CHAPTER I

1. 1 INTRODUCTION

Human immunodeficiency virus (HIV) causes progressive impairment of body's cellular immune system, leading to increased susceptibility to infection and tumor, and the fatal condition Acquired Immunodeficiency Syndrome(AIDS) (Cheesebrough, 2009). Among the special feature of HIV infection is that once infected it is probable that person will be infected for life (Park, 2007).

Ever since its discovery, the HIV has emerged as a global disaster. As of December 2008, the Joint United nations programme on HIV/AIDS (WHO/UNAIDS) estimated that 33.4 million people are infected with HIV.Since the first AIDS case, reported in 1988, the HIV epidemic in Nepal has evolved from a "low prevalence" to a "concentrated epidemics". In 2009, the estimated number of people living with HIV/AIDS was 63,258 with average number AIDS death per year of 4,701. HIV epidemic is largely concentrated among sex workers, injecting drugs users, MSM, and labor migrants to India. The latest cumulative HIV/AIDS cases as of 13 March 2010 were reported as 17, 556 with 578 cases of AIDS (NCASC, 2010).

Furtherance of HIV infection is largely dependent on the interaction between the viral load and host factors. HIV brings about the destruction of CD4+ T-lymphocytes, which are crucial cells in forming immune response to foreign antigens and it is also the primary target cells of HIV (Pattanapanyasat and Thakur, 2005; Paranjape, 2005). The progressive loss of these lymphocytes eventually results in the loss of an ability to mount desirable immune response to any pathogen (Pattanapanyasat and Thakur, 2005) and death of the patients in the terminal stage of HIV infection occurs (Paranjape, 2005). Major cause of morbidity and mortality of such patients are opportunistic infections (Gordon et al., 1992). Among them, the leading cause of their morbidity is various respiratory illnesses. Among the opportunistic infections associated with HIV, disease like pneumonia of bacterial origin occur at a rate many times higher in the HIV infected patients than in general population (Bhalla, 1999).

Patients with HIV infections frequently present with spectrum of pulmonary complications from opportunistic infections and neoplasm that may be associated to high mortality rates. Disease of respiratory tract accounts for half of death from AIDS. Bacterial pneumonia and AIDS can lead to significant morbidity and mortality and are second to *Pneumocystis carinii* pneumonia (PCP) (Orenstein et al., 1985). Since the initial AIDS cases were associated with opportunistic infection such as PCP, the importance of bacterial infection, including bacterial pneumonia, was not recognized at the beginning of the epidemic. However, studies have shown that bacterial infections occur more frequently than other opportunistic infection in patients with HIV (Selwyn et al., 1988; Wallace et al., 1997).

Streptococcus pneumoniae is the commonest cause of community-acquired pneumonia with the second most common being *Haemophilus influenzae* (Janoff et al,1993; Moreno et al, 1991).In comparison, nosocomial pneumonia occurs primarily in patient with AIDS and is usually due to *Staphylococcusaureus*, *Pseudomonasaeruginosa*, and other gram negative bacilli (Hirschtrick et al., 1995; Polsky et al., 1986). Nosocomial infection has high fatality rates.

Resistance of numerous bacterial pathogens to many antibiotics continues to increase globally. Frequencies, pattern, and distributions of resistant bacteria vary significantly with geographic regions and often reflect the usage patterns of antibiotics. Factor that increase in resource-poor and resource-rich nation include total antibiotic consumption as well as under use through lack of access, inadequate dosing, poor adherence, and substandard antimicrobial usage (Spector et al., 1994).

Mycobacterium tuberculosis (MTB) co-infection with HIV increases thirty times the risk of developing active tuberculosis, extra pulmonary tuberculosis (lymphadenopathy, pericardial disease, pleural effusion, and meningitis) and military tuberculosis. Active tuberculosis accelerates the progress of HIV disease. HIV-related tuberculosis is the cause of one third or more of death in those with HIV/AIDS (Cheesebrough, 2009).The incidence of TB among person infected with HIV is increased with CD4 lymphocytes count less than 200 per cubic millimeter.

The progression and outcome of HIV depends on the factors like baseline health, nutritional status, environment, endemic disease and access to therapy. It is important to understand the presentation of HIV disease in the local context to minimize the HIV-related mortility (Kumarasamy et al., 2005). The type of pathogen responsible for Opportunistic Infections (OIs) varies from region to region. Therefore, identification of the specific pathogens is important for management of such cases (Ayyagari et al., 1999). On this perspective it seem important that the causative agent of the endogenous source should be evaluated i.e. their occurrence, prevalence and their responsiveness to the available antibiotics either symptomatic or carrier by using enriched technique. This is especially important in developing counties like Nepal where education and hygiene is substandard and epidemicity of infections is in higher rates, thus emphasizing the need of studies like this in our country.

This present study evaluates the presence of respiratory tract pathogens in HIV/AIDS patients and thus requiring the awareness among clinicians regarding the occurrence of these pathogens in HIV/AIDS patients. The spectrum of bacterial infections and their respective antibiotic susceptibility pattern would be important implication regarding the empirical choice of antibiotics in the management of LRTI among the study population in areas with a high prevalence of HIV infection.

CHAPTER II

2. OBJECTIVES

2.1 General objective:

- To determine the etiological agents of lower respiratory infection among HIV seropositive individuals.

2.2Specific objectives:

- To determine the bacterial pathogen from the sputum sample of HIV seropositive individuals.
- To determine the antibioticsusceptibility pattern for the isolated bacterial pathogens.
- To determine tubercle bacilli by Ziehl Neelsen staining technique.
- To describe distribution factors and risk factors of Lower respiratory tract infection among study population.

CHAPTER III

3. LITERATURE REVIEW

3.1HIV and AIDS

3.1.1Introduction

AIDS, the acquired immunodeficiency syndrome (sometime called “slim disease) is a fatal illness caused by retrovirus known as the human immunodeficiency virus (HIV) which breaks down the body’s immune system , leaving the victim vulnerable to a host of life threatening opportunistic infections, neurological disorders, or unusual malignancies (WHO, 1986). The term AIDS refers only to the last stage of the HIV infection (Park, 2007).

The current case definition for AIDS includes those who

A. Test positive for human immunodeficiency virus (HIV) and

B. Meet one of the two following criteria

1. They have CD4 T-cell number of less than 200 per micro literof whole blood (the normal count is 600-1000 per ml) or CD4 T-cell / total lymphocytes percentage of less than 14 %; or

2. They may have a CD4 T-cell number of less than 200 per micro literand any of the following conditions; fungal diseases including candidiasis, coccidiomycosis, cryptococcosis, histoplamosis, isosporiasis, *Pneumocystitis carinii* pneumonia, cryptosporidiosis or toxoplasmosis of brain; bacterial disease including pulmonary tuberculosis or other *Mycobacterium* infections or recurrent *Salmonella* septicemia; Viral diseases including cytomegalovirus infection, HIV related encephalopathy, HIV wasting syndrome, chronic ulcers, or bronchitis due to herpes simplex; or progressive multifocal leukoencephalopathy, malignant disease such as invasive cervical cancer,

kaposi's sarcoma of the brain, or immunoblastic lymphoma; recurrent pneumonia due to any agent (Madigan and Martinko, 2006).

3.1.2History and Origin

3.1.2.1 History

Acquired immunodeficiency syndrome (AIDS) was first recognized in USA in 1981 amongst homosexual and drug addicts in whom the incidence of Kaposi's sarcoma and Pneumocystis carinii pneumonia were alarmingly high. The causative agent of AIDS was first reported by Luc Montagnier and colleagues at Pasteur Institute, Paris in 1983. They called it lymphadenopathy associated virus (LAV). Robert Gallo and colleagues from National Institute of Health, USA, reported isolation of retrovirus and called it Human T-cell Lymphotropic Virus-III (HTLV-III). In 1986, the International Committee on Virus Nomenclature gave the name Human Immunodeficiency Virus (HIV) to LAV/HTLV-III (Known as HIV-1). In 1986, another virus called LAV-2 and HTLV-IV at that time (now known as HIV-2) was isolated from AIDS patients, which is serologically distinct from HIV-1, but belongs to same family (Weiss et al., 1993; CDC, 1987).

3.1.2.2Origin

HIV in humans originated from cross-species infections by simian viruses in rural Africa, probably due to direct human contact with infected primate blood. Current evidence is that the primate counterparts of HIV-1 and HIV-2 were transmitted to humans on multiple (at least seven) different occasions. Sequence evolution analysis places the introduction of SIVcpz into humans that gave rise to HIV-1 group M at about 1930. Presumably, such transmissions occurred repeatedly over the ages, but particular social, economic, and behavioral changes that occurred in the mid-20th century provided circumstances that allowed these virus infections to expand, become well-established in humans, and reach epidemic proportions (Brooks et al., 2007).

3.1.3 Biology of human immunodeficiency virus

HIV is a member of the genus Lentivirus (ICTV db, 61.0.6), part of the family of retroviridae (ICTV db, 61). Lentiviruses have many common morphological and biological properties. Many species are infected by lentiviruses, which are characteristically responsible for long-duration illness with a long incubation period (Levy, 1993). Lentiviruses are transmitted as single-stranded, positive-sense, enveloped RNA viruses. Upon entry of the target cell, the viral RNA genome is converted to double stranded DNA by a virally encoded reverse transcriptase present in the virus particle. This viral DNA is integrated into the cellular DNA by a virally encoded integrase, along with host cellular cofactor, so that genome can be transcribed. After the virus has infected the cell, two pathways are possible: either the virus becomes latent and the infected cell continues to function or the virus becomes active and replicates, and a large number of virus particle that can then infect other cells are liberated.

There are two species of HIV known to exist: HIV-1 and HIV-2. HIV-1 is the virus that was initially discovered and termed both LAV and HTLV-III. It is more virulent, more infective, and is the cause of the majority HIV infections globally. The lower infectivity of HIV-2 compared to HIV-1 implies that fewer of those exposed to HIV-2 will be infected per exposure. Because of its relatively poor capacity of transmission, HIV-2 is largely confined to West Africa (Reeves and Domes, 2002). Both the types of HIV infects human.

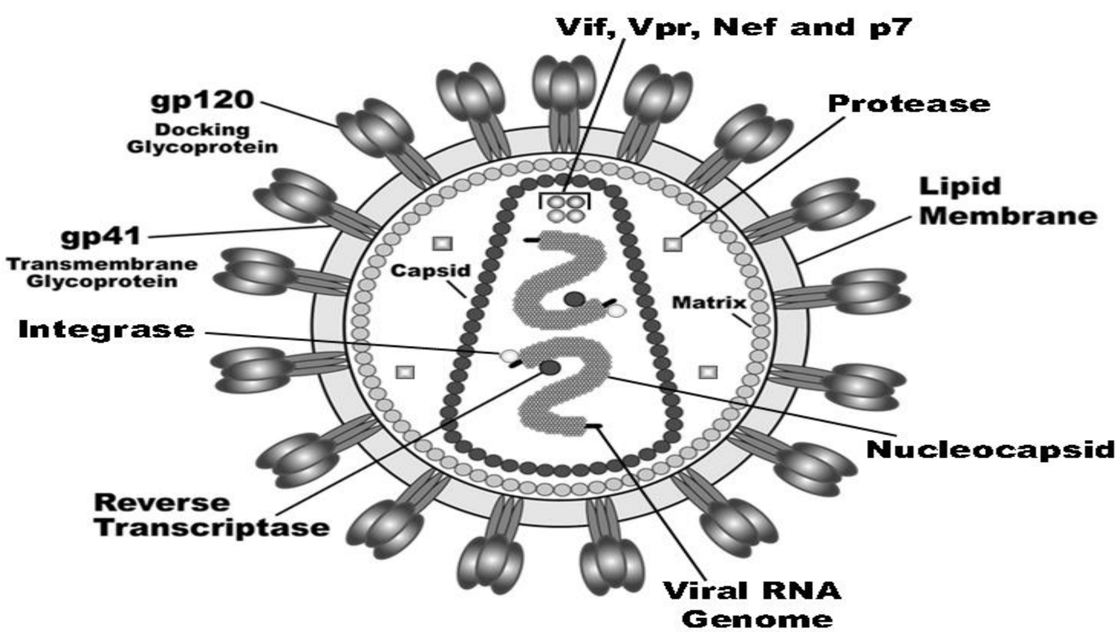


Figure 1: Structure of HIV virus

3.1.4Burden of HIV/AIDS

Ever since its discovery, the HIV has emerged as a global disaster. As of December 2008, the Joint United Nation program on HIV/AIDS (WHO/UNAIDS) estimates that 33.4 million (31.1-35.8 million) are infected with HIV. Since it was recognition in 1981, AIDS has killed more than 25 million people. In 2008 alone, AIDS acclaimed total of 2 million lives of which 280,000 were children less than 15 years. It is estimated that more that 40 million children will have one or both parents dead from AIDS by 2010. People in productive age groups are predominantly affected by AIDS and hence in same countries the impact of AIDS has led to major decrease in Gross National Product (GNP) (WHO, 2009).Sub-Saharan Africa remains by far the worst- affected region. In 2008, an estimated 1.9 million people living in sub-saharan Africa became newly infected with HIV, bringing the total number of people living with HIV to 22.4 million. While the rate of new HIV infections in sub-Saharan Africa has slowly declined-with the number of new infections in 2008 approximately 25% lower than at the epidemic's peak in the region in 1995 (WHO/UNAIDS, 2009).

Asia, home to 60% of the world's population, is second only to sub-Saharan Africa in terms of the number of people living with HIV. Asia's epidemic peaked in the mid-1990s, and annual HIV incidence has subsequently declined by more than half. In 2008, an estimated 330,000 AIDS-related deaths occurred in Asia. While the annual number of AIDS-related deaths in south and South-East Asia in 2008 was approximately 12% lower than the mortality peak in 2004, the rate of HIV-related mortality in East Asia continues to increase, with number of deaths in 2008 more than three times higher than in 2000 (WHO/UNAIDS, 2009).

In Nepal the first case was reported in 1988. Since then the HIV epidemic in Nepal has evolved from a "low prevalence" to a "concentrated epidemic". Nepal's epidemic has the characteristics of a concentrated epidemic where HIV has spread in defined sub-populations but is not well established in the general population and HIV sero-prevalance is consistently over 5% in at least one risk group (UNAIDS/NCASC, 2008). In 2009, the estimated number of people living with HIV/AIDS was 63,258 and overall adultprevalence rate was 0.39% (0.3-0.5%) with average number AIDS death per year of 4,701. Year wise data indicates that the cases of HIV/AIDS have increased sharply since mid-1990s. In 1992, more than double numbers of new cases were reported than the formerly reported cumulative cases. Similarly, high number of cases was reported in 2004 as compared to other years (NCASC, 2010).

3.1.5 Pathogenesis

HIV disease begins with acute infection which is only partly controlled by the adaptive immune response, and advances to chronic progressive infectionof peripheral lymphoid tissues. The incubation period in the acute stage is from 1-2 months. This is preceded by a period of intense, unrestrained viral replication, reflected in the presence of high numbers of viral RNA genomes and p24antigens in the circulation. After entering the body, virus is taken up by cell such as dendritic cells that expresses viral receptors. Within 24-48 hours infected cell are present in the regional lymph node; virus can be detected in the blood and circulating lymphocytes by 5 days. As the immune system responds, both p24 antigen and RNA copy number decrease, sothat by 6-12 months p24 antigen is usually undetectable and the RNA load has been stabilized at a lower level; in some it may be undetectable. The decline in RNA copy number is usually by at least 3-4 $\log_{10}$, and the effectiveness of the immune system in controlling virus will escape control and symptoms appear. The temporary increase in the RNA level can be seen during intercurrent infections, immunizations and pregnancy (Simmonds and Peutherer, 2006).

In peripheral blood, lymphoid tissue and other tissues such as brain where HIV replication occurs, HIV targets CD4+ cells and the cells of the monocyte-macrophage lineage; the latter may act as an important in carrying the virus into the central nervous system across the blood brain barrier (Simmonds and Peutherer, 2006).

The proportion of infected CD4+ cells and the level of circulating virus rise as the infection progresses, reflecting increasing virus replication until the patient become symptomatic. Activation of latently infected lymphocytes can be achieved by contact with foreign antigens and lecitins such as phytohaemagglutinin. Activation of uninfected CD4+ cells is also important in the increasing their sensitivity to infection (Simmonds and Peutherer, 2006).

Destruction of CD4+ cells is caused by:

- Viral replication
- Syncytium formation via membrane gp120 binding to cell CD4 antigen
- Cytotoxic T cell lysis of infected cells
- Cytotoxic T cell lysis of CD4+ cells carrying gp120 released from infected cells
- Natural killer cells
- Antibody-dependent cell cytotoxicity(Simmonds and Peutherer, 2006).

The proportion of infected CD4= cells and the titre of circulating virus rise as the infection progresses until the patients become symptomatic. The RNA copy number will rise and rich high levels (>10^5/ mm^3) and CD4+ cells counts drop below 200/µl. When the count reaches 200/µl the patient is severely compromised and the diagnosis of AIDS is made even in the absence of an AIDS indicator disease. High viral loads and cytopathic phenotype all correlated with disease progression. The number progressing to AIDS have been studied over many years, although the use of specific therapy has changed the outcome. Untreated, from initial infection the results were:

- 5% within 3 years
- 20-25% by 6-7% years
- >50% asymptomatic for more than 10 years

3.1.6 Clinical feature of HIV disease

The Center for Disease Control (CDC) in Atlanta, USA has classified the clinical course of HIV infection in to various groups (CDC, 1987)

1. Group I-Acute HIV infection

The illness is characterized by acute onset of fever, malaise, sore throat, myalgia, arthralagia, skin rash and lymphadenopathy, peripheral blood shows lymphocytosis, viral neculic acid or viral p24 antigen may be detected during acute infection. HIV antibodies are usually negative at the onset of illness but become positive during its course.

2. Group II-Asymptomatic infection

This includes all infected persons who are usually well. They show positive HIV antibody tests, and infectious.

3. Group III-Persistent generalized lymphadenopathy (PGL)

This group is characterized by enlarged nodes (more than 1 cm) at two or more extra genital sites for at least three months.

4. Group IV-Asymptomatic HIV infection

When CD4+ T lymphocytes count falls below 400 per mm^3 the patients may develop symptoms like fever, diarrhea, weightless, night sweats and opportunistic infection. During this phage, some patients develop an illness which is known as AIDS related complex or condition (ARC).

When CD4+ cells falls below 200 per mm^3, the patients are susceptible to various opportunistic infection and malignancies. In addition to opportunistic infections, patients may also develop primary CNS lymphoma and progressive multifocal leukoencephalopathy.

3.1.7Prevention

There is no cure for HIV infection. The only way to prevent HIV infection is through total avoidance of sources of transmission. HIV prevention must be linked to HIV/AIDS care and treatment (UNAIDS/WHO, 2006). Measure required for prevention of HIV infection includes:

- Increased access to HIV testing and social marketing that promotes testing and counseling to person without HIV symptoms who are not accessing services.
- Promotion of HIV transmission prevention strategies in the community.
- Promotion of and increased access to affordable condoms.
- Promotion of strategies to prevent transmission of sexually transmitted diseases (STD) in the community and access to testing and treatment for sexually transmitted infection.

- Scale up comprehensive HIV/AIDS prevention services to injecting drugs users, maintenance and other drug detoxification, peer outreach needle and syringe programs etc.
- Scale up for peer outreach, condoms promotions and treatment of sexually transmitted infection to sex workers and others vulnerable groups (e.g. injecting drug users and men having sex with men (MSM)).

3.1.8Vaccines

Much effort has been devoted to the development of a vaccine to provide protection against infection. By analogy with hepatitis B and other viruses, and understanding of the attachment mechanisms of HIV to cells, most emphasis has been placed on vaccines containing the viral env protein gp160 or gp120 or gp41 prepared by recombinant DNA cloning and expression, as synthetic peptides known to be important epitopes for neutralizing antibodies. Several prototypes are under evaluation (Simmonds and Peutherer, 2006).

3.1.9Treatment

Several drugs have been identified that delay symptoms of AIDS and can significantly prolong the life of those infected. Therapy is aimed at reducing the viral load of HIV-infected individuals below detectable levels. The strategy used to accomplish this aim is called Highly Active Antiretroviral Therapy (HAART) (Madigan and Martinko, 2006).This has been highly beneficial to many HIV-infected individuals since its introduction in 1996 when the protease inhibitor-based HAART initially become available (Palella et al, 1998). Current optimal HAART is accomplished by giving two or more antiretroviral drugs at once to inhibit the development of drug resistance (Madigan and Martinko, 2006).The most commonly used combinations of drugs are: two nucleoside analogue reverse transcriptase inhibitor with one protease inhibitor or two nucleoside reverse transcriptase inhibitors with one non-nucleoside reverse transcriptase inhibitor or two nucleoside reverse transcriptase inhibitor with protease inhibitors.

HAART neither cures the patient nor does it uniformly remove all symptoms; high level of HIV-1, often HAART resistant, return if treatment is stopped (Martinez-Picado et al., 2000; Dybul et al., 2002). Moreover, it would take more than the life time of an individual to be cleared of HIV infection using HAART (Blankson et al., 2002). Despite this many HIV infected individuals have experienced remarkable improvements in their general health and quality of life, which has led to a large reduction in HIV-associated morbidity and mortality in the developed countries (Palella et al., 1998; Wood et al., 2003; Chene et al., 2003). HAART is thought to increase survival time by between 4 and 12 years (King et al., 2003; Tassie et al., 2002). However, current drug regimens are often complicated and expensive, cannot be tolerated by all patients, have toxic side effects and leads to treatment failure due to development of HIV mutants so there is necessary for development of newer drugs for future.

3.2 Respiratory tract infection

Respiratory tract infections (RTI) are very common in the community and are one of the major reasons for appointments to primary care physicians, particularly in the winter season (Macfarlane et al., 1993). The broadest diagnosis of RTI includes the two principle sub-diagnosis of lower respiratory tract infection (LRTI) and upper respiratory tract infection (URTI), although it is often difficult to distinguish between them (Lieberman et al., 2002).

Lower respiratory tract infection comprises of infection in trachea, bronchi, bronchioles and lungs. However, upper respiratory tract infection comprises of infection in epiglottis and surrounding tissues, larynx, nasal cavity, and the pharynx (throat) (Forbes et al., 2007). LTRI is diagnosed in the presence of a cough and at least one of purulent sputum, dyspnea, chest pain or discomfort, wheezing and/or new focal crepitation’s or reduced breath sounds on lung auscultation (Macfarlane, 1999). All other patient having RTI but not meeting the diagnostic criteria for LRTI is defined as URTI (Lieberman et al., 2002).

3.2.1HIV/AIDS and bacterial respiratory tract infections

HIV does not kill those who are infected directly. Instead, it weakens the body's ability to fight disease. Infections which are rarely seen in those with normal immune systems can be deadly to those with HIV. People with HIV can get many infections (known as opportunistic infections, or OIs). Many of these illnesses are very serious and require treatment. Some can be prevented.

OIs are caused either by organisms of low or no virulence which are nonpathogenic in individuals with an intact immune system, or by known pathogens which present in a different way than usual in immunodeficient individuals, e.g. in the form of increased virulence, recurrence, multidrug resistance or atypical presentation. The spectrum of OIs has been found to vary from continent to continent and region to region. With the unprecedented increase in the number of AIDS cases, OIs are also increasing. Several of these OIs are recognized as case-defining entities in HIV/AIDS patients

Lower respiratory tract infections are the most common recurrent infections in PLHIV. They are usually life-threatening and can be caused by bacteria, viruses (rarely) and fungi (also rarely). Patients may present early in the course of HIV infection with bacterial pneumonias, which respond readily to antibiotics. Patients with HIV infection appear to be particularly prone to infections with encapsulated organisms such as *S. pneumoniae*, and *H. influenzae*. Later, and with the onset of immune suppression, patients may develop opportunistic pulmonary infections, the most important of which is pulmonary TB. As the cell-mediated immunity deteriorates, patients may develop life threatening opportunistic infections such as PCP and severe fungal and viral pneumonias.

Pneumocystis carinii pneumonia is the most frequently identified serious opportunistic infection in HIV disease. It is also one of the most common AIDS defining illnesses in the undiagnosed HIV infected individual. The most frequently occurring infection in developing countries like Nepal is tuberculosis. It occurs relatively early, with CD4 cell count in the range 200-400/µL. Besides these, pyogenic bacterial infections occur more frequently in HIV-infected individual. The most common organism includes*S.*

pneumoniae, H. influenzae, and *Morexella catarrhalis.* Infection with *Staphylococcus aureus, Legionella* species and gram negative organism may also occur. *Mycobacterium avium* complex (MAC or MAI) is an extremely common complication of advance HIV infection, although it is not considered as major pulmonary pathogen

Type of infection	**Possible complications (1)**
Bacterial	
Pneumococcal pneumonia	Empyema (2), pleural effusion, Lung abscess
H. influenzae pneumonia	Pleural effusion (2), Lung abscess, empyema
Klebsiella pneumonia	Empyema (2), pleural effusion
Staphylococcal pneumonia	Lung abscess (2), empyema, pleural effusion
M. tuberculosis pneumonia	Pericardial effusion, lung abscess, empyema, pleural effusion
MAC pneumonia	Rare complications: Abscesses especially with IRIS
Viral	
Cytomegalovirus	Pneumonitis (2) (highly lethal)
Herpes simplex virus	Pneumonitis (2) (highly lethal)
Fungal	
Pneumocystis pneumonia	Pneumothorax
Cryptococcosis	
Histoplasmosis	
Aspergillosis	Lung abscess
Other conditions	
Kaposi's sarcoma	Pleural or pericardial effusion
Lymphoma	Pleural or pericardial effusion
Carcinoma (non-HIV- related)	Pericardial effusion

(1)Possible complications are in the order of the frequency they occur.

(2)Complications that occur most frequently.

Lower Respiratory Tract Infections and CD4 Cell Count

CD4 cell count less than 500 cells per μL

Bacterial pneumonia (recurrent)

Pulmonary mycobacterial pneumonia (nontuberculous)

CD4 cell count less than 200 cells per µL

P. carinii pneumonia

C. neoformans pneumonia

Bacterial pneumonia (associated with bacteriemia/ sepsis)

Disseminated or extrapulmonary tuberculosis

CD4 cell count less than 100 cells per µL

Pulmonary Kaposi ’s sarcoma

Bacterial pneumonia (Gram-negative bacilli and *Staphylococcus aureus* increased)

Toxoplasma pneumonitis

CD4 cell count less than 50 cells per µL

Disseminated *H. capsulatum*

Disseminated *C. immitis*

Cytomegalovirus pneumonitis

Disseminated *Mycobacterium avium* complex

Disseminated *Mycobacterium* (nontuberculous)

Aspergillus pneumonia

Candida pneumonia

3.3 *Mycobacterium Tuberculosis*

Tuberculosis (TB) is a specific infectious disease caused by *M. tuberculosis* (Park, 2007). TB is also caused by *M. bovis* and *M. africanum*, all of which are complexed into *Mycobacterium tuberculosis* complex (MAC). Beside the MAC, opportunistic mycobacteria like *M. kansasii, M. malmoense, M. simiae, M. szulgai, M. xenopi, M. avium-intracellulare, M. scrofulaceum, M. chelonei* also causes TB (Cheesebrough, 2002). They primarily affect lungs and causes pulmonary tuberculosis. It can also affect intestine, meninges, bones, and joints, lymph glands, skin and other tissues of body. The disease is usually chronic with varying manifestation (Park, 2007).

3.3.1 Biology of Mycobacteria

Organism belonging to genus *Mycobacterium*, are very thin rod shaped (0.2 to 0.4×2 to 10µm) (Forbes et al., 2007). They are aerobic, non-capsulated, non-motile and acid fast and alcohol fast organisms, sometimes showing branching, filamentous forms resembling fungal mycelium, and hence bear name "Mycobacteria", meaning fungus like bacteria (Grange, 2006). They are acid fast due to the presence of mycolic acid in the cell wall and weakly gram positive.

3.3.2 Pathogenesis

The tubercle bacilli owe its virulence to its ability to survive within the macrophages rather than to production of toxic substance. The immune response to the bacilli is of the cell-mediated type, which if mediated by Th1 T helper cells, leads to the protective immunity, but the presence of Th2 cells facilitates tissue-destroying hypersensitivity reaction and progression of the disease process (Grange, 2006).

Inhalation of single viable organism has been shown to lead to infection, although close contact is usually necessary for acquisition of infection (Forbes et al., 2007). The first infection with M. tuberculosis is known as primary tuberculosis. It is usually sub pleural; often in the mid to upper zone within the hour of reaching the lungs, tubercle bacilli reach the draining lymph nodes at the helium of lungs and few escapes into the blood stream. In the initial reaction the alveolar macrophages ingest the bacillus. The bacteria proliferate inside the cells and macrophages release chemokines and cytokines which attract neutrophils granulocytes, monocytes and other inflammatory cells. The macrophage presents the antigen to the T lymphocytes with the development of cellular immunity. Delay hypersensitivity occurs, resulting in tissue necrosis, and at this stage the classical pathology of tuberculosis (Frew and Holgate, 2009).

The formation of mass of a granulomas surrounding an area of caesation leads to the appearance of the primary lesion in the lungs, referred to as 'Ghon focus'. If the bacilli spread (either by lymph or blood) before immunity is established, secondary foci may be established in other organs including lymph npdes, serous membranes, meninges, bones, liver, kidney and lungs. These foci resolve once an immune response is mounted and the organisms gradually lose viability. However ‘latent bacilli’ may persist for many years (Innes and Reid, 2006).

Post primary TB occurs after a latent period of months or years after primary infections. It may occur by reactivation of dormant bacilli. Reactivation may be in response to triggers, such as weakening of immune system by HIV infection. Post primary infection usually affects the lungs but can involve any part of body (Grange, 1990).

3.3.3 Symptoms of pulmonary TB

The main symptoms of pulmonary tuberculosis in adults are chronic cough with the production of mucopurulent sputum which may contain blood (haemoptysis). In the latter stages of the disease, there is loss of weight, fever, night sweats, fatigue, chest pain and anaemia. Complains include tuberculous pleurisity, pericarditis, and occasionally lung collapse (Cheesbrough, 2009).

3.3.4 Epidemiology

The increasing rate of HIV infection in many countries has had an impact on the TB epidemiology. While TB prevalence has remained stable, TB incidence continues to rise, especially in countries most severely affected by the HIV epidemics as well as those facing political turmoil, migration, poverty and unemployment and where intravenous drug abuse is rampant (Swaminathan and Narendran, 2008).

HIV is the most important known risk factor that promotes progression to active TB in people with *Mycobacterium tuberculosis* infection (WHO, 2004). The life time risk of tuberculosis in immunocompetant persons is 5% to 10%, but in HIV positive individuals, there is a 5% to 15% annual risk of developing active TB disease (Swaminathan et al., 2000).

In 2009, there were an estimated 9.4 million incident cases of TB globally. Most of the estimated number of cases in 2009 occurred in Asia (55%) and Africa (30%), smaller proportions of cases occurred in the Eastern Mediterranean Region (7%), the European Region (4%) and the region of the Americas (3%). India alone accounts for an estimated one fifth (21%) of all TB cases worldwide, and China and India combined account for 35% of all the incident cases in 2009, an estimated 1.0-1.2 million (11-13%) were HIV-positive, with a best estimate of 1.1 million (12%). 1.7% million people died from TB (including 380,000 women) in 2009, including 380,000 people with HIV, equal to 4700 deaths a day (WHO, 2010).

Tuberculosis remains one of the major public health problems in Nepal. About 50% population is infected with TB, of which 60% are adult. Every year 21,827 smear positive infectious TB incidence cases are expected to arise in the country. Although introduction of DOTS has already reduced the numbers of deaths, however 5,000 to 7,000 people still continue to die each year (STAC, 2009).

3.3.5 Mode of transmission

TB bacilli spread from lungs of people with TB when they cough, sneeze, speak or spit. A person inhales the droplet containing TB bacilli through respiration and may become infected with TB. It is actually not spread from food, smoking or drinking alcohol. Neither is it spread by insects or parasites or by heredity (NTC, 2003).

Not everyone who is infected with TB bacilli gets TB. If the infected person has good immunity, the disease may not develop. However if their immunity is weak (e.g. due to malnutrition, HIV infection) the TB can develop soon after infection (Cheesbrough, 2007). Most transmission of disease occurs within households or other environments where individual are close together for long period (Grange, 2006).

3.3.6 Diagnosis

Tuberculosis is diagnosed definitively by identifying the causative organism (*Mycobacterium tuberculosis*) in a clinical sample (for example, sputum or pus). When this is not possible, a probable - although sometimes inconclusive (Konstantinos, 2010) - diagnosis may be made using imaging (X-rays or scans) and/or a tuberculin skin test (Mantoux test). A complete medical evaluation for TB must include a medical history, a physical examination, a chest X-ray, microbiological smears, and cultures. It may also include a tuberculin skin test. The interpretation of the tuberculin skin test depends upon the person's risk factors for infection and progression to TB disease, such as exposure to other cases of TB or immunosuppression (CDC, 2003).

The tuberculin test measures the body's immune system response to an injection to tuberculin Purified Protein Derivatives (PPD). The tuberculosis test has limited value in clinical work, especially where TB is common (DOH, 2000). A positive reaction to the test is generally accepted as evidence of past or present infection by *M. tuberculosis*. The tuberculin test is the only means of estimating the prevalence of infection in a population (Park, 2007).

The definitive diagnosis of tuberculosis is based on the detection of acid fast bacilli in clinical specimens by microscopy, cultural techniques or by Polymerase Chain Reaction (PCR). Most usual specimen for diagnosis of PTB is sputum but, if none is produced, bronchial washings, brushings or biopsies and early morning gastric aspirates may be examined (Grange, 2006). Three sputum specimens are collected for patients suspected to have TB, including one overnight collection. Smear should be stained by Ziehl-Neelen (Z-N) (Convention microscopy) or Auramin rodamine (Fluorescent microscopy) (Swaminathan and Narendran, 2008).With Z-N stain *M. tuberculosis* look slender, straight or slightly curved rod with beaded appearance (Chakraborty, 2006).

Culture (on Lowenstein-Jensen medium) is much more sensitive but takes 6-8 weeks (Swaminathan and Narendran, 2008). Culture of mycobacteria is reference method for the detection of tubercle bacilli but it is probability slow and requires special safety procedure in laboratories (Gebra et al., 1995).

M. tuberculosis is obligateaerobes; grow optimally at 37° C and pH 6.4-7.0. It is a slow growing organism with generation time of 14-15 hours. On solid medium *M. tuberculosis* form a dry, rough, raised, irregular colony with wrinkled surface. The colonies are creamy white initially, becoming yellowish or buff colored later and tough when picked up (Forbes et al., 2007).

3.3.7 Prevention and Control

TB prevention and control takes two parallel approaches. In the first, early case finding and effective treatment of persons with active TB are the most important measures for preventing spread of TB in the community. A thorough contact investigation should be done around every case (American Thoracic Society/CDC, 1983).Contact investigations are usually based upon screening with the tuberculin skin test, followed by chest radiographs for those with skin test reactions greater than or equal to 5 mm (CDC, 1992). In the second approach, children are vaccinated to protect them from TB. No vaccine is available that provides reliable protection for adults. However, in tropical areas where the levels of other species of mycobacteria are high, exposure to non tuberculous mycobacteria gives some protection against TB (Fine et al., 2001).

Many countries use Bacillus Calmette-Guerin (BCG) vaccine as part of their TB control program, especially for infants. According to the WHO, this is the most often used vaccine worldwide, with 85% of infants in 172 countries immunized in 1993 (WHO, 1995). Immunization with an attenuated strain of *M. bovis*, the BCG strain, is routine for prevention of tuberculosis. The live BCG vaccine induces a delayed-type hypersensitivity response, and all individuals who receive it develop a positive tuberculin test, neutralizing the value of tuberculin test as a diagnostic and epidemiologic indicator for the spread of *M. tuberculosis* infection (Madigan and Martinko, 2006).

3.3.8 Treatment

Effective TB treatment is difficult, due to the unusual structure and chemical composition of the mycobacterial cell wall, which makes many antibiotics ineffective and hinders the entry of drugs. The two antibiotics most commonly used are rifampicin and isoniazid. However, instead of the short course of antibiotics typically used to cure other bacterial infections, TB requires much longer periods of treatment (around 6 to 24 months) to entirely eliminate mycobacteria from the body (CDC, 2003). Latent TB treatment usually uses a single antibiotic, while active TB disease is best treated with combinations of several antibiotics, to reduce the risk of the bacteria developing antibiotic resistance (O'Brien, 1994). People with latent infections are treated to prevent them from progressing to active TB disease later in life.

Usually TB patients resistant to Isoniazide and Rifampicin is said to be MDR TB case (NTC, 2003). While drug resistant TB is generally treatable, it requires extensive chemotherapy with second line anti-TB drugs which are more costly than first line drugs, which produces adverse drug reactions that are more severe, though manageable(WHO, 2010). The emergence of extensively drug resistant (XDR) TB, particularly in where TB patients are also infected with HIV poses a serious threat to TB control (WHO, 2010).

To ensure treatment and thus discourage development of antibiotic resistant organisms, DOTS (Direct Observation Treatment Short-course) may be necessary for noncompliant individuals (Madigan and Martinko, 2006).

3.4 *Streptococcus pneumonia*

3.4.1 Introduction

Streptococcus pneumoniae or pneumococcus is an important human respiratory pathogen. *S. pneumoniae*is found in the respiratory flora up to 40% of healthy individuals, and endogenous strains can cause severe respiratory disease in compromised individuals (Madigan and Martinko, 2006).

3.4.2 Discovery

The organism, discovered by Leo Escolar, in 1881, and then known as the pneumococcus for its role as an etiological agent of pneumonia, was first isolated simultaneously and independently in France and USA. The organism was termed *Diplococcus pneumoniae* from 1926 because of its characteristic appearance in Gram-stained sputum. It was renamed *Streptococcus pneumoniae* in 1947 because of its growth in chain in liquid media.

3.4.3 Structure/ Morphology

S. pneumoniae is a Gram-positive, non-motile, elongated (Lancet-shaped) diplococcus. The organism is capsulated and may form short chains, particularly following culture (Cheesebrough, 2009). The cocci are about 1µ in diameter and all freshly isolated strains are capsulated (Ross, 2006).

3.4.4 Types

More than 90 serotypes of *S. pneumoniae* have been identified. Serotypes of pneumococcus are on the basis of differences in polysaccharide (sugar) capsule of the organism. Capsular polysaccharide is a crucial virulence factor. The capsule is phagocytic, inhibiting complement deposition and phagocytosis where type-specific opsonic antibody is present. The serotypes are designated by numbers, and those that are structurally related are grouped together (1, 2, 3, 4, 5, 6a, 6b, etc.). The different serotypes differ in virulence. Thus, about 90% of cases of bacteremia pneumococcal pneumonia and meningitis are caused by some 23 serotypes.

3.4.5 Pneumococcal colonization

S. pneumoniae is commonly found in the nasopharynx (back of nose) of healthy people. The presence of peumococcus in the nasopharynx is referred to as “carriage”. Most people have been carriers of *S. pneumoniae* at some point in their lives. Pneumococcal carriage is more common in young children is usually transient and generally causes no illness**.** Colonization with a given pneumococcal type may be followed by development of type specific anticapsular antibody in the absence of overt sights and clinical illness.

3.4.6 Pathogenesis

HIV infections are responsible for immunological defects not only in T-lymphocytes, but also in β lymphocytes, macrophages, polymorph nuclear cells and cytokine production. Although the primary immunologic defect in cell-mediated immunity depends in the humoral immune system also occurs and are easily immunologic squeal of HIV infection (Terpstra et al., 1989). The numerous abnormalities in the humoral immune system and photolytic cells increase the susceptibility if the HIV-1 infected person to bacterial infections particularly more caused by encapsulated bacteria.

In setting of HIV infection the predisposition to pneumococcal infection is probably due to dysfunctional host defenses rather than to increased colonization. Defect in mucus immunity could play a role in the susceptibility to pneumococcal infections because mucosal IgA may prevent bacterial adherence to mucosal surfaces and colonization with *S. pneumoniae (*Janoff et al., 1992).

The capsule of *S. pneumoniae* renders its resistant to phagocytosis. The ability to invade this important host defense mechanism allows *S. pneumoniae* to survive, multiply and spread to various organs. The cell wall of *S. pneumoniae* consists of teichioc acid and the inflammatory response induced by Gram-positive cell wall differ that induced by the endotoxin of Gram-negative organism, but does include recruitment of PMN neutrophils, changes in permeability and perfusion, cytokine release and stimulation of Platelet Activation Factor (PAF). Other *S. pneumoniae* moieties in virulence is less clear, protein

A, pneumolysis and peptide permease pleural effusion is the most common and emphysema pus in pleural space, one of the most serious complications of *S. pneumoniae*.

3.4.7 Clinical manifestation

Clinical presentation of pneumococcal pneumonia in patient with HIV infection is similar to that in normal host, most patients with pneumococcal pneumonia experience the acute onset of fever and productive cough (Forbes et al., 2007).

Victims of pneumocoaal pneumonia are often found seriously ill. The onset is usually sudden with shaking chills, "stabbing" chest pain (exaggerated by respiration but sometimes referred to the shoulder, abdomen, or flank), high fever, cough and "rusty" sputum and occasionally vomiting. Respirations are grunting and the patient often lies on the affected side in an attempt to splint the chest. Herpes simplex facial lesions are often present.

Breath sounds are suppressed. Later, the classic signs (absent breath sound, dullness, etc.) of consolidation appear. A pleural friction rub or abdominal distention may be present. During resolution of the pneumonia, the signs of consolidation are replaced by tales. During the febrile period complaints of malaise, anorexia, weakness, myalgia and general prostration are extremely common.

3.4.8 Epidemiology

S. pneumoniae can be found as a part of normal upper respiratory flora and this organism is also the leading cause of bacterial pneumonia and meningitis (Forbes et. al., 2007). It normally colonizes the nasopharynx, and the prevalence studies have shown that it can be isolated from 5% to 10% of healthy adults and 20% to 40% of healthy children. The rate of colonization appears to be seasonal, with an increased prevalence seen during the winter.

The rate of pneumococcal bacteremic pneumonia is higher in blacks than in whites and 41 times higher in those with HIV infection than in individuals of same age who are not HIV infected (Marrie, 1999). Pneumococcal disease can occur at any time during the course of HIV infection.

Pneumococcal pneumonia is a serious infection and untreated cases have a mortality rate of about 30%. Even with aggressive antimicrobial treatment, individuals hospitalized with pneumococcal pneumonia have up to 10% mortality (Madigan and Martinko, 2006).*S. pneumoniae* is an exclusively human pathogen and is spread from person to person by respiratory droplets, meaning that transmission generally occurs during coughing or sneezing to others within 6 feet of the carrier. Thus carriers of *S. pneumoniae*, while generally healthy are an important source of infection and disease for others.

3.4.9 Laboratory diagnosis

Diagnosis is generally made by demonstrating the presence of pneumococci in a specimen of sputum, lung aspirate, pleural fluid, CSF, urine or blood by Gram-film and culture, and then identifying the culture in an optochin sensitivity test (Ross, 2006).

Hemolysis should not be used as stringent identification criterion. *S. pneumoniae* can be separated from other β-hemolytic streptococci on the basis of sensitivity to surfactants, such as bile or optochin (ethylhydrocupreine hydrochloride). These agents activate autolytic enzymes in the organisms that hydrolyze peptidoglycan.

S. pneumoniae, which lacks a demonstrable group antigen by the lancefield test, is conventionally identified by the quelling or capsule swelling test that employs type specific anti-capsular antibody.

3.4.10 Prevention and Treatment

As in general population, the treatment of HIV related pneumococcal disease depends on the site of infection and whether or not the organism is susceptible to penicillin.Most strains of *S. pneumoniae* respond to penicillin therapy. However, there are penicillin-resistant strains, especially among strains causing hospital –acquired infections. Thus individual isolates must be tested for penicillin susceptibility. Erthromycin is drug of choice for penicillin-resistant organism, but cephalosporin, fluroquinolone, ceftriaxone, cefotaxime, or vancomycin may also be used. However, some strains have acquired resistance to each of these drugs, and some strains have acquired multiple drug resistance, underscoring the need to test each isolate individually (Madigan and Martinko, 2006).

An effective multivalent vaccine is available for prevention of infection by at least two-thirds of the 90 known strains of *S. pneumoniae*, including all common pathogenic strains. The vaccine consists of mixture of capsular polysaccharides from the most prevalent pathogenic strains. The vaccine is recommended for elderly, health-care providers, individuals with compromised immunity, and others at high risk for respiratory infections (Madigan and Martinko, 2006).

3.5 *Haemophilus* species

Members of the genus *Haemophilus* are obligatory parasites that colonize human and animal mucosae. The type species, *Haemophilus influenzae*, is associated with a range of respiratory and invasive infection, later occurring predominantly in childhood. Other species may be responsible for acute conjunctivitis (*H. aegypticus*), venereal infection (*H. ducreyi*) and, rarely, abscesses and infective endocarditis (*H. parainfluenzae, H. haemolyticus, H. parahaemolyticus, H. aphrophilus, H. paraphrophilus,* and *H. segnis*) (Howard and Ison, 2006).

The haemophilic character of the genus reflects a requirement for either or both of two factors (X and V) generally supplied by the addition of blood to a nutrient agar medium. X factor can comprise protoporphyrin IX, haemin or other iron-containing prophyrins. V factor compromises nicotinamide adenine dinucleotide (NAD) or nicotinamide adenine dinucleotide phosphate (NADP)(Howard and Ison, 2006).

H. infleunzae is separated from most other *Haemophilus* species by its requirement for both X and V factors (Howard and Ison, 2006).

3.5.1 *Haemophilus influenzae*

It forms a part of normal commensal flora of the throat and nasopharynx of between 25 and 75% of healthy persons and acts opportunistically as a secondary invader in a variety of respiratory tract infections. These are often preceded by viral infections. It is frequently responsible for acute otitis media, sinusitis and infections involving the lower respiratory tract in patients with pre-existing pulmonary disease, such as chronic obstructive airways disease, cystic fibrosis or bronchiectasis. *H. influenzae* is commonest bacterial pathogen associated with acute exacerbations of chronic obstructive airways disease (Howard and Ison, 2006).

3.5.2 Morphology

H. influenzae are small, pleomorphic Gram-negative rods or coccobacilli. In clinical specimens *H. influenzae* is most commonly seen as small, uniform coccobacilli. In some cultures, and occasionally in clinical material, longer, filamentous forms may be seen, often in association with large, spherical or fusiform bodies (Slack, 2006).A minority of strains are capsulated. These comprise six types, a-f, based on the antigenic nature of their capsular polysaccharide. One of these, type b, is more virulent than other varieties of this species and is responsible for a number of invasive disease infections which are predominantly seen in children (Howard and Ison, 2006).

3.5.3 Clinical features

Clinical feature does not differ from that in HIV negative patient. The presenting symptoms of fever and productive cough in cases of pneumonia are usually present. One series of 34 cases of *H. influenzae* pneumonia found fever and productive cough in 100%, chest pain in 53%, and dyspnea in 47%. Most patients had an elevated white blood cell count, with a left shift in 65% (Schlamm and Yancovitz, 1989). Of note, a separate report of 12 patients with *H. influenzae* pneumonia found that three patients were a febrile and the majority presented with a sub-acute course, both atypical presentations for a bacterial pneumonia (Moreno et al., 1991).

3.5.4 Epidemiology

H. influenzae type b or Hib, is estimated to be responsible for some three million serious illness and an estimated 386,000 deaths per year, chiefly through meningitis and pneumonia. Almost all victims are children under the age of five, with those between four and 18 months of age especially vulnerable (WHO, 2005).

H. influenzae is the most common bacterial infections occurring in persons infected with HIV.Infections in adults are less common and tend to occur in the elderly with chronic illnesses, or in younger adults with underlying medical conditions associated with impaired immunity (Musher et al., 1983 and Moxon, 1995).

3.5.5 Pathogenesis

Shortly after birth, exposure to *H. influenzae* occurs and upper respiratory tract carriage is common. It is a normal colonizer of the pharynx and can be spread from person to person by airborne droplets or by direct contact with secretions. Host defenses against *H. influenzae* include bactericidal and opsonizing antibody, complement, and cells of the mononuclear-phagocytic system. Anticapsular antibody to type b is acquired in childhood and protects most adults from serious infection, whereas immunity to nontypable *H. influenzae* is less well understood. HIV-infected individuals have defects in humoral immunity as well as cell-mediated immunity, which undoubtedly contribute to the increased rate of invasive disease. Whether this is due to an impaired ability to mount an appropriate immune response or to failure of preexisting immunity is unknown (Casadevall et al., 1992).

3.5.6 Diagnosis

Because history, physical examination, and radiologic studies do not distinguish *H. influenzae* pneumonia, bacteremia, or meningitis from that caused by other bacteria, diagnosis requires a positive culture. Cultures of normally sterile sites such as blood, CSF, or joint fluid confirm the diagnosis. A positive sputum culture alone should be interpreted with caution as *H. influenzae* can colonize the pharynx. A sputum gram stain with polymorphonuclear leukocytes, gram-negative coccobacilli, and few if any epithelial cells is very suggestive in the appropriate clinical setting (Collee et al., 1999).

3.5.7 Prevention and Control

H. influenzae infections occur at an increased rate in HIV-infected adults, and preventive measures including vaccination, prophylactic antibiotics, and intravenous immune globulin have all been considered. *H. influenzae* type b conjugate vaccine is effective in HIV-infected adults with an improved antibody response the earlier it is given in the course of HIV disease (Steinhoff et al., 1991; Weiss et al., 1995).Recent data have demonstrated a transient increase in viral load after pneumococcal and influenza vaccination (Staprans et al., 1995) and after immunization with tetanus toxoid booster. (Stanley et al., 1996) Clearly, the risk versus benefit needs to be carefully considered, because the long-term consequences of the transient increase in immune activation are unknown.

H. influenzae produces beta-lactamases, and it is also able to modify its penicillin-binding proteins, so it has gained resistance to the penicillin family of antibiotics. In severe cases, cefotaxime and ceftriaxone delivered directly into the bloodstream are the elected antibiotics, and, for the less severe cases, an association of ampicillin and sulbactum (Sulbactum is a molecule that is given in combination with beta-lactam antibiotics to inhibit beta-lactamase); cephalosporins of the second and third generation, orfluoroquinolones are preferred. Fluoroquinolone-resistant *H. influenzae* has been observed (Chang et al., 2010).

Macrolide antibiotics (e.g. Clarithromycin) may be used in patients with a history of allergy to beta-lactam antibiotics. Macrolides resistance has also been observed (Roberts et al., 2011).

3.6*Staphylococcus aureus*

Staphylococcus is gram-positive bacteria which includes several species that can cause a wide variety of infections in humans and through either toxin production or invasion. Most of the infection results from an infected but asymptomatic individual to a susceptible individuals(Madigan and Martinko, 2006).

Staphylococci are non-motile, non-spore forming, occasionally capsulate. Most are catalase positive. With exception of one species, *S. saccharolyticus*, which is true anaerobe, staphylococci are facultative anaerobic. Their cell walls contain peptidoglycan and techoic acid, important cell-adherence factors. Their peptidoglycan is linked by pentaglycine bridges (attacked by lysostaphin) (Baird, 2006).

There are currently nearly 30 defined species and subspecies, though half are primarily animal origin. Staphylococci are widespread in nature, their normal habitats being the skin and mucous membranes of mammals and birds. Human skin is densely colonized with several of the coagulase-negative species and to lesser extent with *S. aureus*, whose preferred habitat is anterior nares, and which is carried there by some 40% of adults (Baird, 2006).

S. aureus accounts for about 2% of community acquired-pneumonias and 10-15% of nosocomial pneumonias. Person at particular risk include infants and the elderly; hospitalized and debilitated patients, especially those with tracheostomy, endotracheal intubation, immunosuppression, or recent surgery; children and young adults with cystic fibrosis or chronic granulomatous disease; patients with a bacterial super infection following viral pneumonia, especially one involving influenza A and B viruses; and drug abusers who are prone to staphylococcal tricuspid valve endocarditis with embolic pneumonia.

3.6.1Morphology and cultural characteristics

S. aureus is approximately 1µm in diameter, and divides to form the clusters characteristics of the genus. On blood agar or nutrient agar, incubated in air for 18-24 hours at the optimal growth temperature at 37º C, it forms colonies 1-3 mm in diameter although dwarf colonize forms are not uncommon. Colonies are smooth, low convex, glistening, densely opaque and of a butyrous consistency, sometimes surrounded by narrow zone of haemolysis on blood agar, depending on the strain. Older colonies become translucent and sticky. Occasionally strains are capsulated; their colonies are large, convex ad glistening, becoming so slimy that they run aver the surface of a tilted agar plate**.** Pigmentation is a characteristic of this species when grown aerobically, and ranges from cream through buff to gold(Baird, 2006).

S. aureus is tolerant of concentrations of sodium chloride that inhibits most other bacteria, and on mannitol salt agar it forms 1mm diameter yellow colonies surrounded by yellow medium due to acid formation. On MacConkey or CLED agar, it acquires the appropriate colour of the indicator, depending on whether or not the particular strain ferment lactose (Baird, 2006).

3.6.2Laboratory diagnosis

Depending upon the type of infection present, an appropriate specimen is obtained accordingly and sent to the laboratory for definitive identification. A gram-stain is first performed to guide the way, which should show typical gram-positive bacteria, cocci, in clusters. Second, the specimen is cultured on blood agar. Then, the colonies typical *of S. aureus* on blood agar is cultured on Mannitol salt agar, which is a selective medium with 7-9% NaCl that allows *S. aureus* to grow, producing yellow-colored colonies as a result of Mannitol fermentation and subsequent drop in the medium's pH. Furthermore, for differentiation biochemical tests such as Modified Hugh & Leifson O/F test (fermentative for *Staphylococcus* spp), catalase (positive for all *Staphylococcus* species) coagulase (fibrin clot formation, positive for *S. aureus*), DNase(positive for *S. aureus*) tests are all done. For staphylococcal food poisoning, phage typing can be performed to determine if the staphylococci recovered from the food to determine the source of infection.

3.6.3 Treatment and Prevention

Mortality rate is generally 30-40%, in part due to the serious associated conditions most patients have. Yet, a fulminating course with a lethal outcome sometimes occurs in previously healthy adults who develop this infection after influenza. Response antibiotic tend to be slow, and convalescence is prolonged.

The treatment of choice for *S. aureus* infection is penicillin; but penicillin resistance is extremely common and first line therapy is most commonly a penicillinase-resistant β-lactam antibiotic (for example, oxacillin or flucloxacillin). Combination therapy with gentamicin may be used to treat serious infections like endocarditis (Korzeniowski and Sande, 1982; Bayer et al, 1998), but its use is controversial because of high risk of damage to the kidney (Cosgrove et al., 2009).

Staphylococcus resistant to penicillin is mediated by penicillinase (form of β-lactamase) production : an enzyme that cleaves the β-lactam ring of the penicillin molecule, rendering the antibiotic ineffective. Penicillinase-resistant β-lactam antibiotics such as methicillin, nafcillin, oxacillin, dicloxacillin, and flucloxacillin are able to resist degradation by staphylococcal penicillinase.

Methicillin-resistant strains (MRSA) are considered resistant to all β-lactam antibiotics. Resistance to methicillin is mediated via the mec operon. Resistance is conferred by the mecA gene, which codes for an altered penicillin-binding protein that lowers the affinity for binding β-lactams. This allows for resistance to all β-lactam antibiotics and obviates their use during MRSA infections. As such, the glycopeptide vancomycin is often deployed against MRSA.

Despite this, MRSA generally remained an uncommon finding even in hospital settings until the 1990s when there was an explosion in MRSA prevalence in hospitals where it is now endemic (Johnson et al., 2001).

Prevention of staphylococcal infection is problematic because most individuals are asymptomatic carriers, and disease such as acne and impetigo can be transmitted by simple contact with contaminated fingers. In hospital environments such as surgical wards and nurseries, carriers of unknown pathogenic strains must either be excluded or be treated with tropical or systematic antimicrobial drugs to eradicate the carrier state (Madigan and Martinko, 2006).

3.7Gram-negative bacilli

Gram-negative bacilli accounts for <2% of community-acquired pneumonias but for most nosocomial pneumonias, including fatal ones. The important pathogen is *K. pneumoniae*, which is causes friedlander’s pneumonia. On usual pathogens are *Pseudomonas aeruginosa, Escherichia coli, Enterobacter spp, Proteus spp, Serratia marcescens*and *Acinetobacter sp. Pseudomonas aeruginosa* is a common pathogen in a patients with cystic fibrosis, neutropenia, advanced AIDS, bronchiestasis, and pneumonias acquired in intensive care. Gram-negative bacillary pneumonias are rare in healthy hosts and usually occur in infants, the elderly, alcoholics, and debilitated or immunocompromised hosts, especially those with neutropenia.

3.7.1 *Klebsiella peumoniae*

Throat colonization occurs in 1-6% of normal people and almost 255 of hospitalized patients. *Klebsiella* causes disease in normal population. However, pneumonia due to *K. pneumoniae* is classically thought of as community acquired and occurring in elderly and debilitated population with underlying alcoholism, chronic lung disease or diabetes. Nosocomial infection occurs frequently in patients with malignancy and in patients in intensive care and post-operative. The pneumonia may progress to fulminating pulmonary necrosis resulting in fibrosis, persistent cavities and bronchiectasis (Bordow et al, 1980).

3.7.2 *E.coli*

It rarely causes pneumonia by the aspiration or haematogenous route. Individuals acquiring pneumonias by haematogenous route often have predisposing genitourinary or gastrointestinal infection (Bordow et al., 1980).

3.7.3 *Pseudomonas aeruginosa*

P. aeruginosa has peculiar propensity to grow in liquid media used for sterilizating hospital equipment and in nebulizers attached to mechanical ventilators. It is a frequent commensal organism of respiratory tract in hospital employees and patients. It may colonize and or infect tracheostomy sites, burns, wounds and urinary tract (Bordow et al., 1980).

3.7.4 Acinetobacter *calcoaceticus*

It is a major constituent of flora of soil, water and sewage and within the hospital environment. *A. calcoaceticus* is also found to colonize the skin of the hospitalized patient. They have been implicated as etiological agents of pneumonia (both community acquired and nosocomial) and urinary tract infection (Barons et al., 1994).

Pneumonia caused due to *P.aeruginosa*, *E.coli, and Proteus*spp is considered nosocomial infection in origin. *Serratia marcescens* and *Enterobacter*spp produce similar pneumonias.

3.7.5Diagnosis

Gram-negative bacilli should be suspected in patients with pneumonia who is in one of the risk categories noted above, especially with neutropenia or nosocomial pneumonia. Gram stain of the sputum usually shows numerous gram-negative bacilli; however distinguishing the various species and genera on the basis of morphologic characteristics is impossible.

Most of the commonly sought etiologic agents of lower respiratory tract infection will be isolated on routinely used media: 5% sheep blood agar, MacConkey agar for the isolation and differentiation of gram-negative bacilli. Because of contaminating oral flora, sputum specimens, specimens obtained by bronchial washings and lavage, tracheostomy or endotracheal tube aspirates are not inoculated to enrichment broth or incubated anaerobically (Forbes et al., 2007).

Sputum cultures usually yield the pathogens; false-positive cultures due to organism that colonizes the upper airways are the major problems, especially, in patients previously treated with antibiotics for pneumonia due to other bacteria. Positive culture from blood, pleural fluid, or a transtracheal aspirates obtained before treatment are considered diagnostic.

3.7.6Prognostic and treatment

The mortality for gram-negative bacillary pneumonia is about 25-50%despite the available of effective antibiotics.

Combination therapy with a β-lactam antibiotic plus an aminoglycoside is commonly recommended for gram-negative bacillary pneumonia because (1) the patients involved are usually debilitated and immunocompromised; (2) mortality is high; and (3) the spectrum of antibacterial activity is increased, emergence of resistance may be retarded, and synergistic activity may result. For example, a third-generation cephem antibiotic plus an aminoglycoside can be used for initial treatment of community-acquired gram-negative bacillary pneumonia, and piperacillin or azlocillin plus amikacin can be used for initial treatment of nosocomial infection in which *P. aeruginosa* or some other antibiotic-resistant gram-negative bacillus is more likely to be involved (Levison and Kaye, 1985). Aminoglycosides should not be used alone. Monotherapy with either ceftazidime or imipenem/cilastatin has been shown to be safe and effective alternative to combination therapy for the treatment of serious hospital acquired infections due to *P. aeruginosa* (Norrby et al., 1993).

CHAPTER IV

4. MATERIALS AND METHODS

4.1 Materials

Details of materials, chemicals, equipment's, biological media and reagents used and their methods of preparation are provided in Appendix II, III and IV.

4.2 Methodology

4.2.1 Study site and period

The laboratory investigation part of this study was carried out at National Public Health laboratory (NPHL), Teku, Kathmandu, Nepal from May to December, 2010.

4.2.2 Selection of study subjects

This study included the HIV/AIDS patients visiting/admitted to Sukraraj Tropical Hospital, Teku, Kathmandu, NPHL and rehabilitation centers (Sparsha Nepal, Kathmandu and Youth vision, Kathmandu). All the cases were confirmed HIV/AIDS, with/without respiratory symptoms.

All together 121 individuals were ready for voluntary participation in this study. Verbal as well as written consent were taken from all the study population and only those who were ready to participate in the study were included. Voluntary involvement of the individual was highly encouraged and once the individual rejected for participation, they were then not included in the study, in any period of time. Repeated cases were not included in study.

4.2.3Patient information

Patient's name/code, age, sex, marital status, smoking habit, CD4+ cell count and status of ART taken were recorded, either by interviewing with them or their care takers, or from their case files. Patients under ART for 1 month or more were considered as ART taken else it was considered not under ART. All the information was filled in the questionnaire form (Appendix I).

4.2.4 Specimen collection

Patients were requested for the sputum specimen in a clean, dry, wide-necked, leak proof container. Patients were requested to cough deeply to produce a sputum specimen. Patients were advised to collect 2ml of early morning sputum specimen. Single specimen was collected from each individual.

4.2.5Inclusion criteria for sputum specimen

Deep seated expectorant, free from salivary contamination, properly dispensed in leak proof container was considered. Salivary specimens were discarded and specimen was re-requested.

4.2.6Sample processing

4.2.6.1Direct Microscopic examination

4.2.6.1.1 Grams Smear

Purulent part of sputum was transferred to a clean, grease free glass slide. It was air dried, heat fixed and stained using Gram's technique (Appendix III and V) and observed under microscope.

4.2.6.1.2 Ziehl-Neelsen Smear

A thin smear was made on a clean, grease free slide taking purulent portion of the specimen. It was air dried and followed by heat fix. The smear was stained by Ziehl-Neelsen method. Thus stained slide was then examined under oil immersion and observed for acid-fast bacilli (AFB) that appeared red, straight or slightly curved rods, occurring in single or bundle. Observation of the stained smear was made in different microscopic fields (Appendix III and V).

4.2.6.2Culture of the specimen

The media used in this study to isolate the commonly sought etiological agents of lower respiratory tract infections included Blood agar, Chocolate agar (heated blood agar), MacConkey agar. The specimens were cultured only for aerobic and facultatively anaerobic organisms.

A sterilized loopful of specimen was inoculated into each three set of culture plate i.e. Blood agar, MacConkey agar and chocolate agar. Blood agar and Chocolate agar were incubated at 37ºC for 24 hours in a candle jar (i.e. enriched moist environment). MacConkey agar was aerobically incubated at 37ºC for 24 hours. For interpretation of culture result on specimens contaminated by normal oropharyngeal flora, growth of predominant aerobic and facultatively anaerobic bacteria was reported.

On second day each plate were studied for specimen. On the blood agar, the hemolysis pattern was observed; if β-hemolysis (clear zone around the colony) was suspected then the colony was transferred to a fresh plate and incubated with bacitracin disc at the center of the plate. If α-hemolysis (green zone) suspected, the colony was transferred to fresh plate and incubated with Optochin disc at the center of the plate. If gamma hemolysis suspected, the colony was not further studied.

On Chocolate agar, if watery colony of *Haemophilus* spp was suspected then it was transferred to a Muller Hilton agar and X, V and XV-factor disc were placed and observed for the appearance of the colony.

On MacConkey agar the appearance of colony was observed and if suspected of any commonly sought pathogens, was transferred to biochemical media.

On consecutive days, the colonies were investigated for several biochemical tests demanding the identification of the suspected organism and antibiotic sensitivity pattern.

4.2.6.3 Identification of Isolated organism

For the identification of isolated organisms, following tests were performed.

4.2.6.3.1 Colony morphology/cultural characteristics

Study of colony morphology involved the observation of Shape, Size, Elevation, Margin, Pigmentation, Hemolysis, Opacity, Consistency.

4.2.6.3.2 Biochemical tests used for the Identification of pathogen

Appropriate biochemical tests were done for the identification of isolates. The predominant organism in primary culture was subcultured to obtain pure culture which were inoculated in different biochemical medium for biochemical tests (Appendix III and IV).

i. Catalase

This test is used to differentiate those bacteria that produce the enzyme catalase, such as Staphylococci from non-catalase producing bacteria such as Streptococci.

In a clean test tube, 2-3 ml of 3% hydrogen peroxide solution was poured and with a sterile wooden loop, several colonies of test organism was picked and immersed in H_2O_2 solution. Then, the bubbling of the gas was noted down.

ii. Oxidase

Oxidase test papers were moistened with distilled water and a colony from the fresh culture was picked up with a sterile glass rod and smeared on the paper. Then, the development of violet purple color within 10 second is an indicator of positive test.

iii. Indole production test

Test tubes with SIM (Sulphide, Indole and Motility) were stabbed with fresh culture from nutrient broth and then incubated at 37ºC for 24 hours. 0.5 ml of Kovac's reagent was added and shaken gently. A red color in the alcohol layer indicates a positive reaction.

Iv. Methyl Red (MR) test

The test organisms were inoculated in the MR-VP broth (Glucose phosphate peptone water medium) and incubated at 37ºC for 24-48 hours. After incubation, about 5 drops of methyl red indicator was added in each tube, mixed well. Positive tests were indicated by the development of bright red color and negative tests with yellow.

v. Voges-Proskauer (VP) test

The test organisms were inoculated in the MR-VP broth and incubated at 37ºC for 24-48 hours. After incubation, Barrit's reagent were added to each tube and shaken well and kept for 14 minutes. Reddening of the medium within 15 minutes indicates the positive test.

vi. Citrate Utilization test

Test tube with Simmon's citrate agar medium slant was streaked with a fresh culture from nutrient broth and then incubated at 37ºC for 24 hours. After incubation, growth in the medium was noted as shown by turbidity and change in colour of the indicator from light green to blue, suggesting citrate utilization.

vii. Triple Sugar Iron (TSI) test

Using sterile wire loop, a pure colony of test organism was inoculated to a TSI medium by first stabbing the butt of the tube through center and then streaking the surface of the slant. After incubation, the colour of both slant and butt, presence or absence of gas and H_2S was noted.Following characteristics of TSI are possible

1. Acid/Acid, Gas Acid/Acid, Gas, H_2S	Lactose or Sucrose fermenter
2. Alkaline/Acid, Gas Alkaline/Acid, Gas, H_2S	Glucose fermenter, Lactose or Sucrose non-fermenter
3. Alkaline /Acid	Glucose fermenter only, not aerogenic
4. Alkaline/ No change No change/No change	Glucose, Sucrose and Lactose non-fermenter
5. Acid/No change	Glucose oxidizing

viii. Urease test

Using a sterile inoculating loop, a pure colony of test organism was inoculated to a Christensen's modified urea broth. After incubation at 37ºC for 3-12 hours, observation was made for the presence of pink colour in the medium.

ix. Oxidative-Fermentative test

The organism to be identified was inoculated by stabbing into two Hugh-Leifson tubes, one of which is then overlaid with mineral oil as a barrier to oxygen. Tubes were then incubated at 37ºC and observation was made for the colour change in tubes. Indicator used in Hugh-Leifson's medium is bromothymol blue, which changes from green to yellow.

Ifyellow color was produced in both tubes, the organism was identified as a glucose fermenter (Fermentative) because fermentation can occur with or without oxygen. If yellow colorwas only detected in the open tube, aerobic tube, the organism was characterized as a glucose-oxidizer (Oxidative). As a third possibility, some bacteria do not use glucose as a substrate as no color change is detected in either tube (a nonutilizer).

x. Optochin Sensitivity test

This simple and reliable test distinguishes pneumococci from viridans streptococci. The pneumococci are susceptible to optochin and show a zone of inhibition more than 13mm while other α-hemolytic streptococci grow to the edge of the disk.

A paper disk containing 5mcg of optochin was placed in the primary inoculum of a chocolate agar plate streaked with material from the specimen and the plate was incubated at 37ºC in candle jar for overnight. After incubation, the plate was observed for the zone of inhibition surrounding the disk.

xi. Bacitracin Sensitivity test

Sensitivity to bacitracin is used to assist in presumptive identification of *S. pyogens*. For this, a paper disk containing 0.04 units of bacitracin disc was added to a blood agar streaked with test organism and the plate was incubated at 37ºC for 24 hours. After incubation, the plate was observed for the zone of inhibition around the disk as given by β-hemolytic group A streptococci whereas most other streptococci are resistant.

xii. Bile Solubility test

Solution of 10% sodium deoxycholate was made in distilled water and stored in room temperature. A well isolated suspicious colony from blood agar or chocolate agar was inoculated in 5 ml nutrient broth and incubated at 37ºC for overnight. While still warm, 0.5ml of 10% sodium deoxycholate was added and re-incubated at 37ºC for 15-30 minutes. If the organism was pneumococci, they were lysed within 15 minutes and the initially turbid culture became clear and transparent.

xiii. Satellitism test

A loopful of suspected *Haemophilus* growth was mixed in about 2ml of sterile physiological saline. Using a sterile swab, the organism suspension was swabbed on plate of nutrient agar and blood agar. Pure culture of *S. aureus* was streaked across each of the inoculated plates. Then, both plates were incubated in carbon dioxide enriched atmosphere at 37ºC overnight and in the following morning examination of culture plates for growth and satellite colonies were done.

The colony was of *H. influenzae* if there was growth on blood agar plate but not on the nutrient agar plate. If the satellite colonies were present on both plates, the organism was probably *Haemophilus* species that requires only V factor, such as *H. parainfluenzae.*

xiv. X and V factor test

A very light suspension (MacFarland 0.5) of organism in sterile saline was made. It was taken care not to carry over any X-factor contained in the medium that the organism was taken from. Therefore, a loop, not a swab was used to make the suspension. A sterile swab was dipped into the organism suspension and swab was rolled over the entire surface of nutrient agar plate. Then, the X, V, XV factor disks were placed on the agar surface separately at least 4-5cm apart. Then, it was incubated overnight at 37ºC in ambient air.

If the suspected organism is*H. influenzae* than it would show a halo of growth around the XV disk; the rest of the agar surface shows no growth. *H. parainflunzae* shows halo around XV and V disks and *H. aphrophilus* shows growth over the entire surface of the plate, neither X, nor V, nor XV factors are necessary for growth.

xv. Coagulase test

A drop of coagulase plasma was placed on a clean, dry glass slide and a drop of normal saline was put next to the drop of plasma as a control. With the help of loop, a portion of isolated colony to be tested was emulsified in each drop in slide, inoculating first to the saline. It was then mixed well with wooden applicator stick and rocked the slide gently for 5-10 seconds. The observation was made for the macroscopic clumping in 10 sec or less in coagulated plasma drop and no clumping in saline.

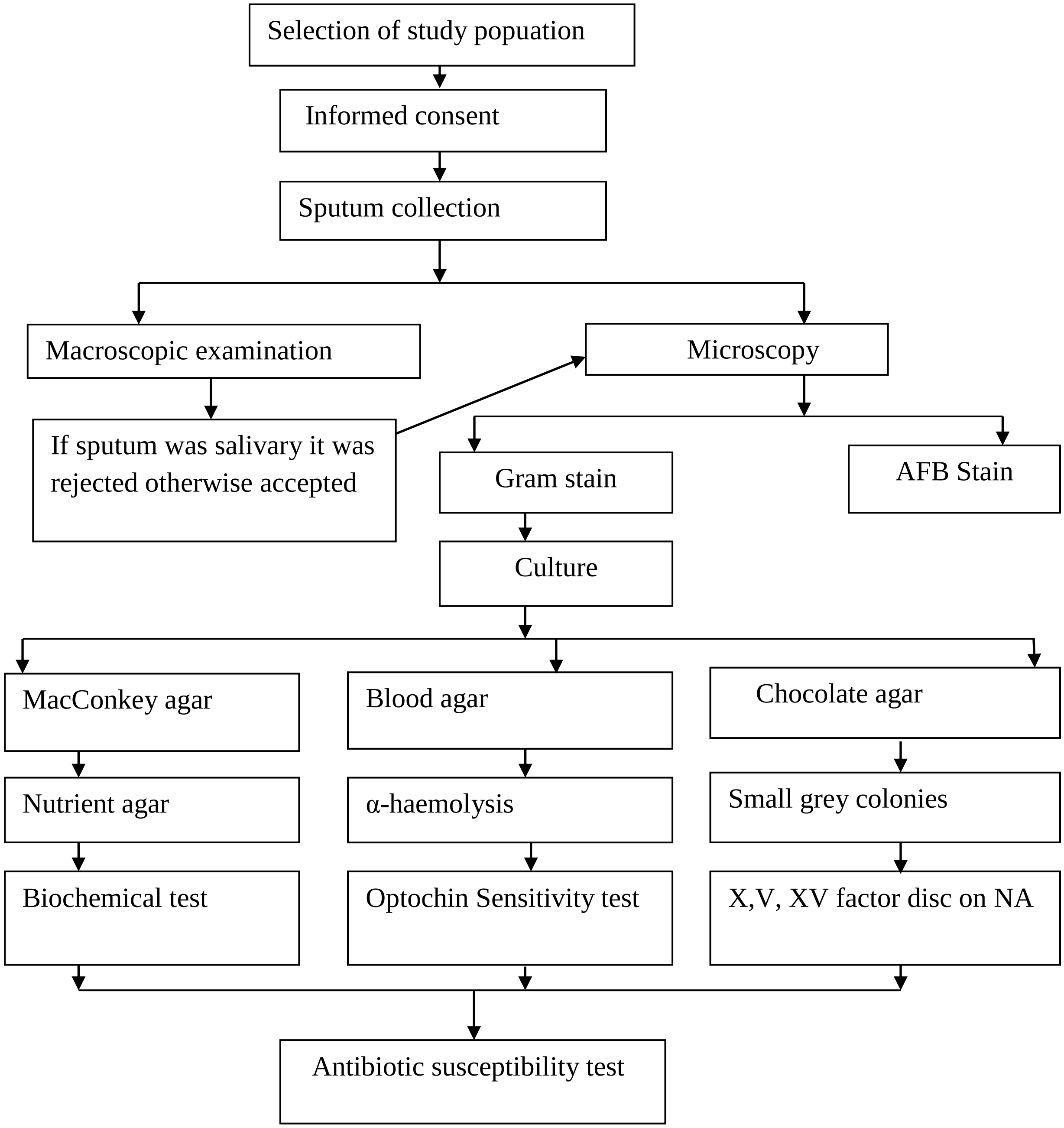
4.2.6.3Antibiotic sensitivity test

Antibiotic susceptibility test, for each isolated organism identified as possible respiratory pathogen, was performed using Kirby-Bauer disc diffusion technique. For the test a broth culture of pure culture of organism was prepared with turbidity equivalent to 0.5 McFarland standard and it was streaked on entire Muller-Hinton agar plate for those organism that were not fastidious, for fastidious organisms like *Haemophilus* spp it was streaked onto Chocolate agar and for *Streptococcus* spp it was streaked onto Blood agar. Six antibiotic discs were placed around the outer edge of the plate and incubatd overnight at 37^{o}C. Diameter of zone of inhibition was measured and CLSI zone diameter criterion was used to interpret the level of susceptibility to each antibiotic. The procedure for the test is given in Appendix V.

4.2.6.4.Data collection and analysis

Along with the Patient's name/code, age, sex, marital status, smoking habit, CD4+ cell count, ART status of patients enrolled for study were recorded in the questionnaire from direct interview or through their case file at the time of sample collection. The data were analyzed using Chi-square test.

4.2.6.5 Flow chart of Methodology



CHAPTER V

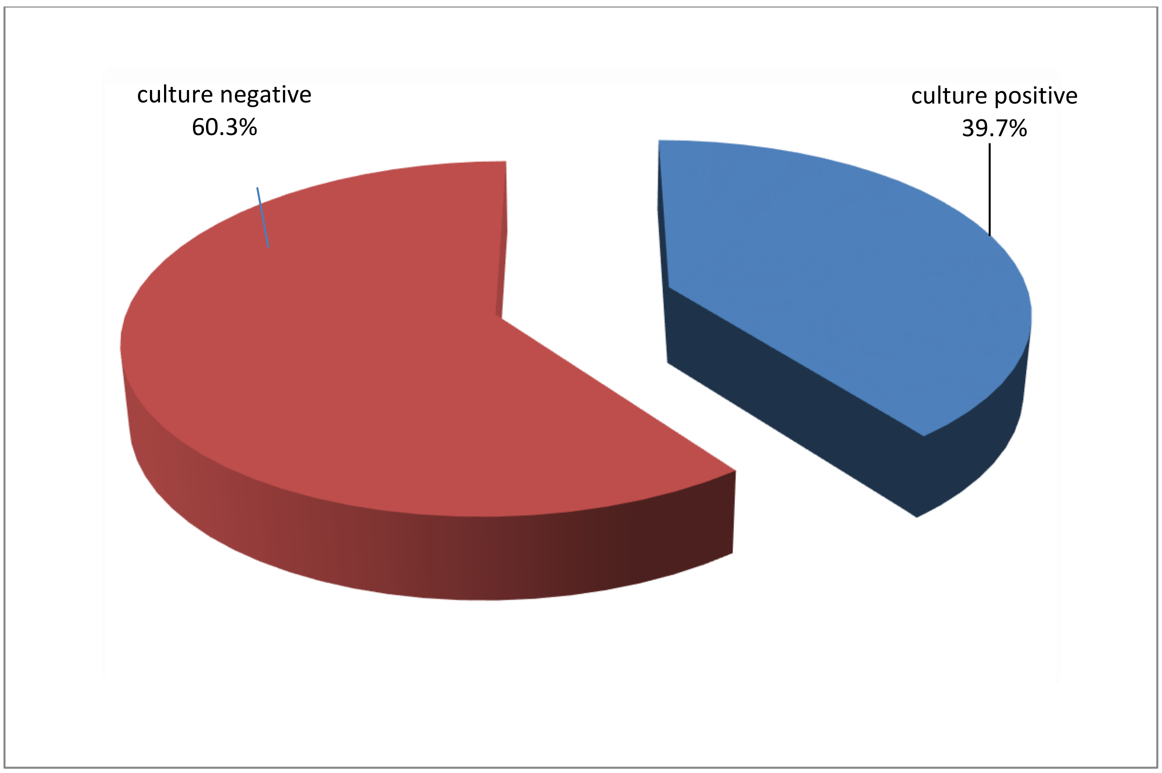
5. RESULTS

The study was conducted among 121 HIV/AIDS patients. Among 121 patients, 60 (49.6%) were from Sukraraj Tropical hospital (SRT), 39 (32.2%) were from National Public Health Laboratory (NPHL), 14 (11.6%) were from Youth Vision and 8 (6.6%) were from Sparsha Nepal.

5.1 Culture positivity among study cases

Of the investigated 121 cases, only 48 (39.7%) were culture positive whereas the remaining 73 (60.3%) were culture negative. (Figure 2)

Figure2: Status of culture among study population



5.2 Distribution of LRTI among genders

Among the total studied cases, 86 were males and 35 were females. Total positive cases were found to be slightly higher in females (54.29%) than in males (51.2%). But this was found to be statistically insignificant (P =0.75)(Table 1)

Moreover, culture positive rate in female (42.9%) was found to be slightly higher than in males (38.3%). This was found to be statistically insignificant (P=0.65).Similarly, AFB positive rate in female (17.1%) was found to be higher than in males (15.1%). But, this too was found to be statistically insignificant (P=0.78).(Table 2)

Table 1: Distribution of LRTIamong genders

Sex	**Cases involved**	**Total Positive cases (Culture as well as AFB)**	**Statistics**
Male	86 (71.0%)	44 (51.2%)	P=0.75
Female	35 (29.0%)	19 (54.29%)	
Total	**121 (100%)**	**63 (52.0%)**	

Table 2: Sputum culture positivity among genders

Sex	**Total**	**Culture positive (%)**	**Statistics**	**AFB (%)**	**Statistics**
Male	86	33 (38.3%)	P=0.65	13 (15.1%)	P=0.78
Female	35	15 (42.9%)		6 (17.1%)	
Total	**121**	**48 (39.6%)**		**19 (15.7%)**	

5.3 Pathogens isolated in LRTI

Altogether 9 different types of bacterial isolates were isolated. Of the total 48 isolates, 19 (39.6%) were Gram positive bacteria and 29 (60.4%) were Gram negative bacteria. Of all the isolates *Klebsiella pneumoniae* was most frequently encountered at 27.0%, followed by *Staphylococcus aureus* 20.8%, *S. pneumonia* 18.8%, *E.coli* 8.3%, *H. influenzae*, *Klebsiella oxytoca*, *P. aeruginosa*, 4.2% each,*Enterobacter* spp 2.1% and unidentified gram negative bacteria 10.4%. (Table 3)

Among 63 positive cases, 4 AFB positive sputum specimens showed growth of other bacteria. Among 4 polymicrobial infection case, AFB was associated with 4 different types of bacteria. (Table 4)

Table 3: Distribution of pathogens in LRTI of HIV/AIDS individuals

Types of isolates	**No. of cases (%)**	**Total %**
Staphylococcus aureus	10 (52.7%)	20.8
Streptococcus pneumoniae	9 (47.3%)	18.8
Total Gram-Positive Bacteria	**19 (100%)**	**39.6**
Klebsiella pneumoniae	13 (44.8%)	27.0
Escherichia coli	4 (13.8)	8.3
Haemophilus influenzae	2 (6.9%)	4.2
Klebsiella oxytoca	2 (6.9%)	4.2
Pseudomonas spp	2 (6.9%)	4.2
Enterobacter spp	1 (3.4%)	2.1
Others gram negative bacteria	5 (17.2%)	10.4
Total Gram-Negative Bacteria	**29 (100%)**	**60.4**
Total no. of bacterial isolates	**48**	**100.0**

Table 4: Frequency of polymicrobial isolation from HIV infected individuals

Pathogens isolated	Frequency (%)
AFB, *S. aureus*	1 (25)
AFB, *S. pneumoniae*	1 (25)
AFB, *E. coli*	1 (25)
AFB, *Pseudomonas* spp	1 (25)
Total	**4 (100)**

5.4 Distribution of LRTI by age

Among age groups, higher prevalence rate of LRTI was observed in age group greater than or equal to 45 years (75.0%) followed by 15-45 years (51.4%). The occurrence of LRTI rate among age group below 15 years was found to be 0 (0%). This was found to be statistically significant (P=0.02). However, higher prevalence was found in 15-45 years age group, 54 (85.7%), followed by age group above or equal to 45, 9 (14.3%), and age group below 15, 0 (0%). (Table 5)

Table 5: Distribution of LRTI by age groups

Age (Yrs)	Cases involved	Total Positive cases	Total %	Statistics
<15	4 (3.3%)	0 (0%)	0	P=0.025
15-45	105 (86.8%)	54 (51.4%)	85.7	
≥45	12 (9.9%)	9 (75.0%)	14.3	
Total	**121 (100%)**	**63 (52.0%)**	**100.0**	

5.5 Distribution of LRTI by smoking habit

Among 38 current smokers, 42.9% had a LRTI, among whom 77.8% were male and 22.2% were female. Similarly, among 34 previous smokers, 31.7% had LRTI, among whom 75%were male and 25% were female. Additionally, among 49 non-smokers, 25.4% had LRTI, among them 50% were male and 50% were female. The data was found to be statistically significant (P=0.01). (Table 6)

Table 6: Distribution of pathogens by smoking habit

Smoking habit	**Cases involved**			**Positive cases***			**Statistics***
	Male	**Female**	**Total**	**Male**	**Female**	**Total**	
Current smokers	29 (76.3%)	9 (23.7%)	38 (31.4%)	21 (77.8%)	6 (22.2%)	27 (42.9%)	P=0.01
Previous smokers	26 (76.5%)	8 (23.5%)	34 (28.1%)	15 (75.0%)	5 (25.0%)	20 (31.7%)	
Non-smokers	31 (63.3%)	18 (36.7%)	49 (40.5%)	8 (50.0%)	8 (50.0%)	16 (25.4%)	
Total	**86 (71.0%)**	**35 (29.0%)**	**121 (100%)**	**44 (69.8%)**	**19 (30.2%)**	**63 (100%)**	

5.6Distribution of LRTI by CD4 cell count

Among different CD4 cell count categories, rate of LRTI was found significantly higher among cases having CD4 cell below 200 (63. 4%), followed by those within 200-500 range categories (53.1%) and those within range above 500 (18.7%). This result was found to be statistically significant (P=0.00). (Table 7)

Table 7: Distribution of LRTI by CD4 cell count

CD4 cell count/µl	Cases involved	Positive cases	Statistics
>500	16 (13.2%)	3 (18.7%)	P=0.00
200-500	64 (52.9%)	34 (53.1%)	
≤200	41 (33.9%)	26 (63.4%)	
Total	**121 (100%)**	**63 (52.0%)**	

5.7 Distribution of LRTI by Antiretroviral therapy (ART) status

Of total cases, 67 cases (55.4%) were under ART whereas 54 cases (44.6%) were not under ART. Prevalence of LRTI was higher among cases not under ART (64.8%) compared those under ART (41.8%). This was found to be statistically significant (P=0.01).**(**Table 8)

Table 8: Distribution of LRTI by Antiretroviral therapy (ART) status

ART status	Cases involved	Positive cases	Statistics
ART taken	67 (55.4%)	28 (41.8%)	P=0.01
ART not-taken	54 (44.6%)	35 (64.8%)	
Total	**121 (100%)**	**63 (52.0%)**	

5.8 Antibiotic Resistance Pattern of bacterial isolates

Resistance pattern of all bacterial isolates towards antibiotics was tested using Kirby-Bauer agar disc diffusion method and zone diameters obtained around each antibiotic disc were compared with those in Zone Diameter Interpretative chart, then it was reported as Sensitive or Resistant. Moreover, Inhibition zone diameter that lied within the Intermediate zone diameter was reported as resistant.

5.8.1 Antibiotic resistance pattern of Gram-positive bacteria

Among the antibiotics used, Gram positive bacteria were found to be most resistant to Co-trimoxazole and Penicillin, 68.4% each, followed by Amoxicllin (47.4%), Chloramphenicol (42.1%), Ciprofloxacin (36.8%), Oxacillin (36.8%) and Azithromycin (31.6%).(Table 9 and 10)

Table 9: Antibiotic resistance pattern of Gram positive bacterial isolates:

Antibiotics Used / Isolate	Penicillin			Oxacillin		
	S	**R**	**S%**	**S**	**R**	**R%**
Staphylococcus aureus	3	7	70.0	7	3	30.0
Streptococcus pneumoniae	3	6	66.7	5	4	40.0
Total	**6**	**13**	**68.4**	**12**	**7**	**36.8**

Table 10: Antibiotic resistance pattern of Gram positive bacterial isolates:

Antibiotics used / Type of Isolates	Amoxicillin			Ciprofloxacin			Co-trimoxazole			Chloramphenicol			Azithromycin		
	S	**R**	**R%**	**S**	**R**	**R%**	**S**	**R**	**R%**	**S**	**R**	**R%**	**S**	**R**	**R%**
S. aureus	4	6	60.0	5	5	50.0	3	7	70.0	7	3	30.0	7	3	30.0
S.pneumoniae	6	3	33.3	7	2	22.2	3	6	66.7	4	5	55.5	6	3	33.3
Total	**10**	**9**	**47.4**	**12**	**7**	**36.8**	**6**	**13**	**68.4**	**11**	**8**	**42.1**	**13**	**6**	**31.6**

Note:-

S -: Sensitive

R -: Resistance

R%-: Resistance percentage

5.8.2 Antibiotic Resistance pattern of Gram-negative bacteria

Gram-negative bacteria were found to be most resistant to Amoxicillin (79.3%), followed by Co-trimoxazole (62.1%), Ciprofloxacin (55.2%), Ceftriaxone (51.7%), Azithromycin (48.3%), Ofloxacin (51.7%) and Gentamycin (60.0%). (Table 11 and 12)

Table 11: Antibiotic resistance pattern of Gram negative bacterial isolates:

Antibiotics Used / Type Of Isolates	Amoxicillin			Ciprofloxacin			Co-timoxazole			Ceftriazone			Azithromycin		
	S	**R**	**R%**	**S**	**R**	**R%**	**S**	**R**	**R%**	**S**	**R**	**R%**	**S**	**R**	**R%**
K. pneumoniae	4	9	69.2	6	7	53.8	4	9	69.2	9	4	30.8	8	5	38.5
E.coli	0	4	100	1	3	75.0	1	3	75.0	1	3	75.0	1	3	75.0
H .influenzae	2	0	0.0	2	0	0.0	1	1	50.0	0	2	100	2	0	0.0
***Pseudomonas* spp**	0	2	100	1	1	50.0	0	2	100	1	1	50.0	0	2	100
K . oxytoca	0	2	100	2	0	0.0	0	2	100	1	1	50.0	1	1	50.0
***Enterobacter* spp**	0	1	100	0	1	100	1	0	0.0	1	0	0.0	1	0	0.0
Other GNB	0	5	100	1	4	80.0	4	1	20.0	2	3	60.0	2	3	60.0
Total	**6**	**23**	**79.3**	**13**	**16**	**55.2**	**11**	**18**	**62.1**	**15**	**14**	**48.3**	**15**	**14**	**48.3**

Table 12:Antibiotic resistance pattern of Gram negative bacterial isolates:

Antibiotics Used / Type Of Isolate	Ofloxacin			Gentamycin		
	S	**R**	**R%**	**S**	**R**	**R%**
K. pneumoniae	6	7	53.8	5	8	61.5
E.coli	2	2	50.0	1	3	75.0
H. influenzae	2	0	0.0	2	0	0.0
***Pseudomonas*spp**	1	1	50.0	0	2	100.0
K. oxytoca	1	1	50.0	1	1	50.0
***Enterobacter*spp**	0	1	100.0	0	1	100.0
Other GNB	2	3	60.0	2	3	60.0
Total	**14**	**15**	**51.7**	**11**	**18**	**62.1**

Note:-

S -: Sensitive

R -: Resistance

R%-: Resistance percentage

CHAPTER VI

6. DISCUSSION AND CONCLUSION

6.1 Discussion

Progression of HIV infection is largely dependent on the interaction between the viral load and host factors. HIV brings about the destruction of CD4+ T-lymphocytes, which are crucial cells in forming immune response to foreign antigens and it is also the primary target cells of HIV (Pattanapanyasat and Thakur, 2005; Paranjape, 2005). The progressive loss of these lymphocytes eventually results in the loss of an ability to mount desirable immune response to any pathogen (Pattanapanyasat and Thakur, 2005) and death of the patients in the terminal stage of HIV infection occurs (Paranjape, 2005). Major cause of morbidity and mortality of such patients are opportunistic infections (Gordon et al., 1992).

Among them, the leading cause of their morbidity is various respiratory illnesses. The spectrum of HIV-related respiratory disease has evolved since initial years of epidemics. With the change in definition of AIDS, shift in demographic and risk factor of HIV infection and PCP prophylaxis, the incidence of pneumonia has matched PCP (Wallace, 1998).

Among the opportunistic infections associated with HIV, disease like pneumonia of bacterial origin occur at a rate many times higher in the HIV infected patients than in general population (Bhalla, 1999). However, the risk factor for bacterial pneumonia and its incidence is not well defined (Hirschtick et al., 1995).

In our study, among the investigated 121 patients, respiratory pathogens were found out in 63 patients (52.0%). In our study only 48 (39.7%) of the total accepted sputum specimen were culture positive whereas 73 (60.3%) were culture negative. Altogether, 9different types of bacteria were isolated and identified andin 5 cases isolates could not be identified in sputum culture.In our study greater number of isolates were gram negative bacteria, 60.4%, whereas, the contribution of gram positive bacteria in lower respiratory tract infection was 39.6%. The rate of gram negative bacterial infection to gram positive bacterial infection was found higher. Similar type of results was obtained in a study conducted by Rasheed and Thajuddin (2011) in which 37.5 % of bacterial isolates were Gram-positive and 62.5% of isolates were Gram-negative. Moreover,Okesola and Ige (2008) recovered 93% of Gram-negative and 7% of Gram-positive organisms from the total of 157 bacterial pathogens of lower respiratory tract. These data are in concur with our study. According to Doddannavar et al. (1985), smoking, alcoholism, prior lung damage and prior antibiotic therapy predisposes patients to gram negative bacterial pneumonia.

In our study out of all the pathogens isolated, *K pneumoniae* was the most common, accounting 13 (27.0%). This was followed by *S. aureus* in 10 (20.8%) and *S. pneumoniae* in 9 (18.8%). The rest were followed by *E. coli* 4 (8.3%), *H. influenzae* 2 (4.2%), *Pseudomonas* spp 2 (4.2%), *Klebsiella oxytoca* 2 (4.2%), *Enterobacter* spp 1 (2.1%) and unidentified gram negative bacteria 5 (10.4%). The decreasing order of causative agent was however not significantly different from general population but with higher incidence in HIV/AIDS population (Sharma et al., 2004).

Sailaja et al.(2004), found 32.26% cases of *K. pneumoniae* , 25.81% of *S. pneumoniae* and 12.90% of *S. aureus*. Tchamran, (1997), in his study on the lung diseases due to common bacteria in HIV infected individuals in African adults, noted 81% of infections due to *S. pneumoniae* and reported it to be the most offending pathogen in HIV reactive patients. Similarly, in a study conducted by Okesola and Ige (2008) *Klebsiella* spp and *Pseudomonas aeruginosa* were the most prevalent (38% and 16.7% respectively) among the Gram-negative pathogens. *S. pneumoniae* was the most prevalent among the Gram-positive organisms (14%) followed by *S. pyogenes* (3.3%) and *S. aureus* (2.7%).Moreover, a study conducted by Raseed and Thajuddin (2011) showed*K. pneumoniae* (26.2%) was the most common pathogen isolated, followed by *Moraxella caterrhalis* (21.4%), *K. oxytoca* (16.7%), *S. aureus* (14.3%), *S. pneumonia* (14.3%), *E. coli* (2.4%), *Nocardia asteroids* (2.4%), *Enterobacter cloacae* (2.4%). All these findings were in accord with our study.

Atypical pyogenic bacteria may also be a causative agent, particularly in patients with advanced HIV disease. For example, *K. pneumoniae*, other members of Enterobacteriaceae family and *Pseudomonas aeruginosa* were present in 13, 10 and 8% of cases, respectively, of confirmed pneumonia in the US cohort study of Hirschtick et al., 1995. Similarly, *P. aeruginosa*, the Enterobacteriaceae family and *S. aureus* were cause of 25, 9 and 10% of community acquired pneumonia respectively (Afessa and Green, 2000).

K. pneumoniae is the major gram negative isolates in our study. *K. pneumoniae* causes disease in normal population. However, pneumonia due to *K. pneumoniae* is classically thought of as community acquired and occurring in elderly and debilitated population with underlying alcoholism, chronic lung disease or diabetes. Nosocomial infection occurs frequently in patients with malignancy and in patients in Intensive care and postoperative.

Concerning *S. aureus*, Levine et al.(1990), recovered this pathogen in 23% of respiratory tract cultures performed in 129 consecutive HIV-infected patients with an episode of respiratory disease. According to them, this presence of *S. aureus* was found to be community acquired pneumonia in 28% of cases, of indeterminate significance in 62% and colonization in 10%. None of the patients with pneumonia were neutropenic or on corticosteroids.

In a French clinical epidemiology database, *S. pneumoniae* and *H. influenzae* were the cause of 52% and 16% of bacteriologically confirmed pneumonias respectively (Abgrall et al., 2000). Similarly, in US cohort study of Hirschtick et al., 1995, *S. pneumoniae* and *H. influenzae* were found in 52% and 15 % of patients with pneumonias respectively. Bacterial pneumonia due to *H. influenzae* occurred less frequently than that due to *S. pneumoniae* and was particularly observed in patients with <100 CD4 T-lymphocyte count per cubic millimeter (Cordero et al., 2000).

E. coli are often present in lower respiratory tract, especially in surgical or otherwise debilitated patients who are being treated with antibiotics to which they are resistant as a result of gastrointestinal or urinary tract infection of elderly patients, with the spread to lung secondary to bacteremia (Berk et al., 1985).

Steinhard et al. (1992) determined the incidence of invasive *H. influenzae* disease in man with AIDS or HIV infection. According to them the cumulative incidence of invasive *H. influenzae* disease in man 20-40 years of age with AIDS and HIV infected men 20-29 years of age without AIDS were 79.2 and 14.6 per 100,000 respectively. Casadevall et al.(1992) reported 10 of 15 cases of adult *H. influenzae* type b bacteremia occurred in-patient with AIDS or who were at risk for AIDS.

P. aeruginosa infection in patients with HIV is often community acquired and is associated with substantial mortility. In a study conducted by Dropulic et al. (1995) on the clinical manifestations of *P. aeruginosa* infection among patients with AIDS found that of the 73 episodes of *P. aeruginosa* infections, 13 were that of pneumonia. In a study carried by Sailaja et al. (2004) found 9.68% of the isolates were *P. aeruginosa.*

In our study 5 (10.3%) of total isolates could not be idenitified but all of them were gram negative coccobacilli. Among the gram negative coccobacilli most often causing lower respiratory tract infection are *Acinetobacter calcoaceticus* and *Moraxella caterrhalis*, *Neisseria* spp.*M. caterrhalis* is generally considered a commensal in the upper repiratory tract of adults, and its isolation from sputum is often reported as "normal flora of the oropharynx" (Shailaja et al., 2004). This appears to be a misconception, as Seghal and Shaimy (1994), reported this organism as the second most common isolate from the patients suffering with lower respiratory tract infections. In a study conducted by Rasheed and Thajuddin(2011), found *Moraxella caterrhalis* in 21.4% of cases which was the second most common bacterial isolate. Even though *Acinetobacter* spp. is implicated ina wide spectrum of infections, pneumonia appears to be the most dangerous one with the highest mortality rates (Fagon et al., 1989; Kollef et al., 1995; Vidal et al., 2003).*Acinetobacter* can cause eithercommunity-acquired or hospital-acquired pneumonia (HAP).A number of risk factors have been shown to be associated with *Acinetobacter* nosocomial infections. They include advanced age, immunosuppression, surgery, previous treatment with broad-spectrum antibiotics, use of invasive devices, burns, fecal colonization with *Acinetobacter*, and prolonged hospital or ICU stays.Immunosuppressed hosts, including neutropenic patients and HIV-infected individuals, especially those with low CD4 cell counts, are at particular risk (Manfredi et al., 2001).

Polymicrobial bacterial etiology was also reported in the present study. Their occurrence was AFB and *S. aureus*, AFB and *S. pneumoniae*, AFB and *E.coli*, and AFB and *Pseudomonas* spp, each occurring in 1 cases. Four (3.3%) patients had polymicrobial bacterial etiology. Okesola and Ige, 2008, found 4.7 % of polymicrobial bacterial etiology. Pulmonary polymicrobial infectionsmostly have been found in HIV infected patients by various studies indicates the severity of the infection in the HIV positive patients (Yoshimine et al., 2001; Eza et al., 2006).

In our study, rate of occurrence of lower respiratory tract infection in female (54.3%) was found to be slightly higher than in male (51.2%). Similarly, culture positive rate in female (42.9%) was also found to be higher than male (38.3%). However, these correlation between gender and infection rate was found to be statistically insignificant (P=0.75). Doddanavar(1985) reported that the difference in the incidence of LRTI between male and female population was probably due to more predisposing factors in male like smoking and alcoholism. However, Prescott et al.(1999) reported that although women generally do not hold jobs, with major exposure to dusts and fumes that potentially cause respiratory disease, yet socio-economic difference in LRTI are found in both sexes and most studies indicates that they are smaller in women.

In our study occurrence of LRTI was observed relatively higher in elderly population (45 years and above), accounting 75.0% of total elderly cases than younger age groups i.e. below 15years and 15-45 years. This data was found to be statistically significant (P=0.02). In particular, cases in age group 15-45 were found to have LRTI in 51.4% and the incidence in age group <15 were found to be nil.

The higher incidence of LRTI in elderly can be attributed to weakening of immune system with age which renders them vulnerable to infection. According to Berk et al.(1985), immune system in older patients becomes less effective due to either malnutrition or underlying degenerative disease such as diabetes mellitus, emphysema, uremia etc. In a study conducted by MacFarlane et al.(1993), confirmed that pneumococcal infection is common in patients over the age of 55, who also have an increasing incidence of LRTI as they get older. Furthermore, socioeconomic differences seems to exist at all ages, i.e. they are present before accumulated occupational exposure has had time to cause an impairment of lung function (Bakke et al., 1995).

In the present study, among 38 current smokers, 42.9% had a LRTI, among whom 77.8% were male and 22.2% were female. Similarly, among 34 previous smokers, 31.7% had LRTI, among whom 75%were male and 25% were female. Additionally, among 49 non-smokers, 25.4% had LRTI, among them 50% were male and 50% were female. The data was found to be statistically significant (P=0.01).

In Nepal, tobacco smoking has been identified as one of the most important risk factors contributing to a high prevalence of chronic bronchitis and chronic obstructive lung disease. In addition, a higher prevalence of tobacco use and wide spread use of unventilated indoor fires for cooking and heating combine to produce high rates of lung diseases in Nepal (Pande et al. (2001). In a study conducted by Hirschtick et al.(1995), tobacco smoking was found to be independent risk factor in the serogroup of HIV-seropositive subjects with <200 CD4+ T-lymphocyte per cubic millimeter.

In our study LRTI was observed significantly higher among cases with CD4 T-lymphocyte count less than 200 per cubic millimeter (63.4%), followed by 200-500 range groups (53.1%). It was found lowest among cases with CD4 T-lymphocyte count more than 500 per cubic millimeter (18.7%). This result was found to be statistically significant (P=0.00).

In US cohort study, acute bronchitis was equally prevalent during all stages of HIV disease, whereas the risk of bacterial pneumonia was clearly related to the entry CD4 count (2.3, 6.8 and 10.8 episodes per 100 person-years in the subgroups of cohort members with >500, 200-500 and <200 CD4 T-lymphocytes per cubic millimeter)

(Wallace et al., 1993; Hirschtick et al., 1995). A similar relationship between a decreased CD4 T-lymphocyte count and increased risk of bacterial pneumonia has been found in European studies (Boschini et al., 1996; Tumbarello et al., 1998). This data is in accord with our result.

The risk of bacterial pneumonia is not same in all HIV-infected people. The most important risk factor for bacterial pneumonia is the degree of immunosuppression, as reflected by the CD4+ T-lymphocyte count (Mayaud et al., 2002).Although primarily the disorder of cell mediated immunity, HIV infection is associated with substantial dysfunction of humoral immunity (Janoff et al., 1992). It predisposes patients to bacterial infection particularly with encapsulated organism such as *S. pneumoniae* and *H. influenzae* (Walsh et al., 1992). Polyclonal hypergammaglobulinemia, impaired B-cell activation and impaired local pulmonary defense are common (Rankin et al., 1988). Therefore it is not surprising that the increased rate of bacterial pneumonia in study participants with fewer than 200 T-lymphocyte per cubic millimeter. Also, HIV positive with fewer than 500 CD4 T-lymphocyte per cubic millimeter also had significantly more episodes of bacterial pneumonia than did HIV negative with similar count suggesting that immune dysfunction occurs even with a minimal reduction in CD4 lymphocyte count (Hirschtick et al., 1995).

In our study, LRTI was found distinctively higher in cases not receiving ART (64.8%), than those receiving ART (41.8)%. This data was found to be statistically significant (P=0.01). This observation showed the decrease of LRTI through the intervention of the ART. In German cohort, during 1992-1996, Brodt et al.,observed a significant decrease in number of cases of bacterial pneumonia, clearly related to the number of antiretroviral drugs administered. Similarly, US cohort study of Dworkin et al. (2001), antiretroviral treatment was an independent factor that contributed to a two-fold decrease in the incidence of pneumococcal disease during 1990-1998. Tumbarello et al. (1999),also observed a decrease in the incidence of bacterial pneumonia in the era of HAART, but this decrease was principally observed for nosocomial pneumonia and remained nonsignificant for community pneumonia.

Even though the incidence of opportunistic LRTI is decreased during the current era of HAART, bacterial pneumonia remains (Mayaud et al., 2002). If, it is borne in mind that bacterial infection remains the leading cause of death at the pre-acquired immune deficiency syndrome stage (Laurichchesse et al., 1998) and the most frequent terminal event in human immunodeficiency virus-infected patients (Afessa et al., 1998), it is clear that their prevention remains a major goal. Thus, pneumococcal vaccination might be given to the patient on highly active antiretroviral therapy with a CD4 cell count reaching 200 cells per millimeter (Mayaud et al., 2002).

In our study more resistant strains were observed than studies conducted few decades back. A large number of bacterial isolates were found resistant to first line antimicrobials. Ciprofloxacin which was considered most effective against Gram-positive bacteria was found to be resistant by 36.8% of the total Gram-positive isolates. Amoxicillin resistance was shown by 47.4% of isolates followed by Chloramphenicol (42.1%) and Azithromycin (31.6%). Gram-positive bacteria were found to be most resistant to Co-timoxazole (68.4%) andPenicillin (68.4%). In addition,Oxacillin resistance was shown by 36.8%of gram positive isolates. Likewise, Gram-negative bacteria was found to be mostly resistant to Amoxicillin (79.3%) followed by Co-trimoxazole (62.1%), Ciprofloxacin (55.2%), Ceftriaxone (51.7%) and Azithromycin (48.3%). Moreover, Gentamycin and Ofloxacin resistance was shown by 62.0% and 51.7% respectively.

In our study, *Pseudomonas* spp was the most resistant whereas*H. influenzae* was found to be the sensitive to nearly all of the antibiotics used, followed by *S. pneumonia* and *S. aureus*.*Pseudomonas* has been reported to develop resistance during prolonged therapy with all antibiotics.Majority of isolates were susceptible to ceftriaxone whereas *H. influenzae* showed 100% resistance followed by *E. coli* (75%) followed unidentified gram-negative bacteria (60.0%). *K. pneumonia*, the most prevalent isolate, showed high resistance to most of the antibiotics except Ceftriaxone and Azithromycin.

The emergence of strains of *S. pneumonia* that are resistant to penicillin is of great concern. Antimicrobial resistance is increasing globally, although patterns and degree of resistance vary by geographic region (Jacobs, 2003; Jacobs et al., 2003; Kaplan, 2004). The resistance to Amoxicillin in *S. pneumoniae* (33.3%) observed in this study is not as high as that observed in other countries such as in South Africa (Nascimento-Carvalho, 2001). This difference in antibiotic resistance pattern may be due to variations in the antibiotic prescribing habits in different geographical regions (Okesola and Ige, 2008). For most non susceptible strains, a second-generation cephalosporin (Cefuroxime) or a third-generation cephalosporin (Cefotaxime or Ceftriaxone) is somewhat more effective than either ampicillin or penicillin, although a high dose of amoxicillin is the preferred treatment for pneumonia in outpatients. The addition of β-lactamase inhibitor conveys no advantage, since the mechanism of resistance in this organism does not involve this enzyme. Vancomycin is rarely needed to treat pneumococcal pneumonia, even in severe cases (McIntosh, 2002).

After the discovery and unlimited use of penicillin for Staphylococcal infection, resistance emerged and rapidly spread possibly due to penicillinase enzyme. Today almost 90% of *S. aureus* are resistant to penicillin. Simultaneously methicillin resistant *S. aureus*(MRSA) were also discovered. MRSA infection in both the hospital and community setting are commonly treated with non β-lactamase antibiotics such as clindamycin and co-timoxazole. Resistance to these antibiotics has led to the use of new, broad spectrum anti-Gram-positive antibiotics such as linezolid because of its availability as an oral drug. First-line treatment for serious invasive infections due to MRSA is currently glycopeptides antibiotics (vancomycin and teicoplanin). Glycopeptides must not be used to treat methicillin-sensitive *S. aureus* as outcomes are inferior (Blot et al., 2002).

The increase in the antibiotic resistant strains of pathogens in recent years could be attributed to their indiscriminate and promiscuous use. According to Hosker (1994), such practice disturbs the oropharyngeal flora and facilitates the colonization or invasion of lung by different organisms or antibiotic resistant strains of the original organisms.

Salmonella, *Pseudomonas* and *Klebsiella* are among the bacteria manifesting high level of resistance-most notable in developing nations.

The key factor influencing the emergence of resistant pneumococci is unnecessary antibiotic use for viral respiratory infections in humans. It is also due to overuse of antibiotics in humans. In some developing countries antibiotics are available without prescription and this potentially facilitates overuse, although the expenses of antibiotics often deter indiscriminate use in these settings. Use of closely related drugs for other condition also plays a role in the spread of resistance (Schrag et al., 2001).Other factors such as reduced drug quality and suboptimal regimen may also play a role in the emergence of pneumococcal resistance. Suboptimal and long-duration regimens increase the opportunity for acquisition and/or amplification of resistant *S. pneumoniae* (Schrag et al., 2001).

In the present study, a substantial resistance was observed to a number of commonly used antibiotics. This may be due to the indiscriminate and inappropriate use of antibiotics that is rampant in Nepal. Hence, it is important to periodically monitor the antibiotic resistance pattern in different regions.

The increasing rate of human immunodeficiency virus (HIV) infection in many countries has had an impact on tuberculosis (TB) epidemiology. While TB prevalence has remained stable, TB incidence continues to rise, especially in countries most severely affected by HIV epidemics as well as those facing political turmoil, migration, poverty and unemployment and where intravenous drug abuse is rampant (Swaminathan and Narendran, 2008).

In this study, among 121 HIV/AIDS, 19 (15.7%) were found to be infected with PTB and 5 (4.1%) were extra PTB cases. PTB was confirmed with sputum microscopy and extra PTB with clinical findings. This prevalence was found to be higher than Serchand et al. (2001), in Kathmandu 6.7% in general population. This suggest that incidence of TB is rapidly increasing among HIV/AIDS patients in Nepal indicating the threat of transmission to whole population.

In our study, among 24 tuberculosis infected (TB) patients, 19 (79.2%) were Pulmonary tuberculosis (PTB) victims and 5 (20.8%) were extra pulmonary tuberculosis victims. Among 19 PTB cases 13 (15.1%) of male cases had PTB and 6 (17.1%) of female cases had PTB. This was found to be statistically insignificant (P=0.78). This result shows equal distribution among male and female population. The possible reason for the equal distribution of male and female may be due to the large number of male participants.

In a study conducted by Dhungel et al. in 2008 reported that out of 17 TB-HIV-co infected case 70% were PTB and 30% were extra PTB. In a study of Napit (2001) at united mission hospital, Palpa, observed 40% of HIV seropositive cases were reported to have tuberculosis out of which 75% had pulmonary tuberculosis and 25% had extra pulmonary tuberculosis. These findings were in accord with our study.

Carvalho et al. (1997); Berhane et al. (1999) (Ethiopia); Diez et al.(2001) (Spain); Shailaja et al.(2004) (India); Rasheed and Thajuddin, (2011) (Ethiopia); found the rate of TB and HIV/AIDS co infection as 26.7%; 54.8%; 17.7%; 42.89%; 39.3% respectively. This variation was again due to geographical distribution of infected group. Nepal being room to TB epidemic has shown a higher TB/ HIV co incident rate.

Prompt diagnosis of TB is crucial for the success of ensuing treatment in any community setting. Conceptually, most of the available standard laboratory and mycobacteriology guideline texts advocate at least three consecutive sputum specimens to detect acid-fast bacilli (AFB) and performing sputum culture on patients suspected to have the disease (Nelson et al., 1998). Considering the huge economic burden imposed on the HIV/AIDS community to avail of expensive antiretroviral drugs and other diagnostic tests, it may be unmanageable for the patients to provide three consecutive specimens.

In the 8th report of the WHO Expert Committee on the Tuberculosis (1964) the opinion is given that a survey of single sputum smear results is the best index of the size of the infection pool, and at the same time identifies the majority of the tuberculosis cases who are dangerous. However, studies have shown that examination of two consecutive specimens (e.g. of on the spot and overnight sputum) is sufficient to detect a large number of infectious cases in the community. In our study specimen was taken only once for the screening of AFB, that could be one of the reasons for lesser percentage of AFB positive findings.

Among the large numbers of reports available, pulmonary tuberculosis is reported more than any other clinical forms in HIV/AIDS patients. HIV infection remains, in global terms, the largest risk factor for the development of tuberculosis (Boyton, 2005). Coinfection with TB and HIV alters the natural history of both diseases. HIV-infected patients are at increased risk of developing active TB from both reactivated latent and exogenous infection (Barnes et al, 1991). HIV seropositive status is also a risk factor for accelerated progression of TB (Daley et al., 1992).

HAART reduces the incidence, recurrence, and mortality rate of TB in HIVinfected patients. The use of HAART has been found to be associated with more than an 80% reduction in the risk of TB (Santoro-Lopes et al., 2002). However, HIV-positive patients on HAART remain at high risk of TB compared to HIV-negative patients, and this risk of remains appreciable even among those with a good response to HAART. A low baseline CD4+ count, 6-month CD4+ count, and 6-month HIV RNA level greater than 400 copies/mL were significantly associated with the risk of acquiring TB after 6 months of HAART. The level of immunodeficiency at which HAART is initiated and the response to HAART are important determinants of the risk of TB (Girardi et al., 2005).

6.2 Conclusion

The current study indicated that there have not been any significant changes in the bacterial involvement in causing LRTI. Smoking habits and CD4 cell count below 200 per µl has remained as the major risk factors. Similarly, the age groups at risk have remained more or less same over a period of time. In contrast, increasing number of drug resistant pathogens in the vicinity truly sets the alarm for an immediate action to be undertaken towards judicial use of antibiotics. Higher prevalence of TB among females observed in the study emphasizes the need of a gender based study in finding TB cases among the study population.

CHAPTER VII

7. SUMMARY AND RECOMMENDATION

7.1 Summary

- A total of 121 sputum specimens were studied from 121 HIV/AIDS patients duringthe period of May to December 2010.Of 121 cases 86 were male and 35 were female.
- Fourty eight (39.7%) were culture positive for bacteria and 73(60.3%) were culture negative.
- Culture positivity among males and females were found to be 38.3% and 42.9% respectively. Moreover, AFB was found among 15.1% of males and 17.1% of females. Altogether 44 (51.2%) of males had LRTI and 19 (54.3%) of female had LRTI.
- *K. pneumoniae* (27.0%) was the most common bacterial isolate followed by *S. aureus* (20.8%), *S. pneumoniae* (18.8%), E. coli (8.3%), *H. influenzae*, *K. oxytoca* and *Pseudomonas* spp(4.2% each) and Unidentified gram-negative coccobacilli (10.4%).
- LRTI was observed relatively higher in elderly population (45 years and above), 75.0% of total elderly cases than age group 15-45 (51.4%) and age group below 15 years (nil).
- LRTI was found at higher rate in current smokers (42.9%) than previous smokers (31.7%) and non-smokers (25.4%).
- LRTI was observed higher among cases with CD4+ cell count below 200/µl than among cases with CD4+ cell count between 200-500/µl and more than 500/µl.
- LRTI was found higher among cases not under ART than those under ART.
- *Pseudomonas* spp was most resistant to antibiotics and *H. influenzae* was the most sensitive. Similarly, ceftriaxone was the most effective antibiotics among gram-positive bacteria whereas Co-trimoxazole and Penicillin were least effective. Among gram-negative ceftriaxone and Azithromycin were most effective whereas Amoxicillin was the least effective.

- **7.2Recommendation**
- More comprehensive study is essential to find other opportunistic infections covering wide range of parasites, fungus, bacteria, virus and helminthes, etc.
- Study of incidence of infection throughout the year can be carried out to obtain the seasonal distribution of the opportunistic infections.
- Similar type of study including control groups (Non HIV seropositive) can be carried to determine the severity of infection among this group (HIV seropositive).
- Along with pulmonary tuberculosis, extrapulmonary tuberculosis can provide elaborated knowledge on incidence of tuberculosis.
- Expanded spectrum of antibiotics resistant organism that cause LRTI among HIV/AIDS patients can be carried out as it will help clinical care of HIV/AIDS.

CHAPTER VIII

8. REFERENCES

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APPENDIX-I

QUESTIONNAIRE FORM

Patient's name/code:

Address:

Sex:

Marital status:

Smoking habit:

Complaints (symptoms if any):

First diagnosis of HIV:

Stage of HIV related syndrome:

Any secondary/ opportunistic infection diagnosed:

Medication:

ART taken status:

Duration of ART taken:

CD4 cell count:

Result of sputum examination

Macroscopic examination:

Color:

Blood:

Microscopic examination:

Gram staining:

AFB staining:

Bacterial culture report:

Choice of drug based on antibacterial susceptibility testing:

Suggestion based on study:

APPENDIX-II

MATERIALS USED

1. Culture media:-

- Blood agarOxoid, UK
- Chocolate agar Oxoid, UK
- MacConkey agarOxoid, UK
- Nutrient agarOxoid, UK
- Nutrient brothOxoid, UK
- Mannitol salt agarOxoid, UK
- Muller Hinton agar Oxoid, UK

2. Biochemical tests:-

- SulphideIndole motility agar Himedia, india
- Media for Methyl Red test (MR)Himedia, india
- Media for VogesPrauskauer test (VP)Himedia, india
- Simmons citrate agar Himedia, india
- Triple Sugar Iron agar Himedia, India
- Urease broth Himedia, India
- Hugh and leifson media Himedia, India

3. Staining reagents:-

- Gram staining reagents
- Zeihl-Neelsen staining reagents

4. Test reagents

- Catalase reagent Oxoid,UK
- Oxidase paper
- Kovac’s reagent
- Methyl Red solution

- Barrit’s reagent

5. Others:-

- Human Plasma

6. Identification discs:-

- Optochin discs MAST, Germany
- Bacitracin disc MAST, Germany
- X, V, XV factor discs MAST, Germany

7. Equipments

- Microscope
- Incubator
- Refrigerator
- Autoclave
- Water bath
- Candle jar
- Biological Safety cabinet (Level III)
- Hot air oven

8. Antibiotic discs MAST, Germany

APPENDIX-III

COMPOSITION AND PREPARATION OF STAINS AND REAGENTS

I. GRAM STAINING REAGENTS

1. Crystal violet

Composition

Solution A

Crystal violet	2.0 gm
95% ethyl alcohol	20.0 ml

Solution B

Ammonium oxalate	0.8 gm
Distilled water	80.0ml

Preparation

Crystal violet was dissolved in ethyl alcohol and similarly ammonium oxalate in distilled water. Then solution A and solution B was mixed well.

2. Gram's Iodine

Composition

Iodine	1.0 gm
Potassium Iodide	2.0 gm
Distilled water	300.0ml

Preparation

Iodine and potassium iodide were dissolved well in distilled water to prepare gram's iodine solution.

3. Decolorizer

70% Acetone

4. Safranin

Composition

Safranin	2.5 gm
Ethanol	100ml
Distilled water	200ml

Preparation

Safranin was well dissolved in ethanol and distilled water was added.

II. ZIEHL-NEELSON REAGENT

1. CarbolFuchsin stain

Composition

Basic fuchsin	10 gm
Absolute alcohol	100 ml
Solution of phenol (5%) in water	1000 ml

Preparation

10.0gm of basic fuchsin dye was dissolved in 100 ml of alcohol. Then the whole content was added to phenol solution.

2. Acid-Alcohol Decolorizer

Composition (to make 1000 ml solution)

Concentrated HCl	30 ml
Ethanol or methanol, absolute	680 ml
Distilled water	290 ml

Preparation:

680 ml of ethanol or methanol was transferred to leak proof container. Then, 290 ml of water was added and mixed well. To this solution 30 ml of conc. HCl was added and mixed well.

3. Methylene blue stain

Composition

Methylene blue	approx 0.5 gm
Ethanol, absolute	30.0 ml
Potassium hydroxide	0.1 ml
Distilled water	100ml

Preparation

0.5 gm of methylene blue was dissolved in 30 ml distilled water. Then, alcohol, potassium hydroxide solution, and water were added to make 130 ml solution.

III. TEST REAGENTS

1. Catalase Reagent (3%H_2O_2)

Composition

Hydrogen peroxide	3 ml
Distilled water	97 ml

2. Oxidase test paper

Composition

Oxidase reagent-

Tetramethy-p-phenylenediaminedihydrochloride	0.1gm
Distilled water	10 ml

3. Methyl Red Indicator solution

Composition

Methyl red	0.1 gm
Ethanol	300 ml
Distilled water	200 ml

Preparation

Methyl Red was dissolved in ethanol and distilled water.

4. Barrit's Reagent

Composition

Solution A

α-napthol	5.0 gm
95% ethyl alcohol	100 ml

Solution B

Potassium hydroxide	40.0 gm
Distilled water	100 ml

Preparation

1 ml of solution B and 3 ml of solution A was added to test suspension.

5. Kovac's Reagent

Composition

n-amyl alcohol	75.0 ml
Conc. HCl	25.0 ml
P-dimethylaminobenzaldehyde	5.0 ml

Preparation

The aldehyde was dissolved in the alcohol and slowly added the acid. It was prepared in small quantities and stored in the refrigerator and shaken gently.

APPENDIX-IV

COMPOSITION AND PREPARATION OF DIFFERENT CULTURE MEDIA AND BIOCHEMICAL MEDIA

1. NUTRIENT AGAR (NA)

Ingredients	gm/liter
Peptone	5.0
Sodium Chloride	5.0
Beef extract	1.5
Yeast extract	1.5
Agar	15.0
Final pH (at 25º C)	7.4±0.2

Preparation

28 grams of media was suspended in 1000ml of distilled water. It was boiled to dissolve the medium completely an sterilized by autoclaving at 121ºC for 5 minutes. It was shaken well before pouring into the sterilized Petridis. It was then allowed to solidify and streaking was done.

2. NUTRIENT BROTH (NB)

Ingredients	gm/liter
Peptone	5.0
Sodium Chloride	5.0
Beef extract	1.5
Yeast extract	1.5
Final pH (at 25ºC)	7.4±0.2

Preparation

13 gram of media was suspended in 100ml of distilled water and boiled to dissolve media completely. Then it was autoclaved at 121ºC for 15 minutes for sterilization.

3. MACCONKEY AGAR (MA)

Ingredients	gm/liter
Peptone	17.0
Protease peptone	3.0
Lactose	10.0
Bile salts	1.5
Sodium Chloride	5.0
Neutral red	0.03
Agar	15
Final pH (at 25ºC)	7.1±0.2

Preparation

51.5 gram of media was suspended in 1000 ml of distilled water. It was boiled to dissolve the medium completely. Then it was sterilized by autoclaving at 15lbs pressure at 121ºC for 15 minutes and it was poured into sterile Petridis.

4. BLOOD AGAR

Ingredients	gm/liter
Blood agar base	28.0
Blood	5%

Preparation

28 gram of Blood agar was suspended in 1000 ml of distilled water. It was boiled to dissolve the medium completely. Then it was sterilized by autoclaving at 121ºC for 15 minutes. Then 5% blood was added aseptically and mixed well before pouring into the sterile Petriplate.

5. CHOCOLATE AGAR

Preparation

The sterilized Blood agar was heated at 75ºC in a water bath with gentle agitation from time to time until the blood becomes brown in colour. The media was poured into plates.

6. MULLER HINTON AGAR

<u>Ingredients</u>	<u>gm/liter</u>
Beef, infusion form	300.0
Casein acid hydrolysate	17.5
Starch	1.5
Agar	17.5
Final pH (at 25ºC)	7.0±0.2

<u>Preparation</u>

38 grams powder was suspended in 1000 ml of distilled water and then boiled to dissolve completely and medium was sterilized by autoclaving at 121ºC (15lbs pressure) for 15 minutes.

8. SULPHIDE INDOLE MOTILITY (SIM) MEDIA

<u>Ingredients</u>	<u>gm/liter</u>
Beef extract	3.0
Peptone	30.0
Peptonized agar	0.2
Sodium thiosulfate	0.025
Agar	3.0
Final pH (at 25ºC)	7.3±0.2

<u>Preparation</u>

36 grams of media was suspended in 950 ml of distilled water. It was boiled to dissolve the media completely. Then it was distributed in tubes to depth of 3 inch and it was sterilized by autoclaving at 121ºC for 15 minutes. The medium was allowed to solidify in vertical position.

9. METHYL RED- VOGES PRAUSKAUER MEDIUM

Ingredients	gm/liter
Buffered peptone	7.0
Dextrose	5.0
Dipotassium phosphate	5.0

Preparation

17.0 grams powder was suspended in 1000 ml of distilled wate. The medium was boiled to dissolve the medium completely. Dispensed in 5 ml tubes and cotton plugged. Then the tubes were sterilized by autoclaving at 121ºC (15 lbs pressure) for 15 minutes.

10. SIMMON'S CITRATE AGAR

Ingredients	gm/liter
Magnesium sulfate	0.2
Ammonium dihydrogen phosphate	1.0
Dipotassium phosphate	1.0
Sodium citrate	5.0
Sodium chloride	1.5
Agar	15
Bromothymol blue	15
Final pH (at 25ºC)	7.0±0.2

Preparation

24.2 gram of media was suspended in 950 ml of distilled water. It was then boiled to dissolve the medium completely and sterilized by autoclaving at 121ºC for 15 minutes. The medium was allowed to solidify making slants in slope.

11. TRIPLE SUGAR IRON (TSI) AGAR

Ingredients	gm/liter
Peptic digest of animal tissue	10.0
Casein hydrolysate	10.0
Yeast extract	3.0
Beef extract	3.0
Lactose	10.0
Sucrose	10.0
Dextrose	1.0
Sodium chloride	5.0
Ferrous sulphate	0.20
Sodium thiosulphate	0.30
Phenol red	0.024
Final pH (at 25ºC)	

Preparation

65 grams powder was dissolved in 1000 ml of distilled water and boiled to dissolve the medium completely. Medium was distributed about 5 ml in test tubes and sterilized by autoclaving at 121ºC (15 lbs pressure) for 15 minutes. After autoclaving tubes containing medium were tilted to form slants with a butt about 1 inch of long.

12. HUGH AND LEIFSON'S MEDIUM

Ingredients	gm/liter
Peptic digest of animal tissue	2.0
Sodium chloride	5.0
Dipotasium phosphate	0.3
Glucose	10.0
Bromothymol blue	0.05

Agar	2.0

Preparation

19.4 grams powder was suspended in 1000 ml of distilled water. The medium was boiled to dissolve the medium completely. Dispensed in 5 ml amounts tubes and cotton plugged. Then the tubes were sterilized by autoclaving at 121ºC (15 lbs pressure) for 15 minutes.

13. CHRISTENSEN UREA AGAR MEDIUM

Ingredients	gm/litre
Peptic digest of animal tissue	1.0
Dextrose	1.0
Sodium Chloride	5.0
Disodium	1.2
Monopotassium phosphate	0.8
Phenol red	0.012
Agar	15.0

Preparation

24 grams powder was suspended in 950 ml distilled water and sterilized at autoclaving at 121ºC (15 lbs pressure) for 20 minutes. After cooling to about 55ºC, 50 ml of 40% urea was added and mixed well. Then, 5 ml was dispensed in test tube and slanted at slope position to make agar slant.

APPENDIX-V

POCEDURE

A. Gram’s staining

- A dried, heat fixed smear was prepared and covered the slide completely with the violet solution for at least 30 seconds to 1 minute.
- Then the stain was washed off with tap water and covered completely with lugol’s iodine solution for at least 20 seconds.
- Again the iodine solution was washed off with tap water. The slide was covered completely with acetone alcohol decolorizer and left for about 10 seconds.
- Next, the slide was washed off with tap wasted and covered with counterstain solution, safranin for about 1 minute.
- Lastly, the counterstain was washed off with tap water, blotted dry with clean blotting paper and observed under microscope.

B. Ziehl-Neelsen Staining

- A dried, oval shaped smear was prepared by means of a wooden stick.
- The smear was then covered with carbolfuchsin solution. The smear was then heated until vapor just start to evaporate. The slide was then left for about 5 min.
- The smear was then washed off with distilled water and decolorized with 20% sulphuric acid until the red stain of carbolfuchsin disappeared.
- Now, the smear was counterstained with Methylene blue for 1 min.
- The smear was again washed off with distilled water, kept in the rack for air dry and observed under oil immersion.

Reporting of sputum smears

If ≥ 10 AFB/field – report as +++.

If 1-10 AFB/field – report as ++.

If 10-100 AFB/10 fields- report as +.

If 1-9 AFB/field – reported the exact number.

C. Antibiotic susceptibility testing

Kirby-Bayer method was used for antibiotic susceptibility test with following procedure:

1. Preparation of plates:

The agar plates were prepared in a way to make the thickness of medium of about 4mm.

2. Preparation of inoculum:

For inoculums preparation, 3-4 pure culture colonies were transferred into a test tube containing 2-3 ml of nutrient broth and were incubated at 37ºC for 2-4 hours to obtain turbidity.

3. Inoculation:

A sterile cotton swab was dipped into the turbid solution and was streaked (by means of swabbing) on the agar surface of MHA plate. The plate was then left 10 minutes at room temperature to dry the inoculums.

4. Application of the disc:

Using a sterile forceps, antibiotic discswere carefully placed on the agar surface of the plate with certain distance in between the discs so that the zones of inhibition do not merge with each other.

5. Within 30 minutes of applying the discs, plate was inverted and incubated it at 35ºC for 16-18 hours.

6. After overnight incubation, plate was examined for zone of inhibition using the Interpretative Chart. Zone sizes were interpretated of each antimicrobial, reporting the organism ‘Resistant’ or ‘Sensitive’. ‘Intermediate zone’ was reported as ‘Resistant’ in our study. The zone of inhibition was interpretated according to the CLSI guideline.