BACTERIAL CAUSES OF RESPIRATORY TRACT INFECTIONS AMONG HUMAN IMMUNODEFICIENCY VIRUS (HIV) SEROPOSITIVE PATIENTS AT KOMFO ANOKYE TEACHING HOSPITAL (KATH), KUMASI

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BY

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DECLARATION

This study was carried out under the supervision of Mr. Patrick Feglo. I declare that this study has not been published by another author to the best of my knowledge. I however accept every responsibility for any error in this write up.

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DEDICATION

This work is dedicated to my parents

MR. EDWARD YAO DOE

and

MRS. ROSINA HIAGBE DOE

who set me on the right path for life

ACKNOWLEDGEMENT

This is how far the ALMIGHTY has brought me and I am very grateful. Thank you LORD.

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LIST OF ABBREVIATIONS

HIV	Human Immunodeficiency Virus
AIDS	Acquired Immune Deficiency Syndrome
HAART	Highly Active Anti Retroviral Therapy
KATH	Komfo Anokye Teaching Hospital
CD4 cells	Cluster of Differentiation Cells
WHO	World Health Organization

ABSTRACT

This study was undertaken from January 2008- June 2008 to investigate the bacterial causes of respiratory tract infection among some selected patients infected with HIV at KATH, Kumasi. Sputum specimen from 200 HIV patients with complaints suggestive of respiratory tract infection and 200 non HIV patients were cultured.

The prevalence of bacterial species responsible for the respiratory tract infections among the HIV seropositive patients was 23.5%. In the HIV seronegative patients used as controls, the prevalence was 22.5 %.

In the HIV seropositive patients, 4 (22%) different Gram negative bacteria and 1(1.5%) Gram positive bacterium was isolated. Also, of the HIV seropositive patients that had isolates in their sputum, 15.9% had dual infections.

Susceptibility patterns revealed that *Klebsiella pneumoniae* were susceptible to ciprofloxacin (95.8%), gentamicin (95.8%), cefotaxime (83.3%), amikacin (79.1%), and cefuroxime (70.8%). Also, *Escherichia coli* were susceptible to amikacin (91.7%), cefotaxime (91.7%), gentamicin (75%), ciprofloxacin (66.7%), and cefuroxime (58.3%). The susceptibility pattern of *Pseudomonas aeruginosa* to gentamicin was 83.3% and to ciprofloxacin was 66.7%. All the *Proteus mirabilis* isolated were susceptible to ciprofloxacin, gentamicin, cefuroxime and cefotaxime. *Staphylococcus aureus* was susceptible to ciprofloxacin, gentamicin, cloxacillin and erythromycin.

This study demonstrated that, bacteria responsible for respiratory tract infections at KATH, Kumasi in both HIV seropositive and seronegative patients were similar. However, *Streptococcus pneumoniae* and *Haemophilus influenzae* which are often reported as the major causes of respiratory tract infections were not isolated.

CHAPTER ONE

INTRODUCTION

1.0 Background

The respiratory system is a system of organs functioning in respiration. It is made up of two parts namely the upper respiratory tract and lower respiratory tract. The upper respiratory tract consists of the nose, nasal cavity, pharynx, and larynx and the lower respiratory tract consists of the trachea, bronchi and the lungs.

The upper respiratory tract contains many normal flora that include *Streptococcus species*, *Haemophilus species, Neisseria species*, *Corynebacterium species*, *Staphylococcus species* and many anaerobes such as *Bacteroides*. Although, the normal bacterial flora is generally harmless and beneficial to the host, they can cause diseases when the host defenses are impaired (Macowicak, 1982).

Bacteria from the upper respiratory tract are washed downwards towards the lower respiratory tract, but the action of the ciliated epithelium and sticky mucus that covers the lining of the bronchial tubes keeps the lower respiratory tract free of these microorganisms (Mizgerd, 2006).

Viruses may however, interfere with the ciliary function, allowing themselves or other microorganism invaders such as bacteria, to gain access to the lower respiratory tract. One of such viruses is HIV, the causative virus in AIDS. This virus decreases the CD4 cells, creating the conditions for other opportunistic organisms to initiate infections in the host (Mohanthy *et al*., 1993). In recent years this virus has infected many people as a result there is a huge increase in the incidence of respiratory tract infections worldwide (Feldman, 2005).

Various defects in immunity occur in the disease process of patients with HIV infection. These defects include humoral immune dysfunction, depressed IgA and IgG levels, and decreased T-lymphocyte cell-mediated antibody-dependent cellular cytotoxicity. Once the immune defect occurs the patient is predisposed to bacterial infections (Lane *et al.*, 1983). Monocytes, macrophages and neutrophils play an important role in nonspecific immunity against opportunistic pathogens by acting as first-line defense against extracellular pathogens, and against intracellular pathogens, but in HIV infection the functions of these defensive mechanisms are reduced (Jones, 1996).

The major causes of morbidity and mortality in HIV infected persons are different opportunistic infections. These infections, responsible for morbidity and mortality, vary from region to region (Ayyagari *et al.,* 1999). The etiology and pattern of respiratory tract infections in HIV infected persons differ between sub-Saharan Africa and industrialized countries (Gilks, 1993). Bacterial pneumonia is responsible for significant morbidity among such patients (Tumbarello *et al.,* 1998). The etiology of bacterial pneumonia among HIV patients was reported to be *Streptococcus pneumoniae, Haemophilus influenzae, Pseudomonas aeruginosa, and Staphylococcus aureus* (McEllistrem *et al.,* 2002). Another study by Mayaud *et al.,* (2002) reported the organisms most frequently associated with bacterial infections of the lower respiratory tract to include *Streptococcus pneumoniae* (52%), *Haemophilus influenzae* (15%), *Klebsiella pneumoniae* (13%), *Staphylococcus aureus 10% and Pseudomonas* (8%).

Bacteria, such as *Legionella pneumophila*, *Mycoplasma pneumoniae*, and *Chlamydia pneumoniae* (Rimland *et al.,* 2002) have been incriminated in pneumonia in HIV/AIDS patients.

Other bacteria incriminated to cause pneumonia in HIV-infected patients include *Rhodococcus equi* and *Nocardia asteroides* (Baughman, 1999).

Bacterial tracheitis and recurrent bronchitis have also been reported in HIV-infected persons (Valor *et al.,* 1992; Chechani *et al.,* 1992). The bacterial isolates associated with tracheitis and bronchitis is *Streptococcus pneumoniae, Haemophilus influenzae, Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Valor *et al.,* 1992; Chechani *et al.,* 1992). These isolates are similar to those found to cause pneumonia by McEllistrem *et al.,* (2002).

In the United States, pneumococcus is reported to be the leading cause of bacterial pneumonia in HIV infected patients (Janoff *et al.,* 1992, Hirschtick *et al.,* 1995). In sub-Saharan Africa, however, the commonest cause of respiratory tract infection in HIV-infected patients has consistently been tuberculosis, although other infections also occur (Batungwanayo *et al.,* 1994). In Ghana, there are no reports on the prevalence of bacteria responsible for respiratory tract infections in HIV infected patients other than *Mycobacterium tuberculosis* in HIV. Frimpong *et al.,* (1997) reported the prevalence of tuberculosis in HIV infection in Kumasi to be at 23%.

Co-trimoxazole also known as trimethoprim–sulphamethoxazole is a widely available, easy to administer, safe and low-cost antibiotic, which is used as a prophylaxis against respiratory tract pathogens in HIV infected individuals (Smilack, 1999). WHO recommends one double-strength tablet or two single-strength tablets per day as the preferred regimen (WHO/UNAIDS, 2000). A study from South Africa showed that co-trimoxazole used by HIV seropositive tuberculosis patients improved survival rates by 53% (Badri *et al.,* 1999). Studies conducted in Cote d'Ivoire had indicated that daily cotrimoxazole will significantly control *Streptococcus pneumoniae* infections (Hart *et al.,* 2000). Alternative antibiotics that would control *Streptococcus*

pneumoniae infections include ampicillin, first generation cephalosporins, macrolide and clindamycin. In patients with higher levels of penicillin resistance, vancomycin or imipenem/cilastatin has been recommended (Fieldland *et al.*, 1994).

1.1 Problem Statement

HAART has greatly reduced deaths from AIDS-related infections by reducing levels of HIV particles in the blood (viral load) and thus strengthening the immune system (Bower *et al.*, 2003). HAART does not cure HIV infection and some degree of immune deficiency remains, despite continued treatment. As a result, the risk of developing bacterial infections of the lung remains higher in people with HIV/AIDS than in HIV negative patients. At KATH, HIV/AIDS patients, are administered HAART after four weeks of counseling. This is after a blood test of the patient confirms that the individual is HIV positive and the CD4 count is less than 250cells/µl. Patients with cough symptoms suggestive of respiratory tract infections are administered two co-trimoxazole tablets, two times a day till their next review. This study seeks to determine the dominant bacteria other than *Mycobacterium tuberculosis*, causing respiratory tract infections and the antimicrobial susceptibility patterns of the isolates obtained in this group of patients, so as to determine the effect of the use of co-trimoxazole as a prophylactic drug against pulmonary infections among HIV seropositive individuals. Organisms which used special requirements for growth in culture were not considered in this study. Therefore, *Legionella pneumophilia*, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* which required special growth media and *Rhodococcus equi* including *Norcardia asteroides* were not sought after in this study.

1.2 Aim of the Study

- To determine the prevalence of bacteria species responsible for respiratory tract infections among HIV seropositive patients at Komfo Anokye Teaching Hospital (KATH).

1.3 Specific Objectives

1. To isolate the bacteria species, responsible for respiratory tract infections among HIV seropositive patients.
2. To determine the similarity or otherwise of organism causing respiratory tract infections among HIV seropositive patients.
3. To determine the antimicrobial susceptibility patterns of the isolates obtained.
4. To determine, if cotrimoxazole had any influence on the isolates in the respiratory tract infections among HIV seropositive patients.

CHAPTER TWO

LITERATURE REVIEW

2.0 Introduction

Human Immunodeficiency Virus (HIV) is a retrovirus that primarily infects cells of the human immune system. The main cell HIV infects is the CD4 cell. Once it attacks the CD4 cells, it takes over and reproduces itself (Ickovics *et al.,* 2001). During the process of reproduction, the infected cell dies and the virus seeks other CD4 cells to infect (Lévy, 1993). Acquired Immune Deficiency Syndrome (AIDS) is a condition in humans in which the immune system begins to fail, leading to life-threatening opportunistic infections (Weiss, 1993).

2.1 The Immune System

The immune system is a network of cells, tissues, and organs that work together to defend the body against attacks by "foreign" invaders. These invaders are primarily bacteria, viruses, parasites, and fungi (U.S. Department of Health And Human Services, 2003). The immune system consists of two components called humoral and the cell mediated systems. The humoral system consists of antibodies (immunoglobulins) which are formed by the B lymphocytes. There are five classes of immunoglobulin (Ig). These are IgG, IgM, IgA, IgE and IgD. In the course of an infection, Immunoglobulin G is the predominate antibody in secondary immune responses and constitutes the defense against bacteria and viruses. Immunoglobulin M is the dominant antibody produced in primary immune responses and is also very effective in defense against bacteria and viruses, while IgA is the main immunoglobulin in secretions of the respiratory tracts. It protects mucous membrane from attack by bacteria and viruses (Sproul *et al*., 2000).

IgE production occurs locally in the nasal mucosa, without the involvement of lymphoid tissue (Takhar, 2005). IgD's function is to signal when the young B cells in the spleen are ready to be activated. Several defects in the HIV infected host's defense against bacterial infections in the respiratory tract have recently been reviewed. In the upper respiratory tract, a reduction in salivary immunoglobulin A may lead to colonization with pathogenic bacteria. In the lower respiratory tract, alveolar macrophages may show impaired function due to direct effects of HIV. In addition, impaired neutrophil function and reduced production of opsonizing antibody by B lymphocytes have been implicated as important factors predisposing to bacterial pneumonia (Davis *et al.,* 1993).

The other component of the immune system is cell mediated immune system which involves the T cells. The cell-mediated immune responses require that the T cells be presented with foreign antigen bound to host proteins (Delves *et al*., 2000). The cell mediated immunity is central in host defense against intracellular pathogens such as viruses (Holtmeier *et al*., 2000).

2.1.1 Neutrophils

Neutrophils are phagocytes that travel throughout the body in pursuit of invading pathogens. They constitute about 55% to 70% of white blood cells. They are the first immune cells to arrive at a site of infection, through a process known as chemotaxis (Zen *et al.,* 2003). They are cells that target bacteria. These cells identify and eliminate pathogens by attaching, engulfing and then killing them (Nathan, 2006).

During pulmonary infection, neutrophils migrate out of the pulmonary capillaries into the air spaces (Burns *et al.,* 2003). These neutrophils extrude antimicrobial proteins which ensnare and kill the invading bacteria (Brinkmann *et al.,* 2004). Bacteria typically enter the lung when

airborne droplets are inhaled. The bacteria can also reach the lung when there is an infection in another part of the body and blood carries it to the lung (Burns *et al.,* 2003). Many bacteria live in parts of the upper respiratory tract, such as the nose, mouth and sinuses, and can easily be inhaled into the alveoli. Once inside the lungs the bacteria may invade the spaces between cells of the alveoli through connecting pores. This invasion triggers the immune system to send the neutrophils, to the lungs. The neutrophils engulf and kill the invading organisms, and also release cytokines, causing a general activation of the immune system.

2.1.2 Lymphocytes –CD4 T-Cells

There are two main types of lymphocytes known as the T cell and B cell lymphocytes. The T cell lymphocytes attack and kill microorganisms, and help regulate the immune system. The B cell lymphocytes make antibodies, special proteins that attack microorganisms. Lymphocytes are normally 20% to 40% of white blood cells.

In HIV infection, it is the CD4 T cells that aid HIV replication. HIV attaches to the CD4 cells, and then enters the CD4 T cells, damaging them in the process. The bulk of CD4 T cell loss occurs during the first weeks of HIV infection, especially in the intestinal mucosa and the respiratory tract which harbors the majority of the lymphocytes found in the body (Mehandru *et al.,* 2004). When the CD4 T-cells are destroyed and its count is less than 200cells/µl, the immune system weakens and cellular immunity is lost and the body is unable to fight infections (Alimonti *et al.,* 2003).

2.2 Respiratory Tract Infections

2.2.1 Infections of Upper Respiratory Tract

Upper respiratory tract infections are usually caused by viruses and are mostly self-limiting. Exposure to many upper respiratory pathogens occurs at a young age. The sections of the upper respiratory tract commonly affected are the pharynx, epiglottitis and the sinuses (Slotar *et al.*, 2002).

2.2.1.1 Pharyngitis

Pharyngitis involves inflammation of the oropharynx or the nasopharynx (Braun *et al.*, 2000). The main symptom is sore throat the severity of which can be judged by the difficulty a patient has during swallowing. Pharyngitis is caused by a range of viruses and bacteria. Viral pharyngitis is generally more common than bacterial pharyngitis and less likely to cause complications. It may be impossible to distinguish between viral and bacterial cause of sore throat (Der Mar, 1992). A number of different bacteria can infect the pharynx. The most common is *Group A streptococcus*, however others include *Corynebacterium diphtheriae , Neisseria gonorrhoeae, Chlamydophila pneumoniae,* and *Mycoplasma pneumoniae* (Bisno,2001).

2.2.2 Lower Respiratory Tract Infections

Causative agents of lower respiratory infections are viral, fungal, parasitical and bacterial. Symptoms include cough, fever, chest pain, and sputum production.

2.2.2.1 Bronchitis

Bronchitis is a lower respiratory tract infection that causes bronchial inflammation (Perlman *et al.*, 1990). It causes a cough that produces phlegm as the bronchial tubes are inflamed and collect

mucus. Bronchitis is caused by viruses in 95% of cases, and is spread from person to person through coughing (Meza *et al.,* 1994). The cough in bronchitis usually lasts for about a week to ten days but in about half of individuals, coughing can last for up to three weeks and 25% of individuals continue to cough for over a month (Falck *et al.,* 1994).

Acute bronchitis, the most frequent HIV related lower respiratory infection, occurs significantly more often in HIV infected patients than in HIV-seronegative patients with similar risk factors. The incidence of acute bronchitis is high in HIV infected people at all stages of the diseempase.

2.2.2.2 Emphysema

Emphysema is a long-term, progressive disease of the lungs that primarily causes shortness of breath due to over-inflation of the alveoli. The condition often follows surgery to the oesophagus. It may be a complication of bronchopleural fistula caused by tuberculosis, actinomycosis or nocardiosis (Light, 2002).

2.2.2.3 Pneumonia

Adaged as the "Captain of the Men of Death," pneumonia continues to be a prominent clinical disease entity. Pneumonia is an illness of the lungs in which the alveoli become inflamed and flooded with fluid (Gennis *et al.,* 1989). Pneumonia can result from a variety of causes, including infection with bacteria, viruses, fungi, or parasites. Pneumonia can also be caused by chemical or physical injury to the lungs. The clinical presentation of pneumonia in HIV seropositive patients is similar to that of HIV seronegative patients who suffer from pneumonia (Cordero *et al.,* 2000). Typical symptoms associated with pneumonia onset are acute with fever, cough, purulent sputum production and chest pain. These symptoms are preceded by a day or two of runny nose and upper respiratory congestion, ending with an abrupt rise in temperature and body shaking chill.

Diagnostic tools used to detect pneumonia include x-rays and examination of the sputum (Syrjala *et al.,* 1998). Pneumonia is a frequent complication of HIV infection and may be a sign of AIDS in persons with HIV. The principal HIV/AIDS associated pneumonias are bacterial pneumonia, and the incidence is significantly higher among persons with HIV infection (Hirschtick *et al.*, 1995).

The high rates of bacterial pneumonia and other respiratory tract infections probably result from multiple factors including qualitative B-cell defects that impair the ability to produce pathogen-specific antibody, impaired neutrophil function or numbers or both (Gilks , 1993).

2.2.2.3.1 Bacterial Causes of Pneumonia

In individuals with HIV infection, almost any organism causes pneumonia. The types of Gram-positive bacteria that cause pneumonia can be found in the nose or mouth of many healthy people. *Streptococcus pneumoniae*, often called pneumococcus, is the most common bacterial cause of pneumonia in all age groups except newborn infants. Another important Gram-positive cause of pneumonia is *Staphylococcus aureus* (Wallace *et al.*, 1997). Some of the Gram-negative bacteria that cause pneumonia include *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Moraxella catarrhalis*. Atypical bacteria which cause pneumonia include *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, and *Legionella pneumophila* (Madhi *et al.*, 2000).

A series of studies investigating bacterial pneumonia in patients with AIDS have shown the predominant role of *Streptococcus pneumoniae* and, to a lesser degree, *Haemophilus influenzae*, in adults as well as children (Witt *et al.,* 1987, Scott *et al.,* 2000,). Hirschtick *et al.,* (1995) reported in their study carried out in the U.S. that 52% *Streptococcus pneumoniae* and 15% *Haemophilus influenzae* were among the bacterial causes of pneumonia whereas Abgrall *et al.,*

(2000) in their studies carried out in France found 52% *Streptococcus pneumoniae*, and 16% *Haemophilus influenzae* to be the other causes of pneumonia.

However, whereas Hirschtick *et al.* (1995) reported in their study in the U.S. that 10% Enterobacteriaceae and 8% *Pseudomonas aeruginosa* were the other bacterial causes of pneumonia, particularly in patients with advanced HIV infection, Afessa *et al.* (2000) found 25% *Pseudomonas aeruginosa*, 9% Enterobacteriaceae and 10% *Staphylococcus aureus* to be the cause pneumonia in Africa.

2.2.3 Laboratory Diagnosis of Respiratory Tract Infection Using Sputum

The laboratory diagnosis of bacterial respiratory tract infections involves an attempt to isolate the infectious pathogen in laboratory cultures of sputum. Routine sputum specimens are typically plated on blood agar, chocolate agar, and MacConkey agar (Reimer *et al.*, 1998). Gram staining of sputum can produce diagnostic useful information in 63-86% of cases within one hour. Culture and identification of the organism require 24-48 hours (Woodhead *et al.*, 2005). The various bacteria that cause respiratory tract infections require either a differential or an enriched medium to grow. Those that require enriched media are fastidious organisms.

Sputum shows a heavy growth of commensal organisms because of the presence of the normal flora in the oropharynx. Attempts to assess respiratory infections by quantitative sputum culture have been reported for more than three decades (Lapenski *et al.*, 1964). Louria, (1962) reported that the pathogen causing pneumonia was present in numbers greater than 10^7 orgs/ml of sputum.

2.2.4 Treatment of Respiratory Tract Infection

Treatment for pneumonia is ideally based on the causative microorganism and its known antibiotic sensitivity. Bacterial pneumonia is treated with antibiotics (el Moussaoui *et al.*, 2006).

Antibiotics administered include vancomycin, third- and fourth-generation cephalosporins, carbapenems, fluoroquinolones, and aminoglycosides (Gonzales *et al.,* 2001). These antibiotics are usually given intravenously. Treatment for acute bronchitis in HIV-infected patients is the same as for people without HIV disease: a broad-spectrum antibiotic-such as amoxicillin, a second-generation cephalosporin, or a macrolide-is taken for 10 to 14 days (McGuinness *et al*., 1997).

2.2.5 Co-trimoxazole as Prophylaxis

Cotrimoxazole is a broad spectrum antimicrobial agent strongly recommended by WHO as a primary or secondary prophylaxis for treatment of HIV infected adults with bacterial infections in Africa (DiRienzo, 2002;WHO, 2005). The antibiotic is effective against many opportunistic infections that occur in individuals with advanced HIV disease, and has been used widely (Kaplan *et al.*, 1997). It was in use to prevent bacterial infections in people with granulocytopenia before HIV was discovered (DiRienzo, 2002).

Two controlled trials of co-trimoxazole in Côte d'Ivoire were published in 1999. One demonstrated that co-trimoxazole reduced mortality by 46% among tuberculosis patients (Wiktor *et al.,* 1999). The other study showed that co-trimoxazole reduced severe morbidity by 47% among HIV patients (Coffin *et al.,* 1986). In both trials, co-trimoxazole was better tolerated than expected. In subgroup analyses both trials showed that the efficacy of co-trimoxazole was not restricted to patients with fewer than 200 CD4cells/µl. Thera, (2005) reported that co-trimoxazole prevented malaria and invasive bacteria. WHO/UNAIDS experts recommended that co-trimoxazole be part of the minimal package of care for African adults with fewer than 500 CD4 cells/µl (UNAIDS, 2000).

CHAPTER THREE

METHODOLOGY AND MATERIALS

3.1 Sampling Population and Ethical Clearance

Patients who reported to the KATH chest clinic with symptoms suggestive of respiratory tract infections were involved in the study. The patients were sorted into two groups, based on whether they were HIV seropositive or HIV seronegative. Ethical clearance was obtained from the ethical committee of KATH and School of Medical Sciences.

3.2 Criteria for Inclusion

For the HIV seropositive patients, the patient should be:

1) Coughing

2) Counseled and tested positive for HIV

3) Consented to participate in the study.

For the seronegative patients, the patient should be:

1) Coughing

2) HIV seronegative and

3) Consented to participate in the study

3.3 Specimen for Study

A wide mouthed sterile leak proof specimen container was given to the patients. They were asked to go to a quiet isolated place, take a deep breath and to cough up sputum. The sputum should be deposited directly into the sterile container provided. The patients were to provide sputum devoid of saliva and to return the sample as early as possible. The sputum specimen was

then transported to the Clinical Microbiology laboratory at KATH for analysis. Patients' demographic data such as age, gender, were also taken.

3.4 Processing of Sputum

The quality of the sputum was assessed macroscopically. A good sputum sample must be viscous, mucoid or purulent. If the sputum sample turned out to be thin, watery and with no purulent matter it was considered unsuitable for processing. Two smears were prepared from each sputum sample. The smears were then Gram stained and also Ziehl Neelsen staining was done (Appendix). Microscopically, the smears were examined under low power field (xl00) magnification. Gram stained smears that contained more than 25 polymorphonuclear cells per low power field with micro organisms were considered to have strong indication of an infection. Samples that had less than 25 polymorphonuclear cells and more than 25 epithelial cells per low power field were discarded and not included in the study.

3.5 Culture of Sputum

Using a sterile microbiological loop a mucopurulent part of the sputum sample was picked and inoculated onto blood agar, chocolate agar and MacConkey agar (Appendix). The plates were incubated overnight at 37^{o}C.

The following day the plates were examined for growth. The colonies were identified using their growth patterns and colonial morphology on the agar plates. On the blood agar and chocolate agar plates, the growths were examined for hemolysis. On the MacConkey agar plates, the colonial appearance and morphology were examined by looking for whether they are lactose fermenters or not.

Growths on the three plates were compared to see if growth occurred on all three or restricted to the blood agar and chocolate agar only. The colonies growing on the plates were then examined by Gram stain again and compared with the Gram stain results obtained from the Gram stain smear prepared directly from the sputum. Depending on the Gram reaction the appropriate biochemical and sugar fermentation tests were employed to identify them.

The organisms were identified by the appropriate biochemical tests (Appendix) such as coagulase tests, catalase tests, optochin test, indole tests, citrate utilization tests, urease test, and oxidase test. The Gram negative organisms were also tested on Kligler iron agar (Appendix) to identify the organism to species level. When large Gram positive oval or spherical cells with buds were seen in the Gram stained slides, then yeast cells were suspected and were identified by Analytical Profile Index (API).

3.6 Biochemical Tests

3.6.1 Kligler Iron Agar Test

This test was performed on colonies which were observed to be Gram negative rods under the microscope and suspected to belong to the Enterobacteriaceae family. The test was performed by picking part of the colony and inoculating on Kligler agar using a straight sterile loop. The setup was incubated overnight. After overnight incubation, the tubes were examined for the following reaction; a red slant, yellow butt, Hydrogen sulphide production and a crack in the medium. The various Gram negative rods reacted differently in this medium and their reaction helped in their identification.

3.6.2 Indole Test

The indole test was done on colonies which were Gram negative rods. To perform the test, loopful of colonies was inoculated into a sterile peptone broth and incubated overnight. The following day, a few drops of Kovacs'reagent were added to the culture broth, using a Pasteur pipette. Appearance of a red layer indicated that the test was positive whereas yellow layer indicated that the test was negative. *Escherichia coli* was suspected if the colonies were lactose fermenting, non mucoid colonies and were Gram negative rods and positive to the indole test.

3.6.3 Citrate Utilization Test

This test was done to determine the ability of an organism to utilize citrate. This test was done by stabbing citrate agar in a test tube with organisms which were Gram negative using a sterile straight loop. After overnight incubation, a change in colour of the agar from green to blue implies a positive test and no change in colour implies a negative test. Among the Gram negative rods, *Klebsiella, Proteus* and *Pseudomonas species* were suspected because these bacteria are able to utilize citrate. The composition, preparation and mode of action of citrate agar are presented in appendix.

3.6.4 Urease Test

This test was used to identify Gram negative rods which have the ability to produce urease. Colonies of the suspected bacteria were stabbed into urea agar in a test tube using a sterile straight loop. After overnight incubation, a change in colour from yellow to pink of the urea agar confirmed the ability of the organisms to produce urease. *Proteus species* was thus suspected, if the colonies were lactose non fermenters on MacConkey and produced swarming on blood agar. The composition, preparation and mode of action of urea agar is presented in appendix.

3.6.5 Oxidase Test

This was done on growths which were non lactose fermenters on MacConkey and identified as Gram negative rods on Gram staining. A slide was used to pick colony from an agar plate. The colony was smeared on a filter paper soaked with the oxidase reagent (Appendix). The filter paper was examined for a colour change after a few seconds. The appearance of a purple / bluish colouration indicated the presence of *Pseudomonas species.*

3.6.6 Coagulase Test

This test was performed on organisms which were Gram positive cocci in clusters after Gram's stain. It was used to differentiate between coagulase positive *Staphylococcus* and coagulase negative *Staphylococcus*. This was done by emulsifying the suspected colonies in a few drops of saline placed on a clean slide. A few drops of plasma were added to the emulsified colonies. The appearance of clumps on the slide after mixing indicated the presence of coagulase positive *Staphylococcus* which is *Staphylococcus aureus*. *Staphylococcus aureus* gives a coagulase positive result which other *Staphylococcus species* are negative for.

3.6.7 Catalase Test

This test is done to differentiate bacteria that produce the enzyme catalase, such as *Staphylococcus* from non catalase producing bacteria such as *Streptococci.* This was done by using a sterile bacteriological loop to pick a colony of the test organism from the blood agar plate and immersing into a test tube containing 3% Hydrogen peroxide. Within one minute, gas bubbles were seen produced and rising from the Hydrogen peroxide indicating that the organism is catalase positive. The test is positive for *Staphylococcus species* and negative for *Streptococcus species*.

3.6.8 Optochin Test

This was done on colonies which produced alpha hemolysis on blood agar and chocolate agar but did not grow on MacConkey agar. Again it was performed on colonies which were Gram positive cocci in chains to differentiate between *Streptococcus pneumoniae and Streptococcus viridans*. Colonies of the test organism were streaked or cross hatched on blood agar using a sterile loop. An optochin disc was placed directly on the cross hatched surface of the agar plate. This was incubated in a Carbon dioxide jar. After overnight incubation, the plates were examined for zones of clearance around the optochin discs. *Streptococcus pneumoniae* is optochin positive; therefore, forms zones of inhibition around the optochin disc whilst *Streptococcus viridans* is optochin negative, hence, no zone of inhibition forms around the optochin disc (Figure 3.1 below).

3.7 Characteristics of the Bacteria Colonies Isolated

Macroscopically, the colonies of the *Klebsiella pneumoniae* isolated on the MacConkey agar looked large, mucoid and pink because of the lactose fermentation. The colonies of *Escherichia coli* isolated on the MacConkey agar looked non mucoid, smooth and pink because of the lactose fermentation and that of the colonies of *Pseudomonas aeruginosa* looked flat, large, and smooth with greenish pigment. The colonies of *Staphylococcus aureus* looked cream, non mucoid and showed beta hemolysis and that of colonies of *Proteus mirabilis* on MacConkey agar no showed swarming.

3.8 Antibiotics Susceptibility Test

The antimicrobial susceptibility test was performed using the modified disc diffusion test method (Kirby-Bauer) recommended by Clinical and Laboratory Standards Institute (CLSI). The following antimicrobial agents were used; ampicillin (10ug), penicillin (10ug), cloxacillin (5ug), cefuroxime (30ug), ceftriaxone (30ug) and cefotaxime (30ug) gentamicin (10ug) and amikacin (30ug), co-trimoxazole (25ug), tetracycline (10ug), chloramphenicol (10ug) ciprofloxacin (5ug), and erythromycin (5ug).

3.8.1 The Disk Diffusion Method (Kirby-Bauer)

To perform the test, a sterile microbiological loop was used to pick 3-5 well-isolated colonies of similar growth appearance on the culture plate and emulsified in 3mls of sterile peptone water in a test tube. The turbidity of the suspension was compared with the turbidity standard (Appendix). If the turbidity standard was denser more colonies were picked with the loop and added to the test suspension and compared again with the standard. However, if the test suspension was denser then more sterile peptone water was added and compared again until turbidity in both tubes were similar. A sterile swab was dipped into the suspension. Excess fluid was removed by pressing and rotating the swab against the side of the tube above the level of the suspension. The swab was then used to inoculate the Muller – Hinton agar (Appendix). The inoculum was evenly spread over the surface of the medium. With the aid of sterile forceps, the filter paper disks containing the antimicrobial agents were evenly placed on the medium.

The set up was then incubated overnight at 37^{o}C. After overnight incubation, the diameters of the zones of inhibition were measured in millimeters with a compass and a ruler. The measurement was compared with the standard interpretative chart provided by Clinical and Laboratory

Standards Institute available at the microbiology laboratory to determine whether the bacterium is intermediate, susceptible or resistant to the antibiotic. The zone of intermediate resistance indicates that some inhibition occurs using that antibiotic but it may not be sufficient inhibition to eradicate the organism from the body. Clinical and Laboratory Standards Institute recommends the use of quality control organisms to see if they too produce the right zone sizes. *Staphylococcus aureus* (ATCC 25923) a Gram positive organism and *Escherichia coli* (ATCC 25922) a Gram negative organism, were used as controls.

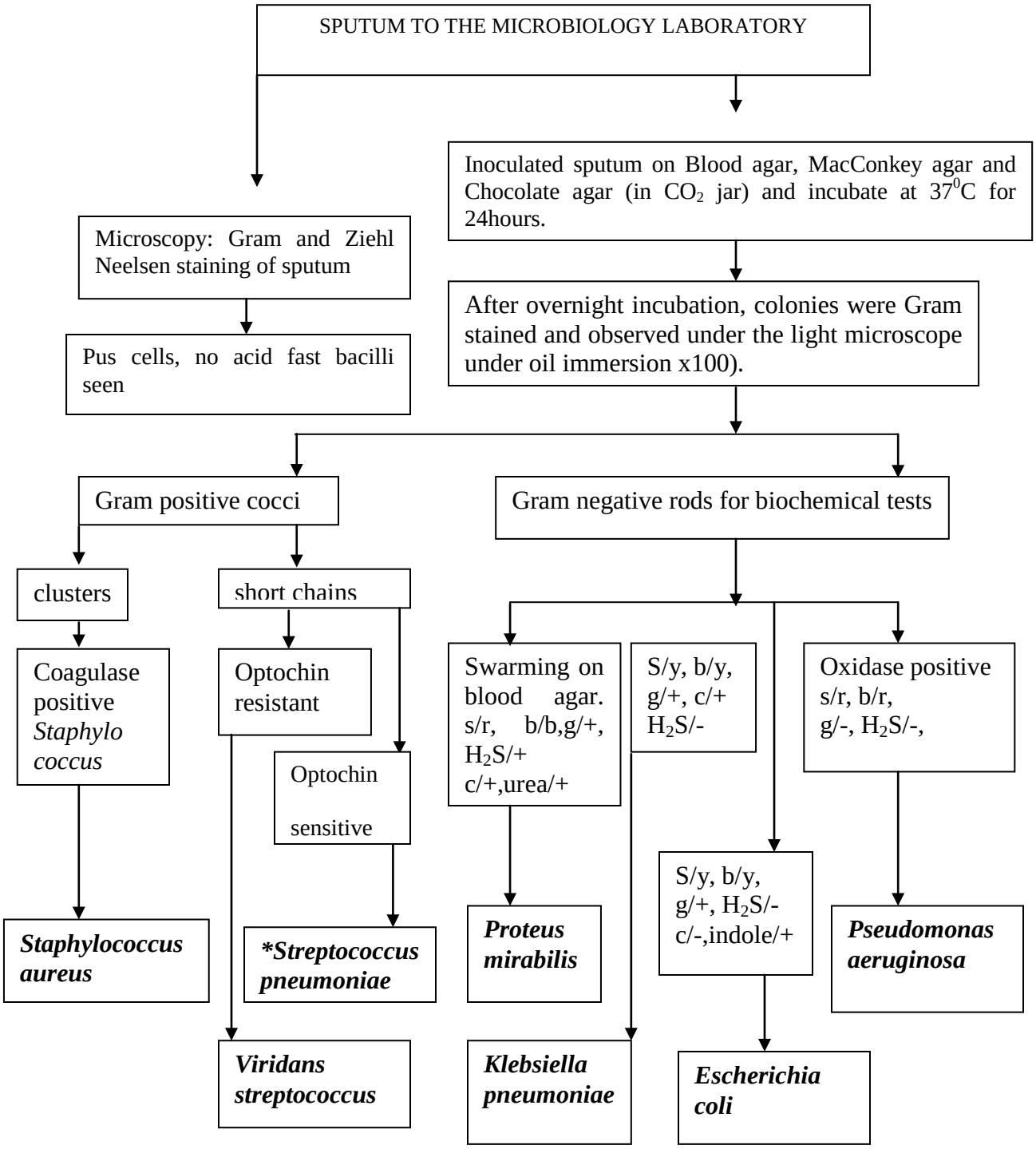

Figure 3. 1 Flow chart for isolation and identification of bacteria in sputum.

Note: S/y--slope/yellow B/y—butt/yellow. B/b--butt/black .S/r--slope/red. B/r--butt/red. G/+ or G/-_gas present or gas absent. H2S/+ or H2S/-_--Hydrogen Sulphide present or Hydrogen Sulphide absent. C/+ or c/-_--citrate present or citrate absent. Indole /+--indole present.
**Streptococcus pneumoniae was not isolated*

CHAPTER FOUR

RESULTS

4.0 Gender Distribution of Subjects

From 12th January to 13th June 2008, 200 HIV seropositive and 200 HIV seronegative patients were tested for bacterial infection of the respiratory tract. These are patients who have cough duration of between one week and six months. Of the HIV seropositive patients, 143 (71.5%) were females and 57 (28.5%) were males and of the HIV seronegative patients, 101 (50.5%) were females and 99 (49.5 %) were males.

4.1 Bacterial Isolates

The Gram stain carried out on the 200 sputum samples in the HIV seropositive patients showed numerous pus cells indicating a level of infection. In the HIV seronegative patients, the pus cells observed were also numerous like that in the HIV seropositive patients. However, out of the 200 HIV seropositive patients, 47 bacterial species were isolated. This was isolated from the sputum of 45 patients. Also, out of the 200 HIV seronegative patients used as controls, 45 bacterial species were isolated from their sputum. The bacterial isolates identified from the sputum of the HIV seropositive patients and their prevalence were *Klebsiella pneumoniae* (12.0%), *Escherichia coli* (6.0%), *Pseudomonas aeruginosa* (3.0%), *Staphylococcus aureus* (1.5%), *and Proteus mirabilis* (1.0%). Those identified from the HIV seronegative patients were *Klebsiella pneumoniae* (15.0%), *Escherichia coli* (6.0%), *Pseudomonas aeruginosa* (1.0%), *and Staphylococcus aureus* (0.5%). *Proteus mirabilis* was not isolated in the sputum of HIV seronegative patients. Results obtained are summarized in table 4.1.

Table 4. 1 Chi-square analysis for the distribution of isolates from the sputum of HIV seropositive and HIV seronegative patients at KATH.

	Observed		
Isolates	**HIV seropositive patients**	**HIV seronegative patients**	**Total**
Klebsiella pneumoniae	24	30	54
Escherichia coli	12	12	24
Pseudomonas aeruginosa	6	2	8
Staphylococcus aureus	3	1	4
Proteus mirabilis	2	0	2
Candida albicans	16	0	16
Total	63	45	**108**

	Expected	
Isolates	**HIV seropositive patients**	**HIV seronegative patients**
Klebsiella pneumoniae	31.5	22.5
Escherichia coli	14.0	10
Pseudomonas aeruginosa	4.66667	3.33333
Staphylococcus aureus	2.33333	1.66667
Proteus mirabilis	1.16667	0.83333
Candida albicans	9.33333	6.66667

($\chi^2 = 0.00176$, $df = 108$, $p = 0.99$)

The commonest bacterial isolate obtained from the HIV seropositive patients was *Klebsiella pneumoniae* contributing 51.1% of the total isolates. The second common bacterium isolated was *Escherichia coli* (25.5%), followed by *Pseudomonas aeruginosa* (12.8%), *Staphylococcus aureus* (6.4%), *and Proteus mirabilis* (4.3%) in that order. Also, in the HIV seronegative patients, the commonest bacterial isolate obtained was *Klebsiella pneumoniae* contributing 67.0% of the total isolates. The second common bacterium isolated was *Escherichia coli* (27.0%), followed by *Pseudomonas aeruginosa* (4.0%), and *Staphylococcus aureus* (2.0%). (Table 4.2)

Table 4. 2 Bacterial isolates relative to one another obtained from the sputum of HIV seropositive and HIV seronegative patients at KATH

Bacteria isolates	HIV Seropositivepatients		HIV Seronegative patients	
	Number	Percentage	Number	Percentage
Klebsiella pneumoniae	24.0	51.1	30.0	67.0
Escherichia coli	12.0	25.5	12.0	27.0
Pseudomonas aeruginosa	6.0	12.8	2.0	4.0
Staphylococcus aureus	3.0	6.4	1.0	2.0
Proteus mirabilis	2.0	4.3	0.0	0.0

The commonest bacteria obtained as single isolate were *Klebsiella pneumoniae* (60.0%) in the HIV seropositive patients and also among HIV seronegative patients, *Klebsiella pneumoniae* was 67.0% relative to other isolates. The other bacteria obtained as single isolate in the HIV seropositive patients were *Escherichia coli* (20.0%), *Pseudomonas aeruginosa* (11.4%), *Staphylococcus aureus* (8.6%) relative to other isolates. *Proteus mirabilis* was isolated together with *Candida albicans*. Results for HIV seronegative patients were similar to those obtained for seronegative patients (table 4.2). Here too *Klebsiella pneumoniae* and *Escherichia coli* were the dominant isolates (table 4.3).

Table 4. 3 Bacterial isolate obtained as single isolates in the sputum of HIV seropositive and HIV seronegative patients at KATH

Bacteria isolates	Seropositive patients		Seronegative patients	
	Number	Percentage	Number	Percentage
Klebsiella pneumoniae	21	60.0	30	67.0
Escherichia coli	7	20.0	12	27.0
Pseudomonas aeruginosa	4	11.4	2	4.0
Staphylococcus aureus	3	8.6	1	2.0
Proteus mirabilis	0	0.0	0	0.0
Total	35	100.0	45	100.0

Of the HIV seropositive patients that had isolates in their sputum, 15.9% had dual infections. Both *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were isolated in one of such patients. In another patient both *Escherichia coli* and *Pseudomonas aeruginosa* were isolated. Dual infections caused by *Escherichia coli* and *Candida albicans* were the most common co-infection, and it was observed in 6.3% of the HIV seropositive patients. Also dual infections with *Klebsiella pneumoniae* and *Candida albicans* were observed in 3.2% of the HIV seropositive patients, so also dual infections with *Proteus mirabilis* and *Candida albicans* were observed in 3.2% of the HIV seropositive patients (Table 4.4). However, in the HIV seronegative patients, no dual infection was obtained.

Table 4. 4 Multiple isolates obtained from sputum of HIV seropositive patients

Multiple occurrence	Number of cases	%
Escherichia coli and **Candida albicans*	4	6.3
Klebsiella pneumoniae and **Candida albicans*	2	3.2
Proteus mirabilis and **Candida albicans*	2	3.2
Klebsiella pneumoniae and *Pseudomonas aeruginosa*	1	1.6
Escherichia coli and *Pseudomonas aeruginosa*	1	1.6
Total	10	15.9

*Note: *A lot of pseudohyphae on smear examination were considered as having significant pathogen (Candida albicans)*

4.2 Bacteria Isolates in Relation to Age of Patients

The mean age of the HIV seropositive patients was 40.0 years SD of 10.2 years with a range of 22 to 75.years. The mean age of the HIV seronegative patients was 41.4 years SD of 16.3 years with a range between 15 and 85 years. Comparison of age by a one way anova showed that there is no statistical significant difference ($p = 0.309$ *at 95% CL*) between the ages of HIV seropositive and seronegative patients involved in this study.

Among the HIV seropositive patients, six bacterial isolates each were obtained from the 20-29 and 60-69 age groups. However, in the HIV seronegative patients, nine and five bacterial isolates were obtained from the 20-29 and 60-69 age groups respectively. Also, in the 50-59 age groups five bacterial isolates were obtained in the HIV seropositive patients whereas in the HIV seronegative patients, five bacterial isolates were obtained from the 10-19 age groups. Further, in

the HIV seropositive patients, no bacteria isolate was obtained from the 10-19 age groups. Whereas seventeen bacterial isolates were obtained from the HIV seropositive patients in the 30-39 age groups, twelve bacterial isolates were obtained from the HIV seronegative patients. Also, in the 40-49 age groups, thirteen bacterial isolates were obtained from the HIV seropositive patients whereas six isolates were obtained from the HIV seronegative patients. Regarding the 50-59 age groups, eight bacterial isolates were obtained from the HIV seronegative patient (tables 4.5 and 4.6).

Table 4. 5 Distribution of the isolates among the age groups of HIV seropositive patients at KATH

Age groups	*Klebsiella pneumoniae*		*Escherichia Coli*		*Pseudomonas aeruginosa*		*Staphylococcus Aureus*		*Proteus Mirabilis*	
	No.	%	No	%	No.	%	No.	%	No.	%
20-29	2	8.3	1	8.3	1	16.7	1	33.3	1	50.0
30-39	8	33.3	6	50.0	1	16.7	1	33.3	1	50.0
40-49	8	33.3	2	16.7	3	50.0	0	0.0	0	0.0
50-59	4	16.7	1	8.3	0	0.0	0	0.0	0	0.0
60-69	2	8.3	2	16.7	1	16.7	1	33.3	0	0.0
Total	24	99.9	12	100.0	6	100.0	3	99.9	2	100

Table 4. 6 Distribution of the isolates among the age groups of HIV seronegative patients at KATH

Age groups	*Klebsiella pneumoniae*		*Escherichia Coli*		*Pseudomonas aeruginosa*		*Staphylococcus aureus*		*Proteus Mirabilis*	
	No.	%	No	%	No.	%	No.	%	No.	%
10-19	5	17.0	0	0.0	0	0.0	0	0.0	0	0.0
20-29	6	20.0	3	25.0	0	0.0	0	0.0	0	0.0
30-39	7	23.0	5	42.0	0	0.0	0	0.0	0	0.0
40-49	5	17.0	1	8.0	0	0.0	0	0.0	0	0.0
50-59	4	13.0	2	17.0	2	100	0	0.0	0	0.0
60-69	3	10.0	1	8.0	0	0.0	1	100	0	0.0
Total	30	100	12	100	2	100	1	100	0	0.0

4.4 Antimicrobial susceptibities of the bacterial isolates

Results obtained in this study shows variable susceptibility levels of the isolates to the antibiotics tested. For instance, whereas 95.8% of *Klebsiella pneumoniae* were susceptible to ciprofloxacin in the HIV seropositive patients, 90% were susceptible in the HIV seronegative patients. In addition, 79.1% *Klebsiella pneumoniae* was susceptible to amikacin in the seropositive patients as compared to 63.3% in the HIV seronegative patients. No *Klebsiella pneumoniae* were susceptible to ampicillin in the HIV seropositive patients. Susceptibility of *Klebsiella pneumoniae* to cotrimoxazole in the HIV seropositive was 20.8% and in HIV seronegative patients was 20%. Also, while 20.8% of the *Klebsiella pneumoniae* isolates were susceptible to chloramphenicol in the HIV seropositive patients, only 6.7% of them were susceptible to chloramphenicol in the HIV seronegative patients. Further, while 20.8% of *Klebsiella pneumoniae* were susceptible to ceftriaxone in the HIV seropositive patients; no isolates were sensitive to ceftriaxone in the HIV seronegative patients. However, while 95.8% *Klebsiella pneumoniae* were susceptible to gentamicin in the HIV seropositive patients, 73.3% *Klebsiella pneumoniae* were susceptible to gentamicin in the HIV seronegative patients. Also, 70.8% of *Klebsiella pneumoniae* were susceptible to cefuroxime in the HIV seropositive patients, and 63.3% of the isolate were susceptible to cefuroxime in the HIV seronegative patients. With tetracycline, whereas 33.3% *Klebsiella pneumoniae* was susceptible to it in the HIV seropositive patients, 6.7% *Klebsiella pneumoniae* was susceptible in the HIV seronegative patients.

In regards to *Escherichia coli* isolates, 66.7% were susceptible to ciprofloxacin in both HIV seropositive and HIV seronegative patients. Likewise, 8.3% of the same isolate was susceptible to ampicillin in both groups of patients. In the HIV seropositive patients whereas 91.7% of *Escherichia coli* was susceptible to amikacin, 41.7% were susceptible in the HIV seronegative

patients. The percentage of *Escherichia coli* susceptible to gentamicin in the HIV seropositive patients was 75% and that in the HIV seronegative patients was 41.7%. Also, whereas 58.3% of the isolate was susceptible to cefuroxime in the HIV seropositive patients, 16.7% were susceptible to cefuroxime in the HIV seronegative patients. In addition, 16.7% of the isolate was susceptible to chloramphenicol in the seropositive patient and 8.0% was susceptible to the same antibiotic in the HIV seronegative patients. In the HIV seropositive patients, 91.7% of *Escherichia coli* was susceptible to cefotaxime whereas in the HIV seronegative patients 58.3% of the isolate was susceptible to cefotaxime. 8.3% of the isolate was susceptible to tetracycline in the HIV seropositive patients and 25% of the same isolate was susceptible in the HIV seronegative patients.

Whereas all the *Pseudomonas aeruginosa* isolated was susceptible to ciprofloxacin in the HIV seronegative patients, 66.7% were susceptible in the HIV seropositive patients. Also, all the *Pseudomonas aeruginosa* isolates were susceptible to the gentamicin in the HIV seronegative patients and that susceptible in the HIV seropositive patients was 83.3%. All the *Staphylococcus aureus* isolates from the HIV seropositive patients were susceptible to ciprofloxacin, cloxacillin and gentamicin. *Staphylococcus aureus* was 66.7% susceptible to erythromycin in the HIV seropositive patients.

All the *Proteus mirabilis* isolated were susceptible to ciprofloxacin, gentamicin, cefotaxime and cefuroxime in the HIV seropositive patients. These distributions are presented in table 4.7 below.

Table 4. 7 Antimicrobial susceptibility pattern of bacterial isolates obtained from HIV seropositive and seronegative patients at KATH.

Antibiotics	Percent sensitivity of pathogens									
	Klebsiella pneumoniae		*Escherichia coli*		*Staphylococcus aureus*		*Pseudomonas aeruginosa*		*Proteus mirabilis*	
	SP	SN	SP	SN	SP	SN	SP	SN	SP	SN
Ciprofloxacin	95.8	90	66.7	66.7	100.0	100.0	66.7	100.0	100.0	-
Gentamicin	95.8	73.3	75	41.7	100.0	100.0	83.3	100.0	100.0	-
Cefuroxime	70.8	63.3	58.3	16.7	0.0	100.0	-	-	100.0	-
Cotrimoxazole*	20.8	20.0	0.0	33.3	0.0	0.0	-	-	0.0	-
Tetracycline	33.3	6.7	8.3	25.0	0.0	0.0	-	-	0.0	-
Ampicillin	0.0	3.3	8.3	8.3	0.0	50.0	-	-	0.0	-
Ceftriaxone	20.8	0.0	0.0	0.0	-	-	16.7	0	50.0	-
Amikacin	79.1	63.3	91.7	41.7	-	-	-	-	50.0	-
Chloramphenicol	20.8	6.7	16.7	8.3	-	-	-	-	0.0	-
Cefotaxime	83.3	66.7	91.7	58.3	-	-	-	-	100	-
Penicillin	-	-	-	-	0	50.0	-	-	-	-
Cloxacillin	-	-	-	-	100.0	100.0	-	-	0.0	-
Erythromycin	-	-	-	-	66.7	0	-	-	-	-

* Administered as a prophylactic antibiotic against respiratory pathogens in HIV infected individuals at KATH.
SP__ Seropositive patients
SN__Seronegative patients
-___Antibiotic not tested on isolate.
0__All isolates were resistant

CHAPTER FIVE

DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS

5.0 Discussion

Over a 6-month period, 400 patients made of 200 HIV seropositive patients and 200 HIV seronegative patients were enrolled in the study. Of the 200 HIV seropositive patients, the majority 143(71.5%) were females. Most of the patients (85.0%) were in their reproductive age group (18-50). This result is similar to studies carried out by Adeleye *et al.,* (2008) where of a total of 100 HIV seropositive patients included in their study, 61 turned out to be females and 39 were males and it was also observed in their study that the majority (88%) of HIV seropositive patients were between the ages of 21 and 40 years.

The prevalence of bacterial species responsible for the respiratory tract infections among the HIV seropositive patients was 23.5%. Amongst the HIV seronegative patients used as controls, the prevalence was 22.5 %. There was no significant difference *($p>0.05$)* between the prevalence in both group of patients. Shailaja *et al.,* (2004) however had a higher prevalence of 44.28% amongst 100 HIV seropositive patients. The prevalence value here is much higher considering the fact that they studied HIV seropositive patients who were not on any antibiotic prophylaxis and also worked on 30 HIV seronegative patients as controls but failed to report the prevalence value in this category of patients. In the HIV seropositive patients, 4 different Gram negative bacteria representing (22%) and one Gram positive bacterium being 1.5% of the total isolates were obtained. Further, in the HIV seropositive patients there were dual infections in 15.9% of

the patients studied. The dual infections encountered were caused by 2 different Gram negative bacteria (*Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) and on another occasion *Klebsiella pneumoniae* or *Escherichia coli* in association with *Candida albicans* isolated in about 16% individuals. This finding was similar to that carried out by Aruna *et al.,* (2005*)* where more than one organism was found in 40 HIV seropositive patients and Shailaja *et al.,* (2004) where more than one organism was found in 8 patients. This the researcher attributed to the immunocompromised state of the patients studied. Difficulties arose in explaining the observation that in this current study there was no significant difference (*p>0.05)* in the number of bacteria isolated from the HIV seropositive patients and the HIV seronegative patients. However, as the CD4 levels of the HIV seropositive patients were not sought, it might be that the levels were not below the value where their immune status would be compromised.

The lungs of Human Immunodeficiency Virus (HIV)-infected patients are a major target for many infections and tumours (Murray *et al.,* 1990; Mitchell *et al.,* 1995*),* it is only gradually that pyogenic bacteria have been recognized to be a major cause of lower respiratory tract infection in HIV infected patients, whatever their level of immunosuppression (Murray *et al.,* 1990; Mitchell *et al.,* 1995; Noskin *et al.,* 1995; Rosen, 1994). *Streptococcus pneumoniae* has been incriminated as the commonest causative organism for community acquired pneumonia and it is common among the elderly (Wilkinson *et al.,* 2004). Contrary to the much publicized reports about the role of pneumococcus as the dominant cause of respiratory infection our study showed that *Klebsiella pneumoniae* was the most frequently isolated organism, followed by *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Proteus mirabilis* respectively (Table 4.2), a study which failed to isolate *Streptococcus pneumoniae* and *Haemophilus influenzae*. This finding is in contrast to other studies elsewhere in which Tchamran (1997) in a study on the

lung diseases due to common bacteria in HIV infected individuals in African adults noted 81% of respiratory tract infections are due to *Streptococcus pneumoniae* and reported it to be the most offending pathogen in HIV seropositive patients. Afessa *et al.,* 2000 and Nuorti *et al*, 2000 have reported *Pseudomonas aeruginosa*, *Streptococcus pneumoniae* and *Haemophilus influenzae* to be the predominant organism causing respiratory tract infection in HIV patients. However, it should be noted that the etiology and pattern of respiratory tract infections in HIV-infected persons differ between sub-Saharan Africa and industrialized countries (Noskin *et al.,* 1995).

In Ghana, Newman *et al.*, (2006) in their Technical Report Series No. 5, Project Number 2001/GD/07 on the Resistance to Antimicrobial Drugs, it was noted that, of the bacteria isolated from sputum from the regions in Ghana, no *Haemophilus influenzae* was isolated (even from the Teaching hospitals) and only four isolates of *Streptococcus pneumonia* were obtained. However, *Klebsiella* spp, *Pseudomonas aeruginosa, Escherichia coli, Proteus* spp and *Staphylococcus aureus* were isolated from sputum from the various hospitals in Ghana. Throughout the 6 months that this study was carried out in 2008, *Streptococcus pneumoniae* and *Haemophilus influenzae* were not isolated in the Clinical Microbiology laboratory of KATH, Kumasi. The record books of the Clinical Microbiology Laboratory, in 2007, had only 2 isolates of *Streptococcus pneumoniae* representing 0.2% of the total isolates obtained and was obtained from 2 male patients aged 18 and 52. Also, no *Haemophilus influenzae* was isolated in the same year. However, in the same year 138(15.1%) *Klebsiella pneumoniae,* 91(10.0%) *Escherichia coli*, 20(2.2%) *Pseudomonas aeruginosa*, 10(1.1%) *Proteus mirabilis,* 6 (0.7%) *Staphylococcus aureus,* 2(0.2%) *Acinetobater baumanii,* 3 (0.3%) and *Enterobacter aerogenes.* were isolated, results which reflect findings in this current study.

The Gram stain carried out on the 200 sputum samples in the HIV seropositive patients showed numerous pus cells indicating a level of infection. In the HIV seronegative patients, the pus cells observed were also numerous like that in the HIV seropositive patients. However, in both cases it was not all the samples that yielded positive isolates. It is difficult to tell whether these indications mean infection with pathogen other than those obtained in this study.

From the study it was noted that the HIV seronegative patients showed no dual infection however, dual infection was observed in the HIV seropositive patients. The organisms causing dual infection in ten of the HIV seropositive patients were a significant finding since this was not encountered in the HIV seronegative patients.

Fungal respiratory tract infections often precede the appearance of other opportunistic infections, but frequently co-exist with other pathogens in the immunocompromised patients (Rosen, 1994). Though pulmonary candidiasis is documented to be a very rare disease, occurring in late stages of AIDS, oral and oesophageal candidiasis is reported as the second most common opportunistic infection among HIV patients, from India (Mohanthy *et al*., 1993). In this study, 10 of the HIV seropositive patients (15.9%) had fungal isolates identified as *Candida albicans* recovered from their sputum. To rule out the possibility of oro-pharyngeal colonization, a common feature among HIV seropositive patients, cases with a lot of pseudohyphae on smear examination were considered as having significant pathogen. Also, none of the HIV seropositive patients included in the study had oral lesions thus, the isolation of *Candida albicans* cannot be attributed to that. No *Candida* species were isolated from the sputum of HIV seronegative patients.

Antimicrobial therapy of bacterial pneumonia must be prompt and adequate, but in HIV infected individuals there are no guidelines for the empirical treatment of bacterial pneumonia (Burack *et al.*, 1994; Huang *et al*., 1996).

This study demonstrated that in the HIV seropositive patients more than 50% of the Gram negative bacteria isolated were susceptible to ciprofloxacin, gentamicin, amikacin, cefotaxime and cefuroxime and the Gram positive bacteria (*Staphylococcus aureus*) was susceptible to penicillin and cloxacillin (Table 4.7). For the *Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa,* and *Staphylococcus aureus* isolated from both group of patients, there was no significant difference in the levels of antibiotic susceptibilities observed ($p > 0.05$). These imply that the HIV status of patients did not influence antimicrobial susceptibility of the bacterial isolates. Since *Proteus mirabilis* was not isolated from seronegative patients, a comparison could not be done.

Cotrimoxazole prophylaxis has been recommended as part of the essential care and support package for symptomatic HIV-infected individuals in sub-Saharan Africa since 2000 (UNAIDS, 2000). In Africa, it has been shown to reduce Human Immunodeficiency Virus (HIV)-associated mortality and morbidity including reductions in the incidence of malaria and pneumonia (Anglaret *et al.,* 1999; Mermin *et al.,* 2004). Despite the favorable outcomes associated with cotrimoxazole use in HIV seropositive people, concerns have been raised that the widespread use of this drug for prophylaxis will contribute to antimicrobial resistance (Gill *et al.,* 2004).

Hamel and colleagues describe the effect of cotrimoxazole prophylaxis on antimicrobial resistance rates among HIV seropositive and HIV seronegative Kenyan adults using a prospective cohort methodology (Hamel *et al.,* 2008). This antibiotic has been found to be 100% potent against *Streptococcus pneumoniae* and *Haemophilus influenzae* (Anglaret, 1999). In Ghana, most (73%) isolates of the Enterobacteria are resistant to cotrimoxazole (Newman *et al.,* 2006).

From the study, most of the isolates were resistant to cotrimoxazole. The use of cotrimoxazole, an antibiotic which is given to the HIV seropositive patients as a prophylactic drug to manage opportunistic infections such as respiratory tract infection, has probably led to development of resistance by the bacteria isolated. Co-trimoxazole has been used widely as treatment for common infections in many resource limited settings and, as a result, co-trimoxazole resistance among these pathogens is high in these settings (Mermin *et al*., 2004).

Data from this study is useful clinically, considering the high prevalence of some respiratory bacterial isolates and their antimicrobial susceptibilities in HIV infection. The antimicrobial pattern may provide guidelines for physicians in treatment, while waiting for antimicrobial susceptibility test results from microbiological laboratories.

5.1 Conclusion

This study was set out to determine the prevalence of bacterial species responsible for respiratory tract infection in HIV seropositive patient other than the *Mycobacterium tuberculosis* at KATH. The prevalence of the bacteria isolated was 23.5%. Subsequently, bacteria isolated from both the HIV seropositive and HIV seronegative patients were found to be similar. Some of the HIV seropositive patients (about 16%) were found to be infected with more than one type of pathogens, that is, have dual infections. Most of the isolates obtained in the study were members of the Enterobacteriaceae and *Pseudomonas aeruginosa*. *Streptococcus pneumoniae* and *Haemophilus influenzae* were not isolated in both the HIV-seropositive and –seronegative patients. The isolates were susceptible to ciprofloxacin, gentamicin, cefotaxime, amikacin and cefuroxime with levels of about 80% and more but were largely resistant to cotrimoxazole with

about susceptibility level of as low as 20.0%. The use of co-trimoxazole may be indicated as a prophylactic drug against *Streptococcus pneumoniae* and *Haemophilus influenzae* but from this study these isolates were not obtained, and cotrimoxazole cannot be recommended for the isolates obtained due the high resistance levels observed.

5.2 Recommendations

Based on these findings, it is recommended that

1. An alternative antibiotic needs to be evaluated to be used as a prophylactic drug for the HIV seropositive patients in view of the high resistance levels of the isolates to cotrimoxazole obtained in this study.
2. A more sensitive diagnostic procedure for the isolation of *Streptococcus pneumoniae* and *Haemophilus influenzae* is suggested.

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APPENDIX

KEY

s/y----slope/yellow
b/y----butt/yellow
s/r----slope/red
b/r----butt/red
g/+ or g/-_---gas present or gas absence
H_2S/+ or H2S/- _------Hydrogen Sulphide presence or Hydrogen Sulphide absence
c/+ or c/-_--------citrate present or citrate absent
indole /+-----indole present

Table A Summary of Results of the Gram Staining Reaction of the Sputum from the HIV Seropositive Patients

ISOLATE	No. OF PATIENTS	GRAM STAIN REACTION	BIOCHEMICAL TESTS
Viridans Streptococci	147	Pus cells, Gram positive cocci in short chains	Optochin resistant
Klebsiella pneumoniae	24	Pus cells and Gram negative rods	s/y, b/y,g/+, H_2S/- c/+
Escherichia coli	12	Pus cells and Gram negative rods	s/y, b/y,g/+, H_2S/- c/-, indole/+
Pseudomonas aeruginosa	6	Pus cells and Gram negative rods	oxidase positive s/r, b/r,g/-, H_2S/-,c/+
Candida albicans	16	Yeast cells	
Staphylococcus aureus	3	Pus cells and Gram positive cocci in clusters	Coagulase positive
Proteus mirabilis	2	Pus cells and Gram negative rods	s/r, b/b,g/+, H_2S/+ c/+,urea/+

Table B Summary of Results of the Gram Staining Reaction of the Sputum from the HIV Seronegative Patients

ISOLATE	No. OF PATIENTS	GRAM STAIN REACTION	BIOCHEMICAL TESTS
Viridans Streptococci	155	Pus cells, Gram positive cocci in short chains	Optochin resistant
Klebsiella pneumoniae	30	Pus cells and Gram negative rods	s/y, b/y,g/+, H_2S/-, c/+
Escherichia coli	12	Pus cells and Gram negative rods	s/y, b/y,g/+, H_2S/- c/-, indole/+
Pseudomonas aeruginosa	2	Pus cells and Gram negative rods	oxidase positive s/r, b/r,g/-, H_2S/-,c/+
Staphylococcus aureus	1	Pus cells and Gram positive cocci in clusters	Coagulase positive
Proteus mirabilis	0	Pus cells and Gram negative rods	s/r, b/b,g/+, H_2S/+ c/+,urea/+

Table A. Statistical analysis of the bacterial isolates in the HIV seropositive and seronegative patients using two-tail test

t-Test: Paired Two Sample for Means		
	HIV seropositive patients	HIV seronegative patients
Mean	4.7	4.5
Variance	20.45	40.25
Observations	5	5
Pearson Correlation	0.993379656	
Hypothesized Mean Difference	0	
Df	4	
t Stat	0.232495277	
P(T<=t) one-tail	0.41378236	
t Critical one-tail	2.131846782	
P(T<=t) two-tail	0.82756472	
t Critical two-tail	2.776445105	

P< 0.05; means significant, P> 0.05; means insignificant

Table B. Statistical analysis of the isolates in relation to the susceptibility of the antibiotics in the HIV seropositive and seronegative patients using two tail test.

t-Test: Paired Two Sample for Means(*Klebsiella pneumoniae)*		
	HIV seropositive patients	HIV seronegative patients
Mean	37.17857	28.09286
Variance	1509.153	1183.639
Observations	14	14
Pearson Correlation	0.969511	
Hypothesized Mean Difference	0	
Df	13	
t Stat	3.378606	
P(T<=t) one-tail	0.002471	
t Critical one-tail	1.770933	
P(T<=t) two-tail	0.004941	
t Critical two-tail	2.160369	

MEDIA

SOLID MEDIA

A. MacConkey Agar

This is a differential medium for the differentiation and isolation of *Enterobacteriaceae*. Swarming of *Proteus* is prevented.

Preparation: This was prepared by suspending 48.5 grams in 1 litre of distilled water. It was then boiled to dissolve completely. Sterilization was done by autoclaving at 121^0C for 15 minutes.

B. Blood Agar

This is an enriched medium.

Preparation: 40g of the powder was suspended in 1 litre of distilled water. It was boiled to dissolve completely. Sterilization was done by autoclaving at 121^0C for 15 minutes. The solution was then cooled to 50^0C and 7% of human blood added. It was mixed thoroughly and poured into Petri dishes.

C. Chocolate Agar

It is an enriched medium for fastidious organisms.

Preparation: 40g of the powder was suspended in 1 litre of distilled water. It was boiled to dissolve completely. Sterilization was done by autoclaving at 121^0C for 15 minutes. 7% of human blood added immediately. It was mixed thoroughly and poured into Petri dishes.

D. Mueller-Hinton Agar

This is an antimicrobial susceptibility testing medium.

Preparation: To prepare the medium 38 g of the powder was dissolved in 1 litre of distilled water. It was boiled to dissolve the medium completely. It was sterilized by autoclaving at 121^0C for 15 minutes.

E. Simmons Citrate Agar

This medium is used in the differentiation of *Enterobacteriaceae.*

Preparation: This is prepared by suspending 24g into 1 litre of distilled water. Mixed thoroughly. It was heated gently to dissolve and distributed into tubes. Sterilization was done by autoclaving at 121^0C for 15 minutes.

F. Kligler Iron Agar

This medium is used for identification of Gram negative enteric bacilli on the basis of glucose, lactose, fermentation and hydrogen sulphide.

Preparation: This is prepared by suspending 65g into 1 litre of distilled water. It was boiled to dissolve and distributed into tubes. Sterilization was done by autoclaving at 121^0C for 15 minutes. After sterilization, the medium was allowed to cool in a slanted position to form a 1 inch butt.

G. Urea Agar Base

This is medium is used for the identification of *Proteus spp.*

Preparation: This is prepared by suspending 2.1g into 95mls of distilled water. It was mixed thoroughly. Sterilization was done by autoclaving at 121^0C for 15 minutes. After cooling to 47^oC 5ml of 40% of urea solution was added and distributed into tubes.

LIQUID MEDIA

A. Peptone Water

Peptone water is used as a growth medium or as the basis of carbohydrate fermentation media.

Preparation:The medium was prepared by dissolving 15 grams in 1 litre of distilled water. It was mixed well and distributed into bijoux bottles in 5ml volumes, and then sterilized by autoclaving at 121^0C for 15 minutes.

B. Kovac's Reagent

This reagent is used for the detection of indole. It is prepared by dissolving 10 grams of 4-dimethylamino-benzaldehyde in 150ml of iso-amyl alcohol. After dissolution 50ml of concentrated hydrochloric acid is added to it. It is then stored in a refrigerator in an amber bottle.

C. Glycerol broth

This was used to store the bacteria isolates.

Preparation: The broth was prepared by weighing 20ml of brain-heart infusion broth. Distilled water and glycerol was added using the ratio of 4:1 distilled water to glycerol. The mixture was stirred until a uniform solution was obtained. A micropipette was used to pipette 1ml of the solution into Eppendorf tubes. The broth was then sterilized at 121^oC for 15 minutes.

D. Turbidity Standard Solution

This is a barium sulphate standard against which the turbidity of the test inocula can be compared. When matched with standard, the inocula should give confluent or almost confluent growth.

Preparation:One percent solution of sulphuric acid was prepared by adding 1ml of concentrated sulphuric acid to 99ml of distilled water. Also, 1% solution of barium chloride was prepared by

dissolving 0.5 grams of dehydrated barium chloride ($BaCl_2.2H_2O$) in 50ml of distilled water. 0.6ml of the barium chloride solution was added to 99.4ml of the sulphuric acid solution and mixed. Small volume was then transferred to a capped tube same as the type used for preparing the test and control inocula.

E. Oxidase test reagent

Mix 1.0g of dimethyl-phenylenediamine hydrochloride in 100ml of distilled water. Preferably, the reagent should be made up fresh, daily. It should not be stored longer than one week in the refrigerator.

STAINS

Gram Stain Reagent

Crystal violet stains

Solution A: Dissolve 2.0g of crystal violet in 20.0ml of ethyl alcohol. Solution B: Dissolve 0.8g of ammonium oxalate in 80.0ml distilled water. Mix solution A and solution B. Gram Iodine-Dissolve 2.0g of potassium iodide in 300mls of distilled water and then add 1.0g iodine crystals. Decolorizer -Ethyl alcohol (95%). Safranin (couterstain)-Dissolve 10mls safranin in 100mls distilled water.

Acid Fast Stain Reagent (Ziehl Neelsen)

Carbolfuchsin stain

Solution A: Dissolve 0.3g of basic fuchsin in 10ml ethyl alcohol. Solution B: Dissolve 5g of phenol in 95mls of distilled water. Mix solution A and solution B. Add 3ml of concentrated hydrochloric acid in 97ml of ethyl alcohol (decolorizer). Dissolve 0.3g of Methylene blue to 100ml distilled water.