. Analysis can be completed in hours, rather than days, for detection of typical pathogens and weeks for detection of atypical pathogens. This approach eliminates concerns about decreased organism viability associated with transport of specimens and the effects of previous antibiotic therapy. Real-time PCR panels that include the common causative pathogens of CAP could substantially increase the diagnostic yield in clinical practice. However, molecular

platforms in this setting are not validated or standardised or widely available. Currently, these assays will supplement culture-based methods for organisms for which antibiotic sensitivity is of concern.

Molecular assays provide high levels of automation, are less labor intensive, and have sensitivities and specificities quite comparable to those of culture-based methods and are cost effective in the long run. Their potential value is that they can expand the expertise of routine microbiological and virological laboratories to increase their detection of viral and bacterial pathogens previously poorly identified, such as rhinoviruses, coronaviruses, and *Streptococcus pneumoniae* (Oosterheert et al., 2005). A higher diagnostic yield for respiratory pathogens could improve management of CAP but may not decrease antibiotic use, length of hospitalisation, or treatment expenses (Oosterheert et al., 2005*).*

One drawback of PCR-based assays to diagnose PCP is the detection of low levels of organisms that might actually represent colonization and not active infection (Larsen et al., 2004).

1.10 Rationale for study

There are numerous reasons for better understanding of the aetiology of CAP. Knowledge of the microbial or viral pathogen in question allows for the use of antibiotics with a narrower-spectrum of activity that will in turn be directed towards a specific microorganism. This approach would limit the use of broad-spectrum antibiotics, avoid antibiotic-related selection pressure and decrease or impede the development of resistance. Early initiation of antimicrobial therapy is important in the management of severe CAP.

Geographic variability is an important factor in the epidemiology of CAP. It requires periodic surveys in multiple areas and is essential to increase background knowledge with regards to the aetiology of CAP.

By collecting information on the various aetiological pathogens responsible for CAP in a particular region and information on their susceptibility patterns, we provide useful background reference for the management of individual cases and to national treatment guidelines. Definitive knowledge of the aetiological pathogen enhances the capacity of the clinician to interpret CAP whether it is to de-escalate empiric therapy or withdraw antibiotics if a viral organism is the aetiological pathogen. Risk factors for specific pathogens and risk factors for antimicrobial resistance often overlap, and there are no definitive data linking risk factors for pathogens or resistance to prognosis.

It is interesting to note that Ewig et al., (2002) reported 82 of 204 (40%) patients with CAP without an aetiological agent, despite a substantial diagnostic effort. A multivariable analysis revealed age, renal and cardiac co-morbidity, and non-alveolar radiological pattern as independent risk factors for an unknown aetiology. Oddly, mortality was not different between patients with and without an aetiology for CAP (Ewig et al., 2002). An interesting confounder, which may have accounted for the number of undiagnosed cases, is incomplete diagnostic work-up. The greatest limitation in the performance of microbiological research is the lack of sensitivity and specificity of current routine diagnostic methods. An extensively broad and fully

encompassing aggressive diagnostic approach may have affected the diagnostic yield.

The aim of the present study was to investigate the aetiology of all bacterial and viral cases of community-acquired pneumonia that occurred in the adult patients that were admitted to the hospital. The study also assessed the incidence of mixed infections, by utilising a new diagnostic PCR platform in addition to conventional microbiological methods. The confounding factor of antimicrobial pretreatment and co-infection with tuberculosis was excluded.

1.11 Study Objectives

1.11.1 Primary

The aim of the study was to describe the microbial aetiology of CAP in adults presenting to an urban tertiary-care hospital in Gauteng, South Africa.

1.11.2 Secondary

To determine by PCR detection the DNA/RNA preserved/ stabilized in PrimeStore™ MTM from nasopharyngeal swabs from patients that met the inclusion criteria of the study prior to their first dose of antibiotics. Samples collected by PrimeStore™ MTM were stored at room temperature for up to 72 hours until they were processed by PCR.

1.11.3 Design

This was a prospective, descriptive, clinical and microbiological investigational study.

1.11.4 Study site

This study was performed at the Charlotte Maxeke Johannesburg Academic Hospital, in South Africa. Acutely ill patients were enrolled as they were admitted to

the casualty, and thereafter to the infectious diseases ward or other medical wards at the hospital. The site of care of each patient was recorded post admission. Patient enrollment was sequentially begun at the beginning of June 2010 and completed at the end of November 2010

CHAPTER 2: MATERIALS AND METHODS

2.1 Definitions

2.1.1 Community Acquired Pneumonia

Refers to a lower respiratory tract infection not acquired at a hospital or a long-term care facility. Pneumonia is an acute infection of the lung parenchyma associated with clinical and radiological evidence (Feldman et al., 1995).

2.1.2 CAP in a community setting

Refers to a complex of symptoms of sweating, fevers, shivers, aches, pains and/or temperature of 38℃ with symptoms of an acute lower respiratory tract illness (for example coughing) and new focal chest signs on examination (Lim et al., 2009).

2.1.3 CAP in patients admitted to hospital (mandatory chest x-ray).

Acute lower respiratory tract infection associated with signs and symptoms that correlate with radiological changes for which there is no other explanation. This disease episode is the primary reason for hospitalisation (Lim et al. 2009).

2.1.4 “Definite” aetiology of CAP

A pathogen is generally considered to be of definite aetiological significance if it is cultured from blood or if the urine antigen assay for *Legionella pneumophila* is positive (Kalin, 1982).

2.1.5 “Probable” aetiology of CAP

The identification of an organism in sputum culture is accepted to be of probable significance (Kalin, 1982). A viral or bacterial aetiology is deemed probable if detection by reverse transcriptase PCR testing for respiratory viruses and PCR for any bacteria is positive.

2.1.6 “Possible” aetiology of CAP

The concept of a “possible” aetiology is based on novel adopted clinical practices for the interpretation of a positive (1→3)-β-D-glucan > 500 as well as a radiological picture of PJP which is suggestive of a fungal aetiology of *Pneumocystis jivorecii* pneumonia.

2.2 Sample population

A total number of 104 patients were screened for the study and 48 patients met the inclusion criteria. The aim was for a sample size of 50 patients but the strict inclusion criteria as well as the cost of materials were limiting factors that affected the final sample size.

2.3 Patient selection

Both male and female adult patients (age ≥ 18 years) with CAP who were examined or admitted to the Charlotte Maxeke Johannesburg Academic Hospital were screened for the study. Patients were informed about the study, and those who gave written informed consent were then further screened according to inclusion criteria based on clinical and radiological grounds. Subsequently, a questionnaire and case report form was completed for each patient.

Ethical approval was obtained from the University of Witwatersrand, Human Research Ethics Committee/R14/49 (Appendix 5). The study details were discussed and a written information sheet (Appendix 2) handed out to all participants prior to obtaining written informed consent (Appendix 1). A clinical data sheet (Appendix 2)

was used to record all relevant clinical details. Study participation did not disrupt the participants' routine diagnostic work-up.

The Charlotte Maxeke Johannesburg Academic Hospital is an accredited central hospital with 1088 beds serving patients from across the Gauteng province and neighboring provinces. The hospital offers a full range of tertiary, secondary and highly specialised services. The hospital is located in Parktown and serves as a referral hospital for a number of hospitals in its referral chain. No walk-in patients are seen. All patients need to be referred via a medical practitioner, clinic or hospital within the referral system.

2.4 Diagnosis of CAP

All patients were assessed by an infectious disease clinician to confirm whether they met the inclusion criteria:

A) Radiological changes consistent with CAP and two or more of the following signs or symptoms:

1. On admission:
    a. Medical history of productive cough with sputum production with or without pleuritic chest pain (not more than a five day history).
    b. Physical examination on day of admission in casualty
        i. Vital signs
            1. Fever >38 ºC or Hypothermia <35 ºC
            2. Confusion/cyanosis
            3. Tachypnoea (>20 breaths per minute)
            4. Blood pressure (<90/60 mmHg)
        ii. Chest examination

1. Dullness to percussion
2. Decreased breath sounds
3. Bronchial breathing
4. Crepitations

c. Laboratory

1. Leukocytosis (>12 000 cells/mL)
2. Leukopenia (<4000 cells/mL)
3. Urea >7 mg/dL
4. HIV (optional on clinician request- this wasn't an exclusion criteria)
5. (1→3)-β-d-glucan serum level if clinically indicated
6. *Streptococcus pneumoniae* serotyping (if blood culture positive)
7. CURB-65 scoring system (confusion, urea>7mg/dL, respiratory rate more than 30 breaths/min, blood pressure 90/60 mmHg and age above 65 years was used to determine severity of pneumonia and admission to the wards or the ICU.

2. Radiological findings as per criteria in Appendix 2

2.5 Exclusion criteria

a. Lack of consent
b. Age <18 years
c. Cystic fibrosis patients
d. Patients with suspected tuberculosis:
   i. Presence of cavitatory lung disease based on chest x-ray at admission and or,
   ii. Presence of acid fast bacilli on sputum microscopy confirmed within 24 hours of current admission.

e. Patients on tuberculosis treatment.
f. Patients transferred from other hospitals with symptoms of pneumonia.
g. Patients re-admitted within 2 weeks of previous admission.
h. Resident of long-term care facility such as old age homes, or other similar institutions.
i. Antibiotic use within the previous 48 hrs (from patient history).

A full clinical and radiological assessment was done at the bedside and recorded on the patient data sheet. All additional routine laboratory testing, as requested by the attending physician was also recorded.

2.6 Microbiological specimen collection and testing

2.6.1. Sputum microscopy, culture and sensitivity

Patients were given instruction on how to produce a deep cough specimen of sputum. They were asked to cough forcefully and spit out sputum into a container. Five milliliters of expectorated sputum was collected in a sterile container and submitted for microbiological diagnosis. Sputum was collected before antibiotic administration and submitted to the microbiology laboratory for microscopy. Samples containing neutrophils and a limited number of squamous epithelial cells were considered acceptable for culture, according to the established criteria of a Bartlett score greater than zero (Bartlett 1974). Antibiotic susceptibilities were determined for *Streptococcus pneumoniae* and *Haemophilus influenzae.* The minimum inhibitory concentration (this is the lowest concentration of an antimicrobial that will inhibit the growth of an organism) of penicillin in *Streptococcus pneumoniae* was determined according to guidelines of a reference body, Clinical Laboratory

Standards Institute (CLSI, 2010). A sputum immunofluorescence assay was used to determine the presence of *Pneumocystis jirovecii*.

2.6.2 Nasopharyngeal swab

Nasopharyngeal swabs were collected and stored in the PrimeStore™ MTM for PCR testing of typical and atypical bacterial organisms: *Streptococcus pneumoniae, Haemophilus influenzae, Mycoplasma pneumoniae, Chlamydophila pneumoniae, Bordetella pertussis and Legionella pneumophila* as well as the following respiratory viruses; adenovirus; influenza A virus; influenza B virus; respiratory syncytial virus A; respiratory syncytial virus B; metapneumovirus; parainfluenzavirus 1, 2 and 3; rhinovirus A/B; coronavirus 229E/NL63, coronavirus OC43/HKU1and bocavirus.

2.6.3 Sputum auramine stain

Another sterile container was used to collect sputum for a fluorescent auramine stain to exclude the presence of AFB (acid-fast bacilli) as it was an exclusion criterion.

2.6.4 Blood cultures

Blood for culture and bacterial identification was collected. Blood was obtained by inserting a needle into a vein in the arm. The drawing site was thoroughly cleaned, usually with an isopropyl alcohol solution, applied in a circular pattern and then allowed to dry. The phlebotomist drew about 20 milliliters of blood and put it into two culture bottles containing broth. These two bottles constitute one blood culture set. The sample was sent for routine bacterial culture, identification and susceptibility testing.

2.6.5 Urine antigen

Urine samples for *Legionella pneumophila* testing were collected and an immunochromatographic membrane test (BinaxNOW *Legionella;* Inverness Medical Innovations) was performed on urine samples for detection of *Legionella pneumophila* serogroup 1 antigen within 24 hours of collection.

2.6.6 (1→3)-β-D-glucan (BDG)

Serum samples were collected from patients with a clinical and radiological picture of PJP.

2.6.7 HIV status

HIV antibody testing was performed on clinical grounds at the discretion of the attending clinician following consent from the patient.

2.6.8 *Streptococcus pneumoniae* serotyping

Streptococcus pneumoniae isolates from blood were sent to the Respiratory and Meningeal Pathogens Research Unit at the National Institute for Communicable Diseases (The Group for Enteric, Respiratory and Meningeal disease Surveillance in South Africa (GERMS-SA)) to undergo Neufeld's Quellung reaction (Lalitha et al., 1996). The high cost and the demanding technical expertise required for this reaction prohibit the adoption of this method for regular serotyping in a routine laboratory.

2.7 Molecular methods

The Seeplex RV5 ACE screening for the detection of adenovirus; influenza A virus; influenza B virus; respiratory syncytial virus A; respiratory syncytial virus B; metapneumovirus; parainfluenzavirus 1, 2 and 3; rhinovirus A/B; coronavirus 229E/NL63; coronavirus OC43/HKU1 and bocavirus was used.

The second kit utilised for the detection of *Streptococcus pneumoniae, Haemophilus influenzae, Mycoplasma pneumoniae, Chlamydophila pneumoniae, Bordetella pertussis* and *Legionella pneumophila* was called the Seeplex Pneumobacter multiplex PCR kit.

2.8 Diagnosis of aetiology

The diagnosis of CAP is conventionally defined as signs and/or symptoms compatible with a respiratory tract infection in the presence of new consolidation on a chest radiograph. The chest radiograph is the gold standard (Hedlund et al., 2005). A pathogen was considered to be of definite aetiological significance if it was cultured from blood, or if the urine antigen assay for *Legionella pneumophila* was positive. Detection of *Legionella pneumophila* by PCR from nasopharyngeal swab was also considered as definite support for the aetiology.

In accordance with previous findings of patients with bacteremic pneumococcal pneumonia, identification of *Streptococcus pneumoniae* in sputum culture was accepted to be of probable significance (Kalin, 1982). A viral or bacterial aetiology

was deemed probable if detection by reverse transcriptase (RT)-PCR testing for respiratory viruses and PCR for any bacteria was confirmed.

2.9 Clinical data collection and analysis

Each patient's medical records were reviewed for clinical data. This descriptive study employed simple statistical analysis including mean, percentage, and correlation between variables. Interviews with the patients or next of kin provided an opportunity to follow a line of questioning that obtained more in-depth information.

The study required little interference on the part of the observer other than routine clinical practices as well as the nasopharyngeal swabbing. Data collection sheets involved recording and timing the events observed, transcribing data into coding sheets and thereafter transferring data from the coding sheets onto a database for data analysis. Data was aggregated, interpreted according to inclusion criteria and thereafter analysed to produce a variety of graphic data representations.

CHAPTER 3: RESULTS

3.1 Patient demographics

The baseline characteristics of the patients are shown in the table 3.1. The study population consisted of 20 males and 28 females. The mean age for males was 43.9 years and the mean age for females was 36.3 years. A total of 48 patients had at least one underlying disease, with HIV infection being the highest co morbidity (60.4%). Five patients were confirmed smokers. The other co-morbidites included five patients with chronic obstructive pulmonary disease (COPD), two asthmatic patients, three patients in cardiac failure, one patient in liver failure, three patients with diabetes mellitus type II and five patients with confirmed alcohol use.

The mean duration of stay in hospital was 5.1 days (range, 1–6.9 days). None of the 48 patients came from a rural background or had resided in a rural area in the past year nor did they have any prior mining employment history. All 48 patients had access to running water and electricity.

Table 3.1 Patient Demographics

Demographic Data	% (No.)
N	48
Males	41.7% (20)
Females	58.3% (28)
Mean Age	39.5 years
Males	43.9 years
Females	36.3 years
Smoking	10.4%(5)
Site of Care	
General pulmonology ward	68.7% (33)
Intensive care unit	31.2% (15)
Co-morbidity	
HIV	60.4% (29)
COPD	10.4% (5)
Asthmatic	4.1% (2)
Cardiac failure	6.2% (3)
Liver failure	2.0% (1)
Diabetes mellitus	6.2% (3)
Alcohol abuse	10.4% (5)
Symptoms	
Productive coughing	85.4% (41)
Fever	60.5% (34)
Confusion	12.5% (6)
Pleuritic chest pain	14.5% (7)
Radiologic appearance	
Consolidation	62.5% (30)
Pleural effusion	14.5% (7)
Reticular infiltrate	12.5% (6.0)
Ground glass appearance	10.4% (5.0)
Negative Urine Leg. Antigen	100% (48.0)

3.2 Bacterial and viral aetiology as determined by different diagnostic methods

The contribution of the different methods in the determination of aetiology is illustrated by the distribution of 80 pathogens among 48 patients (table 3.2). Blood cultures provided a definite aetiology for 8 (16.7%) of 48 patients. A probable diagnosis was established by sputum culture alone for 5 (10.4%) of 48 patients. This probable aetiology increased (as defined previously) when a molecular PCR platform was added to the conventional methods with the use of the PrimeStore™ MTM swabs to 62.5% (30 of the 48 patients).

The most frequently detected bacteria across all assays was *Streptococcus pneumoniae* (23 times in total) and *Haemophilus influenzae* (14 times in total). Human metapneumovirus, rhinovirus A/B and coronovirus were the most frequently detected viruses (8 times in total or each). Only 4 of those 8 instances had those viruses as probable mono-viral aetiology (blood cultures, sputum cultures and bacterial PCR negative).

There were five patients in whom an aetiological pathogen was never ascertained.

Table 3.2 Bacterial and viral aetiology as determined by different diagnostic methods

PATHOGEN	FREQUENCY OF POSITIVE FINDINGS	POSITIVE BLOOD CULTURE	POSITIVE SPUTUM CULTURE	POSITIVE PCR NASOPHARYNGEAL SECRETION SAMPLE (DNA)	POSITIVE PCR NASOPHARYNGEAL SECRETION SAMPLE (RNA)
S. pneumoniae	23	8	4	20	N/A
H. influenzae	14	0	3	14	N/A
Metapneumovirus	8	N/A	N/A	N/A	8 *
Rhinovirus A/B	8	N/A	N/A	N/A	8 *
Coronavirus 229E/NL63/ OC43/HKU1	8	N/A	N/A	N/A	8 *
Influenza A	6	N/A	N/A	N/A	6
Adenovirus	3	N/A	N/A	N/A	3
Influenza B	1	N/A	N/A	N/A	1
Respiratory Syncytial virusA/B	1	N/A	N/A	N/A	1
Parainfluenza1,2,3	1	N/A	N/A	N/A	1
M. pneumoniae	1	0	0	1	N/A
Bocavirus	1	N/A	N/A	N/A	1
***S. pneumoniae and H. influenzae* co-infection**	8	3**	2***	8	3****
Negative PCR and negative cultures but BDG>500	5*****	N/A	N/A	N/A	N/A

(REPRESENTING THE DISTRIBUTION OF 80 PATHOGENS AMONG 48 PATIENTS)

NOTE: All urine *L. pneumophila* Serogroup 1 antigen tests were negative

*****All 3 bands appeared together in all 8 positive samples.

** In these 3 cases blood culture only yielded *S.pneumoniae. H.influenzae* was not cultured**.**

*** In these 2 cases sputum culture only yielded *S. pneumoniae. H. influenzae* was not cultured.

**** Case #1 had a viral co-infection with influenza B. Blood culture positive *S. pneumoniae* only. Sputum culture neg. Case #2 had a viral co-infection with Adenovirus and influenza A. No positive bacterial cultures despite positive Pneumobacter. Case #3 had a viral co-infection with Influenza A. Blood culture positive *S. pneumoniae* and sputum culture negative**.**

***** Only 1 case positive by monoclonobal antibody assay for *Pneumocystis jirovecii* pneumonia

3.3 Microbial aetiology of CAP

A total probable microbial aetiology (as defined previously) of CAP was established for 62.5% (30 of the 48 patients) when the PCR platform for respiratory viruses was added to the conventional methods with the use of the PrimeStore™ MTM swabs (table 3.3). In contrast, the definite bacterial aetiology was 16.7% (8 of 48 patients) when conventional culture methods were used, none of whom had more than one bacterial species identified. There were five patients in whom an aetiological pathogen was never ascertained. The urine *Legionella pneumophila* antigen was negative for every single patient. Of the 16.7% of patients with a definite bacterial aetiology *Streptococcus pneumoniae* was isolated from blood cultures in all patients (table 3.3).

There were five patients in whom blood cultures and sputum cultures were negative but the clinical and radiological reporting was indicative of PJP with a positive (1→3)-β-d-glucan >500. For this group of patients the term possible aetiology was introduced and these patients were categorised as such.

Table 3.3 Microbial aetiology of CAP

PROBABLE MICROBIAL AETIOLOGY		DEFINITE BACTERIAL AETIOLOGY	POSSIBLE AETIOLOGY
62.5% (30)			
SPUTUM CULTURE	**MOLECULAR**	16.7% (8)	10.4% (5)
10.4%(5)	52.1% (25)		

A probable aetiology was ascertained in 5 patients with positive sputum cultures. All 5 sputum cultures yielded the same isolates when bacterial PCR was undertaken. *Streptococcus pneumoniae* was cultured in 3 of those patients on their sputum and thereafter confirmed on PCR. The other 2 patients cultured *Haemophilus influenzae* on sputum that was confirmed by PCR. One of these patients also had three viral bands representing metapneumovirus, human rhinovirus virus and coronavirus and the second patient was co-infected with respiratory syncitial virus A/B.

An additional 25 patients (in whom sputum and blood culture was negative) had a probable aetiology attributed to their CAP with positive PCR results. The PCR was positive for five patients with both *Streptococcus pneumoniae* and *Haemophilus influenzae.* Three of these five patients also had a positive viral PCR. The first one had a positive influenza B band, the second one was co-infected with adenovirus and influenza A and the third patient had a positive band for influenza A. The remaining two patients had a positive PCR with the previously mentioned dual bacteria only.

The PCR was positive for *Streptococcus pneumoniae* for 8 patients in whom sputum, blood cultures and viral PCR were negative (figure 3.3.1).

Haemophilus influenza PCR was positive in 3 patients in whom sputum and blood cultures were negative. Two of these patients were also negative for the viral PCR. One of these 3 patients had positive bands for human metapneumovirus, rhinovirus, coronaviruses and influenza A.

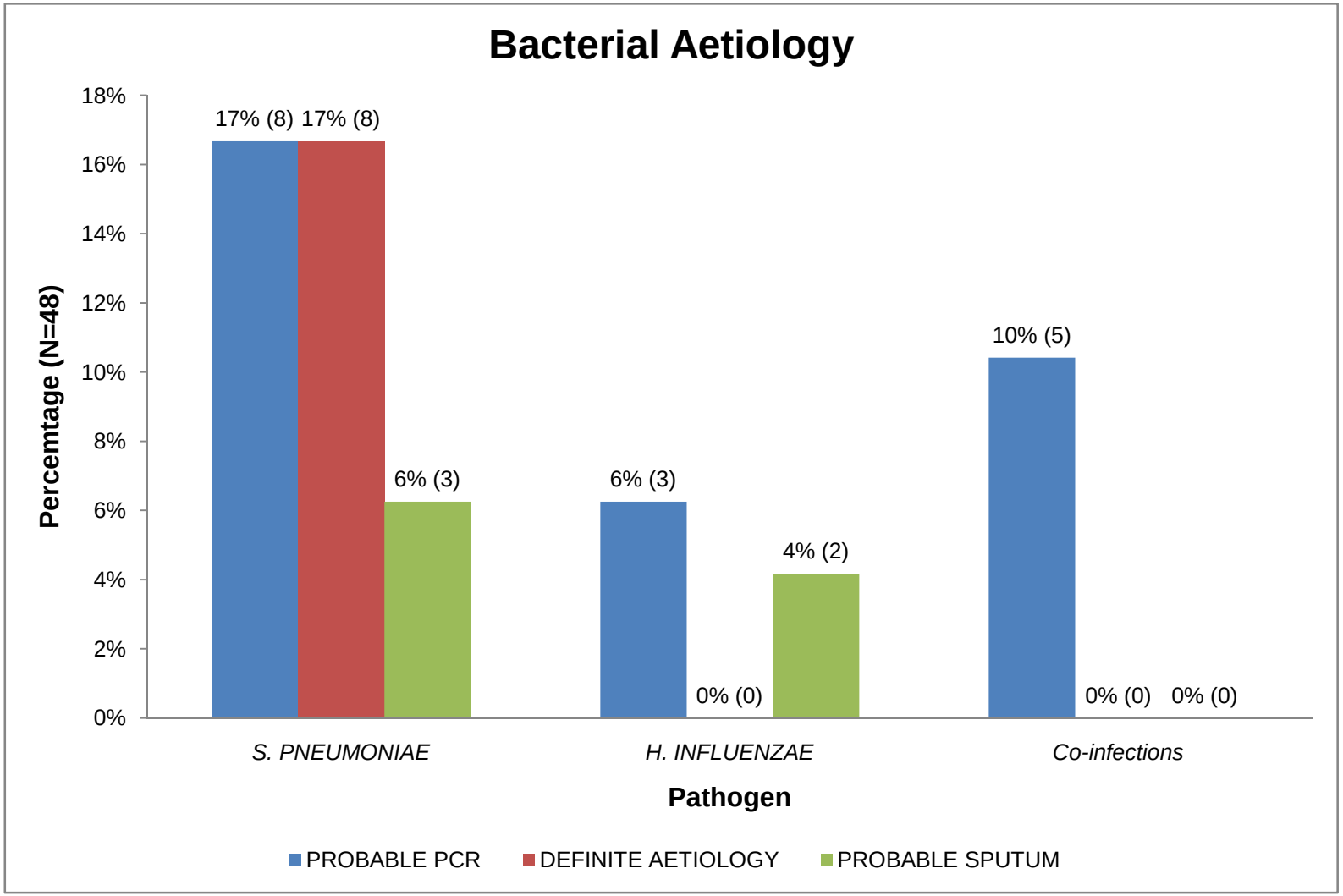

Figure 3.3.1 Bacterial Aetiology

There were nine patients with positive viral PCR's only that were representative of probable CAP aetiologies. From this group of patients, four of them were positive for metapneumovirus, rhinovirus and coronaviruses, three were positive for influenza A and one patient was positive for adenovirus, parainfluenzae virus and bocavirus. The last patient was positive for adenovirus but also had signs indicative of PJP with a BDG>500.

There were five patients in whom blood cultures and sputum cultures had negative tests for other pathogens but the clinical and radiological reporting was indicative of PJP with a positive BDG>500. Out of these 5 positive BDG's only 1 of these patients had a positive immuno-fluorescence assay. One had a mixed infection with a positive BDG and a positive PCR for *Mycoplasma pneumoniae.* Another one of these patients, was a patient mentioned earlier with a positive viral PCR for adenovirus who was also admitted to the intensive care unit and intubated.

Figure 3.3.2 depicts the range of total patients' viral pathogens detected as probable viral aetiology.

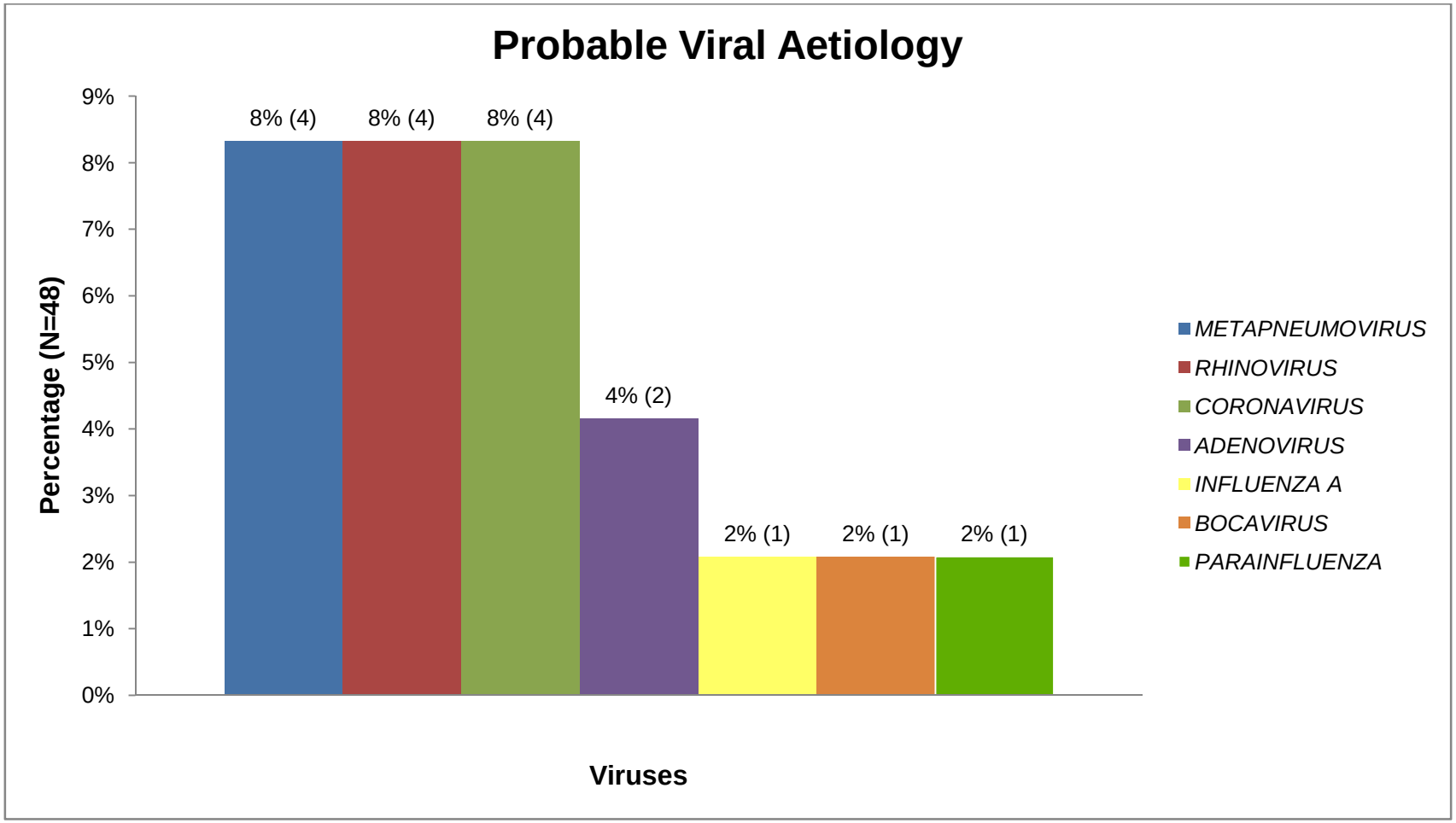

Figure 3.3.2 Probable Viral Aetiology

3.4 Microbial aetiology in the Intensive Care Unit.

Of the 48 patients, five were transferred directly to the intensive care unit from casualty and a further 10 were transferred within 48 hours of admission. An aetiological agent was found in the samples of 12 of these 15 patients (figure 3.4.1).

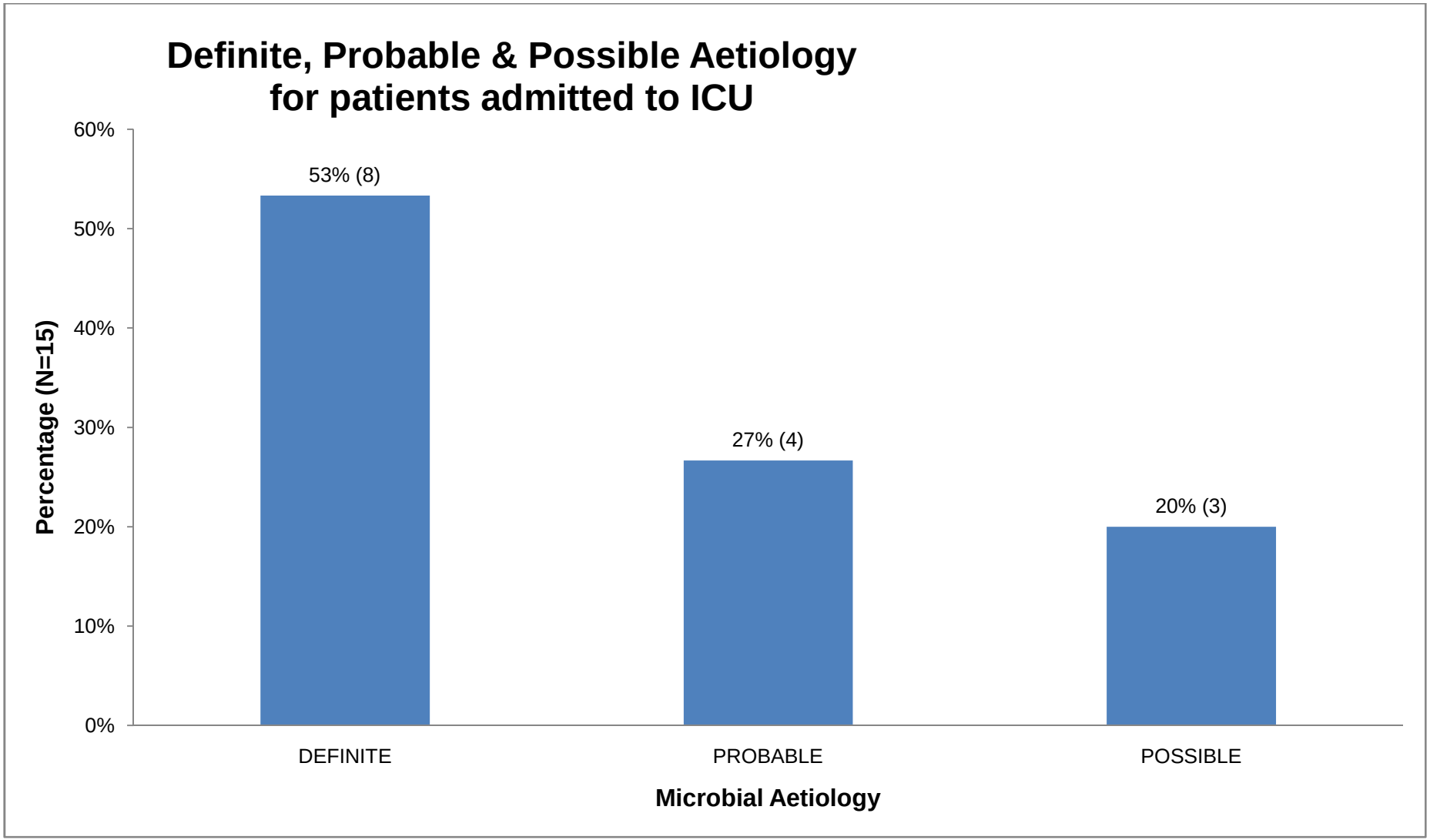

Figure 3.4.1 Definite, Probable & Possible Aetiology for patients admitted to ICU

Eight patients had definite *Streptococcus pneumoniae* as the aetiology of CAP based on positive blood cultures (table 3.4.2). For two of these eight patients, viral PCR was positive for metapneumovirus, rhinovirus and coronavirus as co-pathogens. Bacterial PCR confirmation of *Streptococcus pneumoniae* correlated with another three of these eight definite cases. Another two of these eight patients with positive *Streptococcus pneumoniae* blood cultures had positive bacterial PCR's for the *Streptococcus pneumoniae* and *Haemophilus influenza* as well as positive viral PCR's representing influenza B for the first case and influenza A for the second case. The last patient in this group was only blood culture positive for *Streptococcus pneumoniae*.

In the probable aetiology group of admissions in ICU, two patients were positive for *Haemophilus influenza* PCR but were negative for blood cultures and sputum cultures. For one of these two patients, metapneumovirus, rhinovirus and coronavirus were also detected. The second patient also had a positive *Streptococcus pneumoniae* PCR.

One of the 12 patients admitted to the ICU had a positive PCR for influenza A.

The three patients without a known aetiological agent presented severely tachypnoeic, BDG>500 (positive) with their chest x-rays suggestive of PJP. Only one of these three patients had a positive PJP immuno-fluorescence assay. Another one of these three patients had a positive viral PCR for adenovirus.

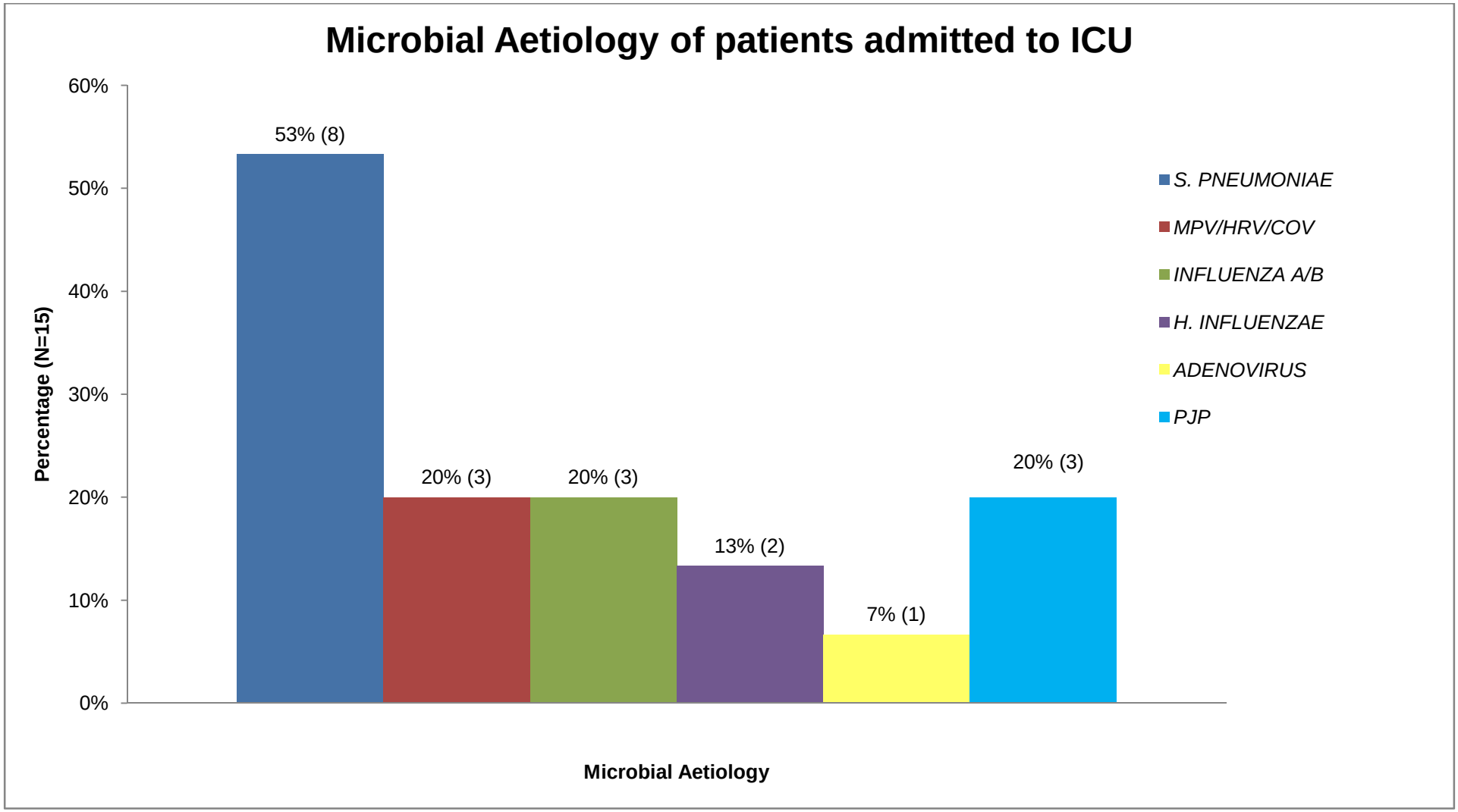

Figure 3.4.2 Microbial Aetiology of patients admitted to ICU

3.5. *Streptococcus pneumoniae* serotypes

All eight-blood culture confirmed *Streptococcus pneumoniae* isolates were from patients admitted to ICU. The serotypes identified were 1, 4, 9N and 19A as summarised in table 3.4. One isolate was missing from the NICD serotyping database. The clinical features of bacteraemic pneumococcal pneumonia were similar in HIV-seropositive (5) and HIV-seronegative (3) patients. All 5 HIV-seropositive patients had underlying risk factors other than HIV infection that may have predisposed them to pneumococcal bacteraemia. These underlying risk factors included the following: diabetes mellitus, chronic pancreatitis secondary to alcohol consumption, COPD and cardiac failure. The predisposing co-morbid factors in the 3 HIV negative patients were COPD, diabetes mellitus and cardiac failure.

Table 3.4 Serotype distribution and penicillin minimum inhibitory concentrations (MIC) of *Streptococcus pneumoniae* isolates from blood cultures.

SEROTYPE	N = 8	ICU	PENICILLIN MIC (µg/ml)
9N	1	1	0.38
8	1	1	0.008
1	1	1	0.38
19A	3	3	0.38(all 3)
4	1	1	0.5
MISSING ISOLATE	1	1	0.5

3.6 Microbial aetiology based on severity of CURB-65 score

The most common pathogen was *Streptococcus pneumoniae*. The frequency of other pathogens decreased with severity (figure 3.6.1). *Streptococcus pneumoniae* was also noted to be the most common pathogen for the two extremes of the CURB-65 scores (figure 3.6.2).

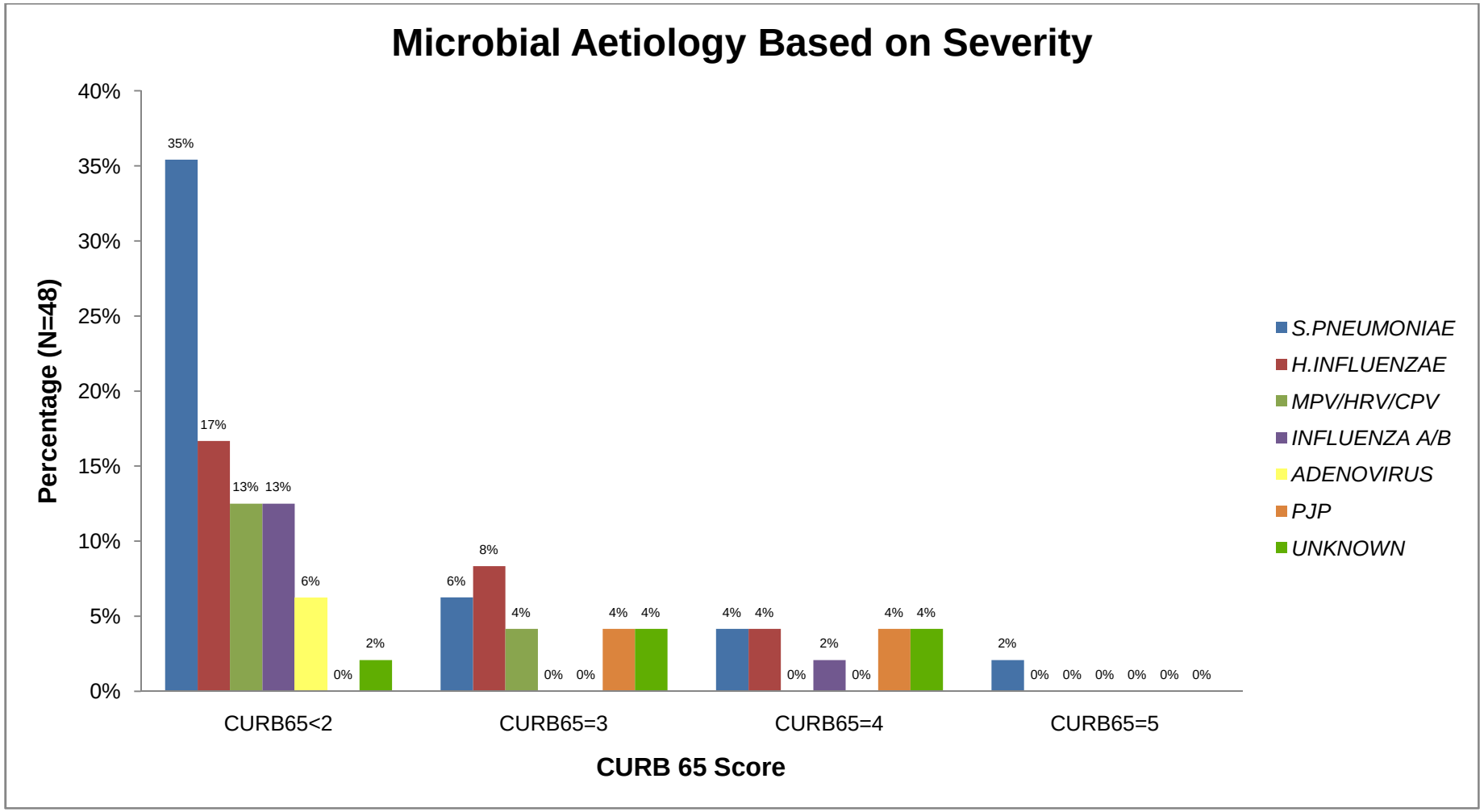

Figure 3.6.1 Microbial Aetiology Based on Severity

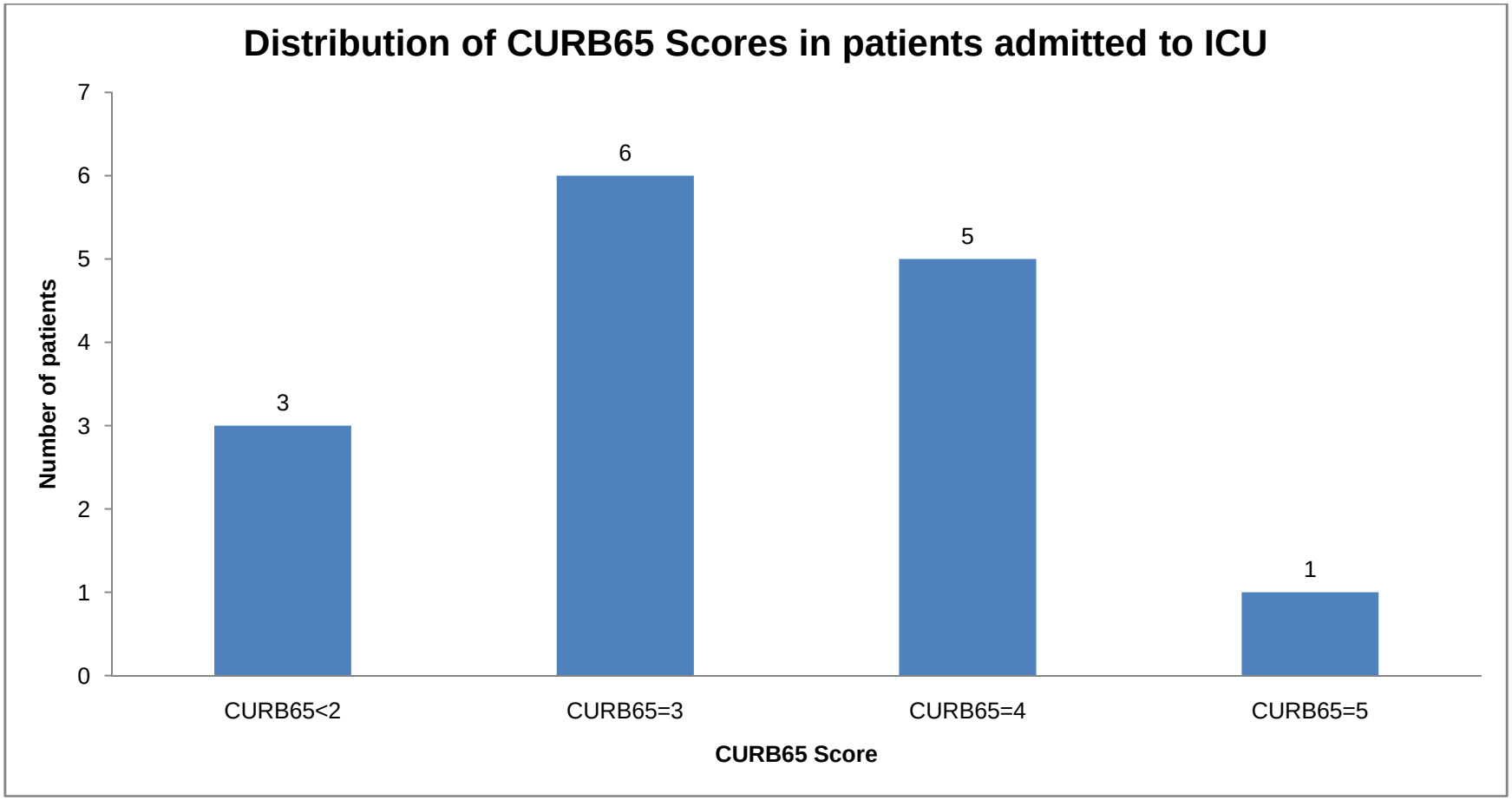

Figure 3.6.2 Distribution of CURB65 Scores in patients admitted to ICU (N=15)

3.7 Initial antibiotic therapy

Empirical antimicrobial treatment was administered in the casualty on admission by the casualty officer. In the cohort of patients in whom empiric antimicrobial treatment was administered, 17 patients received amoxicillin-clavulanic acid and one of fluoroquinolone, 10 patients received piperacillin-tazobactam and an aminoglycoside, six patients received cotrimoxazole, four patients received penicillin G and an aminoglycoside, four patients received ertapenem, four patients received ceftriaxone (three with cloxacillin and one without cloxacillin) and three patients received a fluoroquinolone (table 3.5).

None of the patients received intravenous second-generation cephalosporins and an aminoglycoside or a macrolide.

In HIV-infected patients presenting acutely with bilateral pulmonary ground glass infiltrates suspected to be due to *Pneumocystis jirovecii,* empirical therapy with cotrimoxazole was begun in 6 of the 48 patients with CAP.

Three patients were empirically initiated on an intravenous fluoroquinolone on admission to casualty.

Table 3.5 Initial antibiotic therapy

INTRAVENOUS ANTIBIOTIC	NUMBER OF PATIENTS (N=48)
Amoxicillin-clavulanic acid & a Fluoroquinolone	**17**
Piperacillin-tazobactam & an Aminoglycoside	**10**
Cotrimoxazole	**6**
Penicillin G & an Aminoglycoside	**4**
Ertapenem	**4**
Ceftriaxone ± Cloxacillin	**4**
Fluoroquinolone	**3**

CHAPTER 4: DISCUSSION

Four major findings are reported in this study. Firstly, the total microbial yield was significantly improved in comparison to those reported previously (Jones et al., 2010). An initial aetiology based on culture positivity was present in only 27% of the study (n=13) which increased to 52.1% (n=25) with the use of a molecular platform. The aetiological yield improved with the implementation of nasopharyngeal secretion samples that were then stored in PrimeStore™ MTM for further analysis by PCR for respiratory viruses, bacterial and atypical pathogens. Secondly, given that the major co-morbid factor in this cohort of patients was HIV (60.4%), it was notable that *Streptococcus pneumoniae* was still the leading causative agent of CAP (48%). Thirdly, despite respiratory viruses being found at a high frequency as part of a mixed infection, usually in combination with *Streptococcus pneumoniae* and *Haemophilus influenzae;* they were identified as the single pathogen in only 9 of 48 patients with clinical and radiologically confirmed CAP. Lastly, as the primary objective of this study was to describe the aetiology of CAP, the lack of atypical pathogens in this series was also noted. One patient had a positive PCR for *Mycoplasma pneumoniae* in whom blood cultures and sputum cultures were negative but the clinical and radiological reporting was also indicative of PJP with a positive BDG>500.

Attempting to establish a microbial aetiology for patients with CAP is challenging. Despite numerous studies to determine aetiology, the causative organisms are not found in almost half of the clinically diagnosed cases (Steinhoff et al., 1996).

In the present study, at least one aetiological agent was found in 39 of 48 cases (81.2%) with well-defined probable or definite microbial aetiology. This high yield could be due to the 48 patients having undergone a complete sampling schedule and no antibiotics given prior to hospital admission. This study was conducted during the winter months of the year when viral infections are also more common. The yield improved from 27% to 52.1% with the addition of PCR testing of nasopharyngeal secretion samples to the traditional diagnostic procedures. These had been stored in a medium ideal for clinical collection and transport that preserves the released nucleic acids. In addition to preserving labile RNA for testing it also contains an internal positive control capable of tracking the degradation of the sample from the point of collection. Diagnosis of viral pneumonia can be challenging. Many viruses do not grow easily in culture and serum antibody testing is often not clinically useful. RNA is rapidly degraded after cell death. Traditional culture techniques detects only viable organisms, unlike PCR, which does not need to distinguish between living and dead organisms thus making this novel transport medium a crucial diagnostic entity. It is possible that the higher diagnostic yield for the viruses detected from our patients is due to the material that prevents the degradation of RNA in the PrimeStore™ MTM.

This novel molecular transport medium allows researchers the flexibility to collect specimens and safely ship them to laboratories without expensive and cumbersome cold chain packaging. For future studies and innovations, the PrimeStore™ MTM could facilitate standard sequencing and meta-genomic analysis of samples by improving the quality of the microbial nucleic acids in the collected specimens when they finally arrive in the laboratory. It would be of value to have an active

comparison between our traditional viral nasopharyngeal swabs in order to validate the PrimeStore™ MTM nasopharyngeal swab. The only limitation with this transport medium currently, is the economic implications of its use, especially within a resource constrained setting such as ours. Despite the potential to offer great promise for improving diagnostic speed and accuracy, for tracking community and nosocomial outbreaks, several limitations currently hinder the widespread implementation of PrimeStore™ MTM. The processing of this specific sample is fully dependent on a molecular platform and the initial cost to set up such a platform is expensive and unlikely to be standard in the near future. These issues need solutions before many of these specialised molecular based storage media can be adopted in clinical practice on a regular basis (Stralin et al., 2006).

A limitation of our study was the use of multiplex PCR that analysed multiple pathogens simultaneously. The multiplex approach has been shown to lack sensitivity in comparison with monoplex techniques (Gröndahl et al., 1999). Alternative strategies minimising the competition between the probes have been developed to allow for primer pairs to be mixed in the same reaction tube without loss of sensitivity. Stralin et al., (2006) compared conventional culture techniques to multiplex PCR for *Streptococcus pneumoniae, Haemophilus influenzae, Mycoplasma pneumoniae and Chlamydophila pneumoniae*. In the examination of sputum and nasopharyngeal samples from adults with CAP sensitivity ranged from 58 to 100% and specificity from 42 to 100%, depending on the organism and the type of sample.

Although these molecular based techniques are sensitive compared to traditional culture methods, absolute pathogen detection is relatively low. One study reported

that real-time quantitative PCR identified only 37.7% of pathogens in the patients with clinically diagnosed pneumonia (Kais et al., 2006). PCR multiplex assays have been shown to exhibit an important range of positive results, the greatest difficulty was to recognise which pathogen is the cause of the community acquired pneumonia and whether the association could play a role in the severity of the disease.

Blood samples for culture are not technically demanding and provide a definite microbial diagnosis when the results are positive, but in the present study, they revealed the aetiology of infection for only 16.7% of all patients, a percentage similar to previous studies (Woodhead et al., 1987). Urine specimens are also easy to obtain, and *Legionella pneumophila* antigen assays are generally considered specific. However, this test did not provide a diagnosis for any of our patients. We haven't assessed the *Streptococcus pneumoniae* urinary antigen assay.

Legionella pneumophila infections are relatively uncommon in HIV-positive CAP patients (Casau, 2004). Most HIV positive patients receive cotrimoxazole as prophylactic treatment for various pathogens and the intrinsic antimicrobial activity of cotrimoxazole against *Legionella pneumophila* might account for this low yield in the majority of our patients but it certainly does not explain the poor yield in the HIV negative patients. There are two possible explanations for the low yield in both patient groups. Firstly, they could be false negative results due to *Legionella* species other than *Legionella pneumophila* serogroup 1, the latter accounting for about 80% of all cases of CAP caused by *Legionella* species (Wimberley et al., 1979) and

secondly, the yield is accepted to be low in adults with non-bacteraemic pneumonia (Bartlett, 2011).

The strengths of this study include the study population and complete sampling schedule. The study population reflects the group of patients referred for more complicated disease in which new diagnostic approaches are needed, i.e. HIV positive individuals. It is interesting to note that our study's aetiological spectrum was rather different from the previously mentioned study in South Africa in 1987, yet the antimicrobial guidelines have remained fairly similar. The reason for the differences noted in previous studies is difficult to ascertain. One possibility is that those studies included patients with co-morbid pathology known to be associated with invasive Gram-negative bacteria causing diseases. Another reason could be that the data from 1987 did not reflect a major proportion of the population since this country was still in an apartheid era and only white patients were admitted. It was also before the AIDS epidemic began in South Africa.

In view of the limited size of this study, it provides incomplete information on the burden of CAP. Aetiological data from developed countries may not be applicable to South Africa as a whole, because of country-specific differences in disease management, cost constraints and hospital admission criteria. South Africa has a patient profile which makes us unique. Additionally, data on epidemiology may be skewed if extrapolated for use in our developing setting. Characteristics of patients in this study are not similar to those reported in previous studies. In previous studies, the majority of the patients who developed community acquired pneumonia

were the elderly with heart disease, COPD, renal failure, diabetes and immune-compromised (Niederman et al., 2001).

Due to the unavailability of a routine viral diagnostic laboratory, traditional viral culture methods were not performed in our patients. More research with regards to viral quantification may be useful in cases when a virus is the only potential pathogen detected (Martin et al., 2008). The clinical correlation of the detection of a virus from the nasopharynx can be challenging. The virus originates from an upper respiratory tract infection and may not be the cause of CAP even as detection of virus can generally be assumed to indicate infection of the lower respiratory tract (Pavia, 2011). Viral quantification may be able to determine when a pathogen is associated with severe disease but more data using standardised methods are needed to validate cutoff values (Martin et al., 2008).

Although it was mentioned earlier that CAP caused by *Streptococcus pneumoniae* has been reported in severely ill patients with higher CURB-65 scores (Rello, 2008) no such association could be found in our analysis by CURB-65 score. Also of concern are patients initially triaged with low CURB-65 scores who subsequently deteriorated and required ICU admission. With regards to *Streptococcus pneumoniae* as the main pathogen detected on blood culture, it was noted that in all cases they were initially triaged with low CURB-65 scores but subsequently deteriorated and required ICU admission.

This finding may reflect an underestimation of the significance of the pathogen detected with regards to its potential for virulence or possibly that some elderly and

severely disabled patients were not admitted to the ICU due to prognostic considerations despite a moderate CURB-65 score which was mistaken since the prognostic score cannot replace clinical insight.

It is possible that we are failing to recognise the important influence of the aetiological agent on the severity of disease (irrespective of severity index score) as well as repeatedly failing to evaluate aetiology as a prognostic indicator requiring extra clinical judgment. The recognition of the aetiological pathogens of CAP should thereafter be alerting the clinician to the need for appropriate empiric antibiotic therapy.

In all instances overall clinical judgment is crucial. Social factors and patients' wishes also influence where to manage a patient. Whether the CURB-65 score should be applied in conjunction with different management strategies to improve clinical outcomes and health service utilisation in our resource constrained setting requires further study. For example a low serum albumin has been previously identified as an independent prognostic variable in addition to the CURB-65 score (Lim et al., 2003). This would be an interesting outcome to analyse in view of our patients' predominant HIV seropositivity co-morbid factor.

In view of South Africa's introduction of PCV 7 (conjugate polysaccharide vaccine), which contains seven serotypes (4, 6B, 9V, 14, 18C, 19F and 23F), to the Extended Programme of Immunisation, despite this study's limited size it was interesting to note that the majority of this study's *Streptococcus pneumoniae* serotype distribution did not include the accepted virulent vaccine strains. This could indicate that with

the use of PCV7 in South Africa we are already witnessing the increase carriage of and disease from serotypes not included in the vaccine. Replacement has occurred in trials of pneumococcal vaccines (Lipsitch, 1999). One of the blood culture confirmed isolates was missing from the NICD serotyping database. It is possible that the isolate was not sent for further typing.

In order to limit the development of resistance, the South African guidelines explicitly state that fluoroquinolones should not be used as routine first-line therapy for CAP, but rather be reserved for patients with proven *Streptococcus pneumoniae* CAP with severe allergy to standard beta-lactam agents and for known or suspected cases of infection with highly penicillin-resistant pneumococci.

Upon review of patient records, none of the patients were treated according to South African guidelines. It was worrying information that the chosen fluoroquinolone was ciprofloxacin and not one of the newer agents.

Not a single patient that was empirically initiated on an aminoglycoside was followed up with trough serum drug levels despite 11 of the 17 (65%) patients being admitted in frank renal failure and two of the 17 patients being over the age of 70. The South African guidelines clearly state that aminoglycosides should be discontinued if organisms other than Gram-negative bacteria are isolated. All 17 patients received a minimum of 5 days of aminoglycosides. Ertapenem was inappropriately initiated as empiric treatment in 4 patients who had not failed standard first-line antibiotic therapy for CAP and with no microbiological guidance. None of the patients that met the criteria for suspected atypical pneumonia were treated with a macrolide.

Guidelines are useful if they are followed and shown to alter prognosis in patience outcome . It was concluded that non adherence to guidelines when selecting empirical antibiotic therapy, particularly amongst patients classified as having severe pneumonia, was associated with a higher mortality (Menendez et al., 2005). Another study concluded that guideline implementation decreased the number of low risk patients that are hospitalised but might lead to patients being managed inappropriately in the community with some reports of over 25% of patients being treated with inappropriate antibiotic therapy (Yealy et al., 2005). These varying practices are fully dependent on the maturity and insight of physicians as well as the hospital rules and regulations with disregard to guidelines. The adherence rate amongst intensive care units is quoted as low as 67% (Menendez et al., 2005).

CHAPTER 5: CONCLUSION

Collection and transport of samples are key components of determining the aetiology of CAP. Effective treatment and management relies on rapid, high quality PCR results with improved safety, whilst at the same time maintaining high sensitivity and specificity. The reliable rapid testing significantly improves the quality and suitability of care that could be provided; both for inpatient and outpatient management.

Regarding diagnostic tests they need to be utilised properly. The availability of a more sensitive assay does not guarantee that it will be placed into practice appropriately. The clinician must be able to interpret the results in the context of the diseases and thereafter determine management appropriately.

Streptococcus pneumoniae as an aetiological agent of CAP may indicate severity of disease and need for critical care but this crucial point may be missed if the low/moderate CURB-65 score is the sole index for severity. This study highlights the need to evaluate aetiology as a prognostic indicator since it is unclear what these severity scores truly reflect in isolation.

The use of antibiotics may depend more on financial constraints than on available aetiological epidemiological data. In cases in which the infecting pathogen can be identified, directed therapy should be employed. The early and rapid initiation of empiric antimicrobial treatment should be based on an epidemiological approach, and these factors are essential for the adequate management of CAP.

APPENDIX 1

INFORMED CONSENT:

I hereby confirm that I have been informed by the study doctor, Dr Parastu Meidany about the nature, conduct, benefits and risks of clinical study THE MICROBIAL AETIOLOGY OF COMMUNITY-ACQUIRED PNEUMONIA IN ADULTS IN JOHANNESBURG

- I have also received, read and understood the above written information (Participant Information Leaflet and Informed Consent) regarding the clinical study.
- I am aware that the results of the study, including personal details regarding my sex, age, date of birth, initials and diagnosis will be anonymously processed into a study report.
- In view of the requirements of research, I agree that the data collected during this study can be processed in a computerised system by National Health Laboratory Services) or on their behalf.
- I may, at any stage, without prejudice, withdraw my consent and participation in the study.
- I have had sufficient opportunity to ask questions and (of my own free will) declare myself prepared to participate in the study.

PARTICIPANT:

Printed Name	Signature / Mark or Thumbprint	Date & Time

I, herewith confirm that the above participant has been fully informed about the nature, conduct and risks of the above study.

STUDY DOCTOR:

Printed Name	**Signature**	**Date & Time**

TRANSLATOR / OTHER PERSON EXPLAINING INFORMED CONSENT……. (DESIGNATION):

Printed Name	Signature	Date and Time

WITNESS (If applicable):

Printed Name	Signature	Date & Time

APPENDIX 2

COMMUNITY ACQUIRED PNEUMONIA STUDY

QUESTIONNAIRE AND DATA SHEET FOR PATIENTS ADMITTED WITH CAP

Patient's demographic data

Date of admission (DD/MM/YYYY)

☐☐/☐☐/☐☐☐☐

Date of birth (DD/MM/YYYY) ☐☐/☐☐/☐☐☐☐
Age ☐ Y ☐ M ☐ Unknown Gender: ☐ Female ☐ Male ☐ Unknown
Patient Hospital Number ☐☐☐☐☐☐☐☐☐☐
Start date of interview (DD/MM/YYYY): ☐☐/☐☐/☐☐☐☐
Diagnosis of CAP Confirmed by chest X-ray ☐ Not confirmed by chest X-ray ☐

Date of specimen collection: (MM/DD/YYYY)

☐☐/☐☐/☐☐☐☐

Where were you born? (Please circle)

☐Urban (town-with electricity and running water)

☐Rural (village no running water and no electricity)

1. Soweto

2. JB city
3. Gauteng – (Please specify)
4. Other (Please specify)
5. Nursing home resident (exclusion criteria)

What is the highest standard you passed at school? (Please tick)

Grade 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, none

☐☐☐☐☐☐☐☐☐☐☐☐☐

Are you? (Please tick)

Living alone Living with long-term partner

☐ ☐

Are you employed? (Please tick)

Unemployed Temporarily Permanently Part time Full time

☐ ☐ ☐ ☐ ☐

Describe your usual or past occupation.

Describe what your usual workplace does or did?

Patient's social history

Have you ever smoked cigarette or pipe regularly? (Please circle)

Yes now	**In the past**	**Never**

If yes: in the past five to ten years, how many would you usually smoke in a day?

Cigarettes	Cigar	Pipes
Pack/s:	Number:	Yes/no

How old were you when you first started smoking regularly?

Year old:

About how much wine, beer or spirits did you drink on average each week?

Beer				Wine		Other	None
1 large glass	2 large glasses	3 large and more	Other	1 glass	2 glasses		

ON EXAMINATION

Vitals on day of admission

Confusion: Yes ☐ No ☐

Blood pressure: systolic =

Blood pressure: diastolic =

Pulse rate=

Respiratory rate=

Temperature=

CURB 65 score =

ICU ADMISSION: Yes ☐ No ☐

Date of ICU Admission:

Blood pressure at the time of admission:

Inotrope Use: Yes ☐ No ☐

Mental State:
Alert: Yes ☐ No ☐
Disorientated: Yes ☐ No ☐
Stuporous / Coma: Yes ☐ No ☐

Radiological findings-criteria

1. Classification of findings
a. Significant pathology* ☐ Yes ☐ No
b. If yes, end-point consolidation (consolidation containing air bronchograms) ☐ Yes ☐ No
c. Parenchymal consolidation ☐ Yes ☐ No
d. Reticular or reticulonodular infiltrate ☐ Yes ☐ No
e. Broncovascular bundle thickening ☐ Yes ☐ No
f. Cavitation ☐ Yes ☐ No
g. Other (non-end-point) infiltrate (interstitial infiltrate and minor patchy infiltrate) ☐ Yes ☐ No
i. Unilateral ☐
ii. Bilateral ☐
iii. Single lobe ☐
iv. Multiple lobe ☐
h. Pleural effusion ☐ Yes ☐ No
i. Adenopaties ☐ Yes ☐ No

*Presence of consolidation, infiltrate or effusion if none no further recording.

Underlying conditions (Please tick):

Conditions	YES	NO
Diabetes mellitus		
COPD		
Heart failure		
Renal failure acute		
Renal failure chronic		
Steroids use		
Liver failure		

Malignancy		
If yes malignancy specify which		
Splenectomy		
Other immunosuppresive therapy		

HIV status:
Positive Negative Unknown
☐ ☐ ☐
CD4 count: Date CD4 count performed: (DD/MM/YYYY)
☐☐/☐☐/☐☐☐☐
Viral load: Date Viral load performed: (DD/MM/YYYY)
☐☐/☐☐/☐☐☐☐

MICROBIOLOGY RESULTS

SPECIMEN TYPE TICK

Sputum MC&S	
Nasopharyngeal swab	
Urine Legionella Ag	
Blood culture MC&S	
BDG	
Blood for U&E and FBC	

Sputum microscopy result:

Bartlett score:

Organisms seen:

Culture results:

Organism isolated	Specimen type

1	
2	

SENSITIVITY PATTERN OF GRAM POSITIVE ISOLATE/S (MIC Value)

1. Organism: 2: Organism:

Penicillin		Penicillin	
Cephalosporin 3rd		Cephalosporin 3rd	
Erythromycin		Erythromycin	
Clindamycin		Clindamycin	
Tetracycline		Tetracycline	
Ciprofloxacin		Ciprofloxacin	
Moxifloxacin		Moxifloxacin	
Levofloxacin		Levofloxacin	
Linezolid		Linezolid	
Vancomycin		Vancomycin	
Synercid		Synercid	
Bactrim		Bactrim	
Chloramphenicol		Chloramphenicol	
Rifampicin		Rifampicin	
Gentamicin		Gentamicin	
Fusidic acid		Fusidic acid	
D zone (yes/no)		D zone (yes/no)	

SENSITIVITY PATTERN OF GRAM NEGATIVE ISOLATE/S (MIC value)

1. Organism 2. Organism

Ampicillin		Ampicillin	
Cefuroxime		Cefuroxime	
Augmentin		Augmentin	
TZP		TZP	
Cefazolin		Cefazolin	
Tetracycline		Tetracycline	
Ciprofloxacin		Ciprofloxacin	
Cefriaxone		Cefriaxone	
Ceftazidime		Ceftazidime	
Levofloxacin		Levofloxacin	
Cefepime		Cefepime	
Gentamicin		Gentamicin	
Amikacin		Amikacin	
Bactrim		Bactrim	
Tobramicin		Tobramicin	
Ertapenem		Ertapenem	
Imipenem		Imipenem	
Meropenem		Meropenem	
ESBL POS		ESBL POS	

Antimicrobial treatment

Antibiotic	Route	Date started	Date stopped

TB treatment: Y/N

Empirical Treatment: Y / N MONO Rx / COMBO Rx

Definitive treatment: Y / N MONO Rx / COMBO Rx

Date of Change:

Definitive Therapy within 48 H after Blood Culture: Y / N

Persistence of fever for 48 H after definitive therapy: Y / N

APPENDIX 3

CURB-65 AND CRB-65 SEVERITY SCORES FOR COMMUNITY-ACQUIRED PNEUMONIA

Clinical factor	Points
Confusion	1
Blood urea nitrogen > 19 mg per dL	1
Respiratory rate ≥ 30 breaths per minute	1
Systolic blood pressure < 90 mm Hg or Diastolic blood pressure ≤ 60 mm Hg	1
Age ≥ 65 years	1
Total points:	

CURB-65 score	Deaths/total (%)*	Recommendation†
0	7/1,223 (0.6)	Low risk; consider home treatment
1	31/1,142 (2.7)	
2	69/1,019 (6.8)	Short inpatient hospitalization or closely supervised outpatient treatment
3	79/563 (14.0)	Severe pneumonia; hospitalize and consider admitting to intensive care
4 or 5	44/158 (27.8)	

(Lim et al., 2003)

APPENDIX 4

PNEUMONIA SEVERITY INDEX FOR COMMUNITY-ACQUIRED PNEUMONIA

Risk factor	Points
Demographics	
Men	Age (years): ___
Women	Age (years) - 10: ___
Nursing home resident	+10
Comorbidities	
Neoplasm	+30
Liver disease	+20
Heart failure	+10
Stroke	+10
Renal failure	+10
Physical examination findings	
Altered mental status	+20
Respiratory rate ≥ 30 breaths per minute	+20
Systolic blood pressure < 90 mm Hg	+20
Temperature < 95°F (35°C) or ≥ 104°F (40°C)	+15
Pulse rate ≥ 125 beats per minute	+10
Laboratory and radiographic findings	
Arterial pH < 7.35	+30
Blood urea nitrogen > 30 mg per dL	+20
Sodium < 130 mmol per L	+20
Glucose ≥ 250 mg per dL	+10
Hematocrit < 30 percent	+10
Partial pressure of arterial oxygen < 60 mm Hg	+10
Pleural effusion	+10
Total points:	

(Niederman et al., 2001)

APPENDIX 5

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Perovic

CLEARANCE CERTIFICATE	**PROTOCOL NUMBER M070345**
PROJECT	The Microbial Aetiology of Community-Acquired Pneumonia in Adults in South Africa
INVESTIGATORS	Dr O Perovic
DEPARTMENT	Molecular Medicine & Haem
DATE CONSIDERED	07.03.30
DECISION OF THE COMMITTEE*	APPROVED UNCONDITIONALLY

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 07.06.14 **CHAIRPERSON** .....
(Professors PE Cleaton-Jones, A Dhai, M Vorster, C Feldman, A Woodiwiss)

*Guidelines for written 'informed consent' attached where applicable

cc Supervisor : Dr O Perovic

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10005, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

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