CHAPTER - I

1. INTRODUCTION

Lower respiratory tract is the part of the respiratory tract below the vocal cords. While often used as a synonym for pneumonia, the rubric of lower respiratory tract infection can also be applied to other types of infection including lung abscess and acute bronchitis. Lower respiratory tract infection (LRTI) describes a range of symptoms and signs, varying in severity from non-pneumonic LRTI in the young healthy adult through to pneumonia or life-threatening exacerbation in a patient with severe disabling chronic obstructive pulmonary disease (COPD).

Acute respiratory infections (ARI) are one of the most important causes of morbidity and mortality in children throughout the world. Globally, about 4.2 million ALRI deaths are estimated to occur among all age groups; of these 1.8 million are estimated to occur among children (World Health Organization 2008). The majority of ARI-related mortalities have been attributed to severe acute lower respiratory infections, especially pneumonia, of bacterial etiology (Walter 1992). According to Natural Health Blog Archive, 2010, LRTI causes more than 4 million deaths every year. In all, these diseases account for nearly half or more than half of fatalities worldwide every year. In spite of being a preventable disease, deaths due to ARI among children is around 4 million each year, with 90% of them occurring in developing world according to an estimate by the WHO.

According to the report of Ministry of Health, Government of Nepal 3.4% of morbidity is caused by acute RTI. It is among top ten diseases of Nepal. There is an increasing trend of the incidence of ARI and pneumonia in children greater than 5 yrs of age in Nepal in past three years. The national average of the incidence of ARI and pneumonia per 1,000 under 5 yrs aged children was 408 (Nepal Ministry of Health and Population, Kathmandu, 2004). The mortality rate among children aged <5 years is very high, at 91 deaths per 1000 live births, with pneumonia as the leading cause of death. Nepal's low rate of treatment of suspected pneumonia cases is to blame for the high fatality rates. Only 15%-18% of all patients with pneumonia who reside in rural or hilly areas are brought by caretakers to health care facilities, according to Ministry of Health estimates (Nepal Ministry of Health: Fact sheet, 2006 and 2007).

It is estimated that Bangladesh, India, Indonesia and Nepal together account for 40% of global acute respiratory infection mortality. As an infection of lungs pneumonias is one of the major causes for ARI (WHO 2000). About 90% of ARI deaths are due to pneumonia, which is usually bacterial in origin (WHO 1999).

On the other hand, many of the bacteria that cause ARI can be isolated as part of the normal flora of healthy people. To have a precise etiological diagnosis it would be necessary to have access to clinical diagnostic laboratories capable of isolating and identifying nonbacterial and bacterial agents, using worldwide standardized procedures. As these conditions are not frequently available, especially in the less developed countries, the magnitude of the global and specific problems of ARI could be even more extensive than currently available data show.

There are a number of acute and chronic infections that can affect the lower respiratory tract. The two most common infections are bronchitis and pneumonia. Pneumonia is the main lower respiratory tract infection, with characteristics much more severe than most of the upper ARI. The etiological agents of pneumonia acquired in the community vary according to the age and previous state of health of the patient, but most cases are considered to be bacterial. Determining the etiologic agent of childhood pneumonia is a particular diagnostic problem, since appropriate representative specimens can rarely be obtained and sensitivity of blood cultures is generally low.

Less information is available from developing countries. Most studies have been hospital based, and they suggest that *Staphylococcus aureus*, *Klebsiella* spp. are the most frequent causes in the first month of life, and that very soon after *Streptococcus pneumoniae* and *Haemophilus influenzae* become the dominant pathogens. *S. aureus* and *Klebsiella pneumoniae* were reported to be the most frequent bacterial pathogens in certain third world communities (Johnson 1993), which is probably related to the indiscriminate use of antibiotics. High prevalence of malnutrition can also be a contributory factor to the emergence of these necrotizing agents of pneumonia.

Woodhead et al., UK found an incidence rate of pneumonia of 4.7 cases per 1000 (aged 15-79) per year, in patients who consulted their general practitioner with pneumonia. As well as high mortality rates, LRTIs also pose a socioeconomic problem. For example, in the USA, the direct costs of LRTIs such as acute

exacerbations of chronic bronchitis(AECB) have been estimated as US$1.2 billion for patients aged 65 years and US$419 million for patients < 65 years (Bartlett 2000).

Respiratory tract infection is the leading cause of morbidity and mortality in critically ill patients in developing countries (Kumari 2007). It is notable that they cause more disease and death than any other infection in the United States and there has been reported little change in mortality caused by respiratory tract infection for more than five decades (Mizgerd 2008). In India, ARI is responsible for one million deaths. Out of these 10-15% is due to acute lower respiratory tract infections (Reddiah 1988).

Inappropriate antibiotic therapy may increase a patient's stay in the hospital and predispose them to increased antibiotic resistance. A direct correlation exists between antibiotic resistance and patient outcomes, including mortality, length of hospital stay and healthcare costs (Cosgrove 2006). Therefore, there is a great need for surveillance of antimicrobial resistance not only to provide early warning of emerging problematic resistances, but also to contribute towards designing appropriate strategies to manage antibiotic resistance. However during the last few years, the increase in the rates of antibiotics resistance amongst the major microbial causes of the respiratory infections in the community has compromised the selection of empirical treatment for some respiratory tract infections. The consequences of increased drug resistance are far-reaching since bacterial infection of the lower respiratory tract is a major cause of death due to infectious disease (Kumari 2007).

In Nepal there are few reports about antibiotic survey. Often, antibiotic are prescribed for illnesses that do not require them. However to ensure appropriate therapy in RTI, current knowledge of the organisms that cause RTI and their antibiotic susceptibility is mandatory. In addition, the emergence of resistance as a major problem has drawn attention to a need for better diagnostic techniques and newer drugs to allow more specific therapy. Hence, the present study was undertaken to define the common bacterial profile in LRTI and to study the resistance patterns to common antibiotics. The essence of this study, therefore, is to analyze the various isolated organisms obtained from sputum with a view to identifying the organism, to test their susceptibility and sensitivity to available antibiotics in use in the hospital and environment.

CHAPTER – II

2. OBJECTIVES

In this study the results of investigations on lower respiratory tract infections (LRTIs) in suspected patients are presented. LRTI comprises different kinds of pulmonary infections. They range from mainly self-limiting bronchitis to pneumonia.

General objectives:

To find out the spectrum of etiological agents causing lower respiratory tract infection and their antibiotic susceptibility pattern.

Specific objectives:

1. To find the incidence of lower respiratory tract infection in the patients visiting Bir Hospital.
2. To find the bacterial pathogens involved in lower respiratory tract infections.
3. To analyze the antibiotic susceptibility pattern of the isolated organisms.
4. To estimate the incidence of Multi Drug Resistant organisms among respiratory isolates.

CHAPTER - III

3. LITERATURE REVIEW

3.1 Definition

3.1.1 Clinical definition

Clinicians use a wide range of disease definitions, such as tracheitis, acute bronchitis, bronchiolitis and pneumonia, depending on the symptoms and signs they observe. Lower respiratory tract infection (LRTI) is defined as the inflammation of the respiratory tract starting from the trachea to the alveoli secondary to entry and subsequent multiplication of an infectious agent. Acute lower respiratory tract infection is defined as an acute illness present for less than 21 days of onset, with cough as the cardinal symptom; and at least one other respiratory tract symptom (sputum production, dyspnoea, wheeze, chest discomfort/pain) (Mac Farlane 2001).

3.1.2 Microbiological definition

Microbiologists use definitions that are based on the causative pathogens, as identified by laboratory investigations into blood, sputum and other material from patients who are ill and have symptoms befitting LRTI. Terms like Pneumococcal infection, mycoplasma infection, bacterial infection and viral infection are commonly used.

3.2 Anatomy of lower respiratory tract:

The shape and structure of the respiratory tract

The respiratory tract is divided into an upper and a lower respiratory tract. The respiratory tract is mostly a membranous structure that allows the passage and diffusion of air when the processes described above keep it open to the surrounding air. The lower respiratory tract is tubular in shape. Proximally, the lower respiratory tract consists of the larynx, trachea, and bronchial system, which all have cartilage in their walls. Distally, the lower respiratory tract consists of the respiratory bronchioles.

A. Trachea

The trachea extends downward anterior to the esophagus and into the thoracic cavity, where it splits into right and left bronchi. The inner wall of the trachea is lined with ciliated mucous membrane with many goblet cells that serve to trap incoming particles. The tracheal wall is supported by 20 incomplete cartilaginous rings**.**

B. Bronchial Tree and the Alveoli

The trachea divides into two main bronchi which enter the lungs. Each bronchus in turn divides several times into narrower and narrower bronchi. The trachea and bronchi have cartilage support. The larger bronchi divide into smaller bronchi, which divide further into the bronchioles. The bronchioles are very narrow and have a diameter of 1mm or less. The bronchioles eventually lead into small sac-like structures called the alveoli. Each lung has about 300 million alveoli. Each alveolus is surrounded by a capillary blood vessel. A continuous exchange of gases takes place between the alveoli and the capillary blood vessels that surround them.

C. Lungs

The right and left soft, spongy, cone shaped lungs are separated medially by the mediastinum and are enclosed by the diaphragm and thoracic cage. The top portion is known as the apex, and the bottom is known as the base. The apex of each lung rises above the clavicles a few centimeters and the base rests against the diaphragm. The bronchus and large blood vessels enter each lung. A layer of serous membrane, the visceral pleura, folds back to form the parietal pleura. The visceral pleura are attached to the lung, and the parietal pleura line the thoracic cavity; serous fluid lubricates the “pleura cavity” between these two membranes. The right lung has 3 lobes: upper, middle, and lower. The left has two lobes: upper and lower. Each lobe is composed of lobules that contain air passages, alveoli, nerves, blood vessels, lymphatic vessels, and connective tissues.

D. Diaphragm

The diaphragm is the major muscle of ventilation. It is a dome-shaped musculofibrous partition located between the thoracic and abdominal cavities. It is

composed of two muscles: the right and left hemi diaphragms. The diaphragm allows the esophagus, the aorta, several nerves, and the inferior vena cava to exit through it.

3.3. Blood supply to the lung

The lung has two sources of blood circulation, the bronchial and the pulmonary vessels.

3.3.1 Bronchial system

The bronchial system supplies the structures of the larger airways and the large pulmonary vessels with oxygen saturated arterial blood. It also warms and humidifies the incoming air. Sympathetic nerves regulate blood flow in the bronchial system.

3.3.2 Pulmonary system

The pulmonary system develops from the artery of the right 6th pharyngeal arch, and supplies the capillary network around the alveoli where the gas exchange occurs. The capillary network looks more like a thin film of blood, spread out over the outside surface of the alveoli. The film is just thick enough to allow the passage of red blood cells. Pulmonary artery blood is arterial blood with a low oxygen saturation that comes directly from the right ventricle. The pulmonary veins take the oxygenated blood from the lungs directly into the left atrium. 10% of the total blood volume is pooled in the pulmonary circulation. The fraction of blood in the lung is 40-50% of its total weight.

3.4 Physiology of respiratory tract

3.4.1 Airways and airflow:

The conducting airways consist of a series of branching tubes which become narrower, shorter and more numerous as they penetrate deeper into the lung. Since the conducting airways have no alveoli they do not take part in gas exchange but constitute the anatomical dead space. Its volume is about 150 ml but it varies because airways are not rigid. During inspiration, respiratory tubes are lengthened and dilated, especially in deep breathing. The alveolated region of the lung includes respiratory bronchioles and alveolar ducts. This zone is called respiratory zone and the gas exchange occurs here. The distance from the terminal bronchiole to the distal alveolus

is only a few mm, but the respiratory zone makes up most of the lung, its volume being about 2.5 to 3 L.

3.4.2 Physiology of breathing and gas exchange

3.4.2.1 Mechanics of breathing

During inspiration, as the volume of the thorax increases, the pressure within the chest decreases. As a result air moves into the lungs from the atmosphere. During expiration, the diaphragm and other respiratory muscles passively return to their normal position; thereby, decreasing the size of the thorax. As the size decreases, pressure increases and air moves out of the chest into the atmosphere.

Pressure-Volume relationships: In the pulmonary physiology absolute pressure means atmospheric pressure (760 mm Hg at sea levels). The pressures and the pressure differences of the respiratory system are expressed as relative pressures to the atmospheric pressure. When it is said that alveolar pressure is zero, it means that alveolar pressure = atmospheric pressure.

Compliance and Resistance

Compliance is the ability to stretch. Elasticity is the ability to return to normal shape. Abnormalities of compliance and/or elasticity result in alterations in ventilation. Pulmonary resistance is impedance of airflow in the lung. Resistance is related to lung compliance, diameter and length of the airways, and the turbulence of airflow. (Guyton 2000)

3.4.2.2 Gas exchange

Gas exchange is actually a combination of two separate processes: ventilation and respiration. Ventilation is the process of moving air between the atmosphere and alveoli. Respiration is the diffusion of gas across the alveolar-capillary membrane to maintain proper concentration of oxygen (O_2) and carbon dioxide (CO_2) in blood. When the partial pressure of oxygen is higher in the alveolar air than it is in the capillary blood, oxygen will diffuse into the blood. When the partial pressure of carbon dioxide is greater in the blood than in the alveolar air, carbon dioxide will diffuse out of the blood and into the alveolus. The alveoli are composed of two types of cells: Type I cells and Type II cells. Type I cells function in gas exchange and also

produce surfactant, the loss of which can result in alveolar instability, collapse, and impairment of gas exchange.

3.4.3 Gas transport: Over 98% of oxygen is carried in the blood bound to hemoglobin of red blood cells, producing oxyhemoglobin. Oxyhemoglobin is unstable in areas where the concentration of oxygen is low, and gives up its oxygen molecules in those areas. CO_2 may be transported dissolved in blood plasma, as carbaminohemoglobin or as bicarbonate ions.

3.4.4 Surface tension: The surface tension arises because the attractive forces between adjacent molecules of the liquid are much stronger than those of between the liquid and the gas. At the interface between the liquid and the alveolar gas, intermolecular forces in the liquid tend to cause the area of the lining to shrink. The surface tension contributes to the pressure-volume behavior of the lungs. The most important component of this liquid film is surfactant. It is produced by type 2 alveolar epithelial cells and its major constituent is dipalmitoyl phosphotidylcholine (DPPC), a phospholipid with detergent properties. It increases the compliance of the lung, reduces the work of expanding of the lung with each breath stabilizes the alveoli and keeps it dry.

3.4.5 Ventilation/Perfusion relationships: When the number of alveoli that are ventilated equals the number of alveoli that are perfused, ventilation and perfusion are equally matched. This is normal. If more alveoli are perfused than are ventilated, a ventilation-perfusion (V/Q) mismatch called shunting results. Pulmonary shunting results from problems that prevent air exchange in the alveoli (e.g. Atelectasis). If more alveoli are ventilated than are perfused, a V/Q mismatch called dead space results. Pathologic pulmonary dead space results from problems that interfere with blood flow to the alveolar capillaries. Ventilation/perfusion relationships vary within portions of the lung, but the normal lung has evenly matched ventilation and perfusion on average. (Berne 1998)

3.5 Host Defence:

3.5.1 First line of defense – physical & chemical barriers

Physical barrier and mechanical host defence mechanisms, and chemical host defence mechanisms are constantly available for immediate action in the healthy host.

Particle size in part determines fate, as particles and microbes generally exceeding 5 mm can be entrapped as air flows through the tortuous channels of the nasopharynx and by nasal hairs, and through inertial forces are impacted along the tonsilar pillars, glottis, trachea and branching bronchi and bronchioles. Entrapped particles and microbes may be expelled through coughing and sneezing mechanisms. The complex glycoprotein mucins lining the airway epithelial surfaces facilitate particle entrapment, and promote elimination by the respiratory epithelial cell ciliary movement that allows expectoration or swallowing of mucin-entrapped pathogens. In general, particles and potential pathogens smaller than 5 mm can bypass these mechanical obstacles and gain access to the terminal bronchioles and alveoli. In addition, the respiratory epithelium serves as a critical barrier function.

A number of secreted airway products also contribute to antimicrobial functions by several mechanisms including direct antimicrobial activity, opsonization and agglutinization. Microbes that pass the physical and mechanical barriers may be eliminated by a range of chemical mediators that are constantly expressed and may be further induced. These mediators include molecules capable of direct antimicrobial effect (ex. lysozyme, lactoferrin, complement, α-, β- defensins and cathelicidins), agents that inhibit microbial growth (ex. transferrin), and molecules that serve as opsonins that facilitate host cell recognition (ex. complement, fibronectin, collectins, IgA and IgG).

3.5.2 Second line of defense

Once beyond the protective outer barrier of the body, the invading microbes will encounter a series of nonspecific cellular and chemical defense mechanisms.

Innate immunity in the lungs

The principal cellular components of lung innate immunity include alveolar macrophages, neutrophils, NK cells, dendritic cells and eosinophils. Alveolar macrophages represent the predominant immune cell in the alveolar airspace, accounting for >85% of mobile cells in the alveoli. Neutrophils and eosinophils are generally not present in the alveoli but are recruited in response to chemotactic signals. Natural killer (NK) cells participate in early innate defence through cytotoxic activity against pathogen-infected cells and secretion of cytokines and chemokines

that modulate subsequent steps in the adaptive immune response. Recognition of microbial products by dendritic cells triggers functional dendritic cell maturation and leads to initiation of antigen specific adaptive immune responses. Innate immune cells such as alveolar macrophages recognize potential pathogens through surface recognition receptors such as mannose receptors, β-glucan receptors, scavenger receptors and Toll-like receptors (TLRs). Classical pathway complement proteins C3, C4, C1q and alternative complement pathway component factor B are expressed in the lung alveolar fluid and can provide opsonization of microbes in the respiratory tract. Alveolar epithelial cells, in addition to providing a physical barrier function, also contribute to the innate immune response in the lungs. Epithelial cells express defensins HBD-1 and HBD-2 and defensins also stimulate IL-8 production by epithelial cells. (Martin 2005)

3.5.3 Adaptive immunity in the lungs

Generation of adaptive immunity requires the somatic rearrangement of lymphocyte receptors (including B-lymphocyte and T-lymphocyte receptors) that confers antigen specificity directed against specific epitopes expressed by pathogens, amplifying the immune response against pathogens expressing the specific antigen or epitope, and promotes immune memory that allows enhanced immune response upon future rechallenge with the pathogen expressing the same antigen or epitope. For persistent infectious challenge, antigens are presented to lymphocytes by lung dendritic cells in regional lymph nodes. As alveolar macrophages are poor antigen presenting cells, alveolar macrophages may transport antigen to interstitium and/or regional lymph nodes where antigen can be processed by dendritic cells, or perhaps antigen may be processed by alveolar dendritic cells that then may be transported to the regional lymph nodes. Once in the regional lymph nodes, dendritic cells present antigen to responsive T- and B-lymphocytes in the context of MCH molecules to activate the adaptive immune response. B-lymphocytes are activated to produce antibodies directed against specific epitopes, and CD8+ T-lymphocytes target infected cells expressing specific epitopes on the cell surface. Whereas T-lymphocyte receptors can interact with processed or cleaved foreign antigens, B-lymphocyte receptors can interact directly with intact foreign antigens.

3.5.4 Predisposing factors on LRTI

i) Malnutrition, breast feeding and vitamin A deficiency: Malnutrition produces alterations in the immune and non-immune host defenses, respiratory muscle weakness, diminished energy stores, and impairment in the recovery of normal pulmonary tissue from inflammation. A causal effect of breastfeeding is plausible given the maternal–infant transfer of innate immune effectors and influences of breast milk on immune-system maturation. Vitamin A is considered an important element in the maintenance of epithelization of the respiratory tract and the pulmonary recovery process and also plays a role in the host immune system.

ii) Increased frequency of low birth weight and prematurity: The biological plausibility of the association between LBW and respiratory morbidity is supported by observations of impaired immunocompetence in infants born small and the association of fetal growth restriction with structural alterations that may affect lung anatomy and function.

iii) Smoking and alcohols: Cigarette smoke has been found to attract inflammatory cells into the lungs and stimulates the release of the proteolytic enzyme elastase from these cells. While smokers often produce more mucus in response to smoking, they are less able than nonsmokers to move the mucus out of their respiratory system. This happens because cigarette smoking paralyzes and eventually destroys cilia. Smoking impairs lung growth and lung tissue. Alcohol intake directly inhibits the ability of resident lung immune cells to kill bacteria.

iv) Viral infection: Viral infections in the lung enhance inflammation and predispose to bronchial hyper reactivity. Once COPD is established, repeated infective exacerbations of airflow obstruction, either viral or bacterial, may speed up the decline in lung function.

v) High-risk age groups: Children younger than 5 years, particularly aged 2 years or younger are at an increased risk of pneumococcal infections. Adults older than 55-65 years are also at an increased risk of disease.

vi) Gender: When smoking and occupational exposure is taken into account, the relative risk of developing COPD is not significantly higher in men than women. With

smoking on the increase in women, it is possible that women may catch up with men in terms of absolute numbers.

vii) Air pollution and occupational exposure: Studies from the UK have shown a relationship between levels of atmospheric pollution and respiratory problems (particularly cough and sputum production) in both adults and children, and similar studies from the USA have confirmed these findings.

Occupations at risk include coal miners, construction workers who handle cement, metal workers, grain handlers, cotton workers and workers in paper mills. Pollution from the burning of biomass fuels or passive smoking alters the integrity of the epithelial lining of the respiratory tract, disrupts mucociliary function, may make pulmonary tissue more susceptible to damage by the inflammatory response, and may compromise the pulmonary recovery process.

viii) Host-related risk factors:

Host related risk factors include preexisting conditions such as immunosuppression, chronic obstructive lung disease, and acute respiratory distress syndrome. Other host-related factors include patients' body positioning, level of consciousness, including psychological stress, exhaustion, number of intubations, and medications, including sedative agents and antibiotics. Generally accepted risk factors that predispose an individual to nosocomial pneumonia include the presence of underlying diseases such as HIV infection, chronic lung disease, congestive heart failure, or diabetes mellitus; age >70 years; mechanical ventilation or intubation; previous antibiotic treatment; a long preoperative stay; and prolonged surgical procedures.

ix) Conditions associated with decreased pulmonary clearance functions:

These include asthma, chronic bronchitis, chronic obstructive pulmonary disease (COPD), viral infections, and active/passive cigarette smoke exposure. Pulmonary edema, Hypoxemia, Uremia and Pneumothorax are also the contributing factors in LRTI.

x) Prior antibiotic therapy:

Normal flora of the nasopahrynx and oropharynx help to prevent colonization of the respiratory tract by pathogenic microorganisms by their antagonism relationship. Prior antibiotic therapy is a major factor in changing the pattern of offending organisms.

3.6 Pathogenesis

3.6.1 Transmission: Microorganisms can infect the LRT by four possible routes:

a) Aspiration of oropharyngeal content

It is likely that most pathogens first colonize the surfaces of the oral cavity or pharyngeal mucosa before aspiration (Currie DC 1987). These pathogens can colonize from an exogenous source or emerge following overgrowth of the normal oral flora after antibiotic treatment. Common potential respiratory pathogens such as *S. pneumoniae*, *Mycoplasma pneumoniae*, and *H. influenzae* can colonize the oropharynx and be aspirated into the lower airways. Aspiration, resulting from either a seizure disorder or a semiconscious state resulting from excessive consumption of alcohol or other drugs, may lead to lung abscesses caused by organisms typically residing in the oral cavity. Persons with abnormal swallowing, such as those who have depressed consciousness, respiratory tract instrumentation and/or mechanically assisted ventilation, gastrointestinal tract instrumentation or diseases, or who have just undergone surgery, especially thoracic and/or abdominal surgery, are particularly likely to aspirate (Torres A 1990).

b) Inhalation of infectious aerosols/ Air borne transmission

Aerosols are a suspension of solid or liquid particles in a gas, with particle size from 0.001 to over 100 mm. A droplet nucleus is the airborne residue of a potentially infectious aerosol from which most of the liquid has evaporated.

The short-range airborne infection route depends on the close proximity of the infected source and susceptible host. Exhaled air from both nose and mouth is able to enter and mix with air in the breathing zone of another person standing nearby. Thus,

short-range transmission implies that air flows between individuals may interact to infect one another (Bjorn E 2002).

Long-range aerosol transmission refers to the potential for agents to be carried long distances by air flows to cause infection, and includes the traditional terms ‘small-droplet’ or ‘droplet nuclei’ and ‘airborne’. A commonly encountered source is the patient with flu-like symptoms who is coughing, sneezing and dispersing the organism. A sneeze can generate up to 40,000 droplets, which can evaporate to produce droplets of 0.5-12 µm in diameter. A cough can generate about 3,000 droplet nuclei, the same number as talking for 5 minutes. During normal breathing, exhalation can project droplets up to 1 m in room air, which may be inhaled by another person nearby whereas sneezing can project droplets several meters.

c) Spread of infection from contiguous sites

The high incidence of Gram-negative bacillary pneumonia in hospitalized patients appears to be the result of factors that promote colonization of the pharynx by Gram-negative bacilli and the subsequent entry of these microorganisms into the lower respiratory tract (Lowy FD 1987).

d) Hematogenous spread from extrapulmonary sites of infection

Rarely, bacterial pneumonia can result from hematogenous spread of infection to the lung from another infection site, e.g., pneumonia resulting from purulent phlebitis or right-sided endocarditis. Entry of bacteria from the gut with spread through the bloodstream to the lungs has also been proposed for the pathogenesis of Gram-negative organisms (Fiddian-Green 1991), but such bacteria are uncommon etiological agents of pneumonia in immune-competent children.

3.6.2 Bacterial interactions with mucus and cilia

The first interaction of inhaled bacteria with the airway mucosa is with mucus. *H. influenzae*, *P. aeruginosa* and *S. pneumoniae* have a high affinity for mucus in vitro, although this is not true for all bacteria that have been investigated. Bacterial adherence to mucus probably involves both specific (adhesin-receptor) and nonspecific interactions (Barsum 1995). The affinity of bacteria for mucus, and their relative lack of adherence to healthy epithelium may explain why they do not infect

normal airways, which have efficient mucociliary clearance. Whereas in chronic bronchitis, bronchiectasis and cystic fibrosis, mucociliary clearance is delayed, giving bacteria that have adhered to mucus time to produce virulence factors in sufficient quantities to establish the infection. Bacterial infection attracts leucocytes into the airways, many of which eventually degenerate releasing deoxyribonucleic acid (DNA) into the secretions making them more viscous and difficult to clear (Konstan 1994).

Some bacteria produce factors which disturb the mucociliary system by slowing and disorganizing the beating of cilia. Some of these cilioinhibitory factors have been characterized: *P. aeruginosa* produces pyocyanin, 1-hydroxyphenazine and rhamnolipid; *H. influenzae* produces low molecular weight glycopeptides; and *S. pneumoniae* produces pneumolysin.

3.6.3 Adherence, invasion and cell damage

Cell damage might remove defence mechanisms, such as ciliary beating, and might also expose new receptors to which bacteria can adhere on damaged cells. A number of bacterial products have been shown to damage epithelial cells, such as the protease enzymes of *P. aeruginosa*. Bacterial adherence to mucosal features occurs *via* specific interactions between adhesin structures on the bacterial surface and receptors on the mucosal surface. These adhesins can be proteins found on the bacterial cell wall / membrane or they can be collected together on structures that project from the cell surface, for example fimbriae and pili, which seem to be expressly aimed at increasing the chance of adherence. Pili have been identified as an important adhesin of *P. aeruginosa* and other adhesins such as exoenzyme S and alginate have been identified. After colonization, organisms may gain access to areas of the upper and/or lower respiratory tracts by direct extension.

3.6.4 The effect of chronic inflammation on bacterial interactions with the mucosa

The multiplication and spread of bacteria within the bronchial lumen, and consequent damage to the epithelium, stimulates the host to mount an inflammatory response. If this fails to clear the bacteria and bacterial infection continues, the inflammatory response becomes chronic. Large numbers of activated neutrophils are

attracted into the airway by host (*e.g.* complement factor 5a (C5a), leukotriene B4 (LTB4), interleukin-8 (IL-8)) and bacterial chemotactic factors (Ras 1990). Activated neutrophils spill proteinase enzymes and reactive oxygen species. Proteinase enzymes and reactive oxygen species both cause epithelial damage, and stimulate mucus production. These changes promote continued bacterial infection by impairing mucociliary clearance. Neutrophil elastase present in secretions attracts additional neutrophils into the airway by inducing production of the powerful chemoattractant IL-8 by epithelial cells and may impair phagocytosis by cleaving complement receptors from neutrophils and complement components from bacteria (Berger 1989). *S. pneumoniae* cell wall components, along with the pneumococcal capsule, activate the alternative complement pathway; antibodies to the cell wall polysaccharides activate the classic complement pathway. Cell wall proteins, autolysin, and DNA released from bacterial breakdown all contribute to the production of cytokines, inducing further inflammation.

3.6.5 Toxins and other virulence factors

Some bacterial toxins disable the inflammatory response, for example by inhibiting phagocyte function or cleaving antibodies, whilst others enhance inflammation, for example by inactivating α1-antiproteinase or enhancing neutrophil oxidative metabolism. Similarly infection may result in the release of a mixture of pro- and anti-inflammatory cytokines. Bacterial factors which disable host defences may compete with pro-inflammatory host factors early in the infectious process, whereas later, when airway damage has occurred and chronic bacterial infection is established, pro-inflammatory bacterial factors may subvert the host defences to promote continued bacterial infection by increasing inflammation, which causes lung damage.

Pneumococcal isolates produce few toxins, however, all serotypes produce pneumolysin, which is an important virulence factor that acts as a cytotoxin and activates the complement system. In addition, pneumolysin causes a release of tumor necrosis factor-alpha and interleukin-1. Other potential virulence factors include cell surface proteins such as surface protein A and surface adhesin A and enzymes such as autolysin, neuraminidase, and hyaluronidase.

3.6.6 Avoiding destruction by alveolar macrophages

Inhaled microorganisms reaching the alveoli encounter alveolar macrophages which destroy most of them, but one or two pathogens have learnt either to avoid phagocytosis or to avoid destruction after phagocytosis. Tubercle bacilli, for instance, survive in the macrophages, and respiratory tuberculosis is thought to be initiated in this way. In a pneumococcus-naive host (or in the absence of antibody to pneumococcal capsule), host-cell phagocytosis is severely limited because of the inhibition of phagocytosis and the inhibition of the activation of the classic complement pathway.

3.6.7 Production of disease

3.6.7.1 Local effects: Certain bacteria cause diffuse infections by producing enzymes that enable them to breakdown the cells and intracellular matrices of host tissues. Streptococci produce the enzyme hyaluronidase which hydrolyses hyaluronic acid, a polysaccharide component responsible for binding cells together. Many bacteria produce different kinases, lecithinases and proteases with varying effects upon host tissues. On the other hand, some bacteria produce more localised infections with the production of pus in the affected tissues; bacteria that do this are described as pyogenic. *Staphylococcus aureus* produces an enzyme coagulase which coagulates fribrinogen and abscesses are particularly likely to develop.

3.6.7.2 Distant effects: There are over 220 known bacterial toxins and they can be divided up into two groups. **Endotoxin** is a component of the cell wall of all gram negative bacteria whilst the **exotoxins** include a huge number of proteins that are secreted from the bacterial cell and which are mostly, but not exclusively, produced by gram positive bacteria.

Endotoxin is the lipid portion of lipopolysaccharide, lipid A, and is a potent inducer of interleukin-1 (IL-1) from macrophages, which resets the hypothalamus to produce fever, and tumour necrosis factor (TNF) from phagocytes, which induces severe shock.

3.6.7.3 Evasion of host defences: Bacteria, such as *S. pneumoniae*, *H. influenzae* and *Klebsiella pneumoniae* produce a glycocalyx capsule that inhibits phagocytosis. *Mycobacterium tuberculosis* accomplishes this by the insertion of waxes into its cell

wall and *Streptococcus pyogenes* has the M protein in its cell wall to decrease phagocytosis. *Staphylococcus* and *Streptococcus* use leukocidins to destroy leukocytes and macrophages and haemolysins to disrupt erythrocytes.

3.6.7.4 Choosing a new victim: The final stage in the infection cycle is for the agent to find a new host to infect which usually ties in with how the present host first acquired the disease. The many agents that use the respiratory route for infection tend to produce symptoms such as nasal discharge, coughing and sneezing which enhance the production of aerosols and therefore the potential for transfer to new hosts.

3.7 Classification of lower respiratory infections

When discussing lower respiratory tract infections, it is important to look at three different groups of patients:

i) Patients with community-acquired infections;

Common agents of community-acquired lower respiratory tract infections include pneumoniae, especially in the elderly; *K. pneumoniae*, especially in alcoholics; *Mycoplasma pneumoniae*, especially in school-age students through young adulthood; *S. pneumoniae*, *H. influenzae*, *S. aureus*, and *Moraxella catarrhalis* may specifically cause bronchitis and/or pneumonia secondary to viral pneumonia in adults.

ii) Patients with nosocomial infections;

Nosocomial pneumonia due to methicillin-resistant *S. aureus* and multi-drug-resistant gram-negative bacilli such as *P. aeruginosa* is a common problem in intubated patients. The potential for outbreaks of pneumonia due to *Legionella* spp. is a constant threat because of this bacterium's ability to survive within hospital water and air conditioning systems.

iii) Patients with underlying lung disease and immunocompromised individuals, especially those with AIDS

Patients with chronic obstructive pulmonary disease brought on by smoking frequently develop bronchitis. *S. pneumoniae*, *Moraxella catarrhalis*, *H. influenzae*,

and *P. aeruginosa* are frequent causes of this type of infection. Chronic airway infections are primarily responsible for the premature death of patients with cystic fibrosis. *S. aureus* and mucoid *P. aeruginosa* are the most important agents of such chronic airway disease.

3.7.1 Laryngotracheal bronchitis or croup

Membranous laryngotracheobronchitis is characterized by diffuse inflammation of the larynx, trachea, and bronchi with adherent or semiadherent mucopurulent membranes in the subglottic trachea and in the upper trachea distal to the conus elasticus. Syndrome is characterized by Barklike cough, Hoarseness, Inspiratory stridor, Respiratory distress of varying severity. It is preceded by coryza and low grade fever for 12 to 72 hrs. Bacterial tracheitis, laryngotracheo bronchitis, and laryngotracheo bronchopneumonitis are usually due to a primary viral infection with secondary bacterial growth. The most common bacteria implicated are *Staphylococcus aureus, Streptococcus pneumoniae, Hemophilus influenzae, and Moraxella catarrhalis*. (Cherry 2008)

3.7.2 Bronchiolitis

Bronchiolitis occurs predominantly in the first year of life and with decreasing frequency in the second and third years. The clinical features are rapid breathing and lower chest wall indrawing, fever in one-third of cases, and wheezing. Inflammatory obstruction of the small airways, leads to hyperinflation of the lungs, and collapse of segments of the lung occur. RSVs are the main cause of bronchiolitis worldwide and can cause up to 70 or 80 percent of LRIs during high season (Cherian 1988).

3.7.3 Acute Bronchitis (Chest Cold)

Bronchitis is produced by an excessive mucus production with a productive cough. Other signs and symptoms could just be colds associated with sputum production, hoarsening, and dyspnoea, which take progressively longer to resolve.

Acute bronchitis is characterized by a persistent cough and occasionally fever and chest pain. It often follows an upper respiratory tract infection, is self-limiting and typically lasts 7-14 days. In previously healthy patients bacterial infection is

uncommon. If the illness is severe or persists longer than seven days, then the bacterial infection secondary to a viral infection can be assumed. Most cases of acute bronchitis (95% by some estimates) are caused by viruses. Viruses, especially influenza virus, cause the vast majority of cases in studies that establish an etiology. *Bordetella pertussis,* the agent of whooping cough, is now recognized as a cause of acute bronchitis in adults. Infection by either *Mycoplasma pneumoniae or Chlamydia pneumoniae* accounts for many of the stubborn cases in which symptoms fail to resolve or recur soon after treatment has been discontinued (Bartlett 2001). *S. pneumoniae, H. influenzae,* and *M. catarrahalis* cause chest cold in otherwise-healthy persons is unclear, but there is little support for the concept of "acute bacterial bronchitis" as a community-acquired disease.

3.7.4 Acute Infectious Exacerbations of Chronic Bronchitis

Chronic bronchitis is defined by the American Thoracic Society (ATS) as excessive sputum production with cough, present on most days for at least 3 months a year and not less than 2 successive years, without an underlying etiology such as tuberculosis or bronchiectasis. This common disorder, affecting up to 25% of the adult population, can lead to full-blown chronic obstructive pulmonary disease (COPD), the fourth-leading cause of death in the United States. Viruses have been found in as few as 7% to as many as 64% of cases. Cultures of sputum often show non-typable strains of *H. influenzae*, *S. pneumoniae*, and/or *M. catarrhalis*. Evidence suggests that repeated episodes of bacterial infections especially when caused by *H. influenzae* contribute to deterioration of pulmonary function. *S. aureus* and aerobic gram-negative rods occasionally cause exacerbations of chronic bronchitis. The pathogens associated with "atypical pneumonia" such as *M. pneumoniae, C. pneumoniae,* and *L. pneumophila* probably cause fewer than 10% of exacerbations. (Dalvi 1983)

3.7.5 Pneumonia

Both bacteria and viruses can cause pneumonia. Bacterial pneumonia is often caused by *S. pneumoniae* or *H. influenzae,* mostly type b (Hib), and occasionally by *S. aureus* or other streptococci. Other pathogens, such as *M. pneumoniae* and *C. pneumoniae,* cause atypical pneumonias. Predisposing factors to bacterial and viral

pneumonia include chronic illness and debilitation, cancer particularly lung cancer, abdominal and thoracic surgery, atelectasis, common colds, or other respiratory infections. Microorganisms can enter the lungs by aspiration, inhalation, or by way of the bloodstream (hematogenous pneumonia).

The five cardinal symptoms of early bacterial pneumonia are coughing, sputum production, thoracic chest pain, shaking chills, and fever. Physical signs vary widely ranging from diffuse, fine rales to signs of localized or extensive consolidation and pleural effusion. Complications include respiratory failure, pleural effusion, empyema, lung abscess, and bacteremia.

Pneumonia accounts for an estimated 45,000 deaths in the United States each year. It is the 6th most common cause of death and the most common infectious cause of death. Estimates suggest that 4 million cases occur each year, prompting 10 million physician visits and 600,000 to 1.2 million hospitalizations and adding $23 billion to health care costs. However, the mortality rate is 1% or less for patients managed as outpatients versus 14% to 25% for those admitted to the hospital. Vuori-Holopainen and Peltola's (2001) review of several studies indicates that *S. pneumoniae* and Hib account for 13 to 34 percent and 1.4 to 42.0 percent of bacterial pneumonia, respectively.

Types of Pneumonia

(A) Acute Community-acquired Pneumonia (CAP):

The microbial cause of community-acquired pneumonia is usually difficult to determine. In prospective studies of patients requiring hospitalization, a cause is found in only 40% to 70% of cases. In primary care practice, a far greater fraction of cases are never diagnosed. Published data concerning the causes of pneumonia vary from one region to another, but some generalizations are possible. *S. pneumoniae* is still considered the major cause of CAP. *S. aureus* pneumonia, when community-acquired, tends to be an acute, fulminant about 1% of cases, except during influenza epidemics. *K. pneumoniae* is a relatively common cause of pneumonia in patients suffering from alcoholism. The relative contribution of agents such as *M. pneumoniae* and *C. pneumoniae* depend upon the published series, whether there was a community

outbreak at the time of the study, and the diagnostic method used (Bartlett 2000). Adults with compromised host defenses are likely to have pneumococcal pneumonia, but can also have pneumonia due to *H. influenzae*, *M. catarrhalis*, *S. aureus*, or aerobic gram-negative rods. Classical bacterial pneumonia begins with sudden onset of fever, chills, pleuritic chest pain, and productive cough. Chills occur in about 50% and chest pain in about 30% of patients. The respiratory rate is usually increased. Atypical pneumonia, on the other hand, usually begins gradually. (Fang 1990)

(B) Nosocomial Pneumonia

Pneumonia is the most frequent nosocomial infection (30 to 33% of cases) among combined medical-surgical intensive care units participating in the National Nosocomial Infections Surveillance System. In the intensive care unit setting, 83% of cases of pneumonia are associated with mechanical ventilation. *S. aureus* is the most frequently reported isolate at 17%. Fifty-nine percent of reported isolates are aerobic gram-negative species, the most common of which is *P. aeruginosa* (15.6%), followed by *Enterobacter* species (10.9%) and *K. pneumoniae* (7.0%). Frequently, infection is polymicrobial (Richards 2000).

Risk is increased by the presence of underlying disease and by various interventions and procedures. Patients with critical illnesses requiring prolonged mechanical ventilation are susceptible to multi-resistant *P. aeruginosa* and *Acinetobacter* species. Aerobic Gram-negative bacilli, including members of the Enterobacteriaceae (such as *Klebsiella* and *Enterobacter* species) and *P. aeruginosa* are implicated in up to 60% of cases (Donowitz 2000). Intravascular catheters and nasal carriage are risk factors for pneumonia caused by methicillin resistant *S. aureus* (MRSA). Although qualitative culture and Gram stain of endotracheal sputum samples are the least invasive tests, they have the same pitfalls for hospitalized patients as for patients in the community, that is, poor predictive values. Both pathogens and nonpathogens alike may be recovered. (Celis 1988)

3.8 Pathogen specific information:

3.8.1 *Streptococcus pneumoniae:*

S. pneumoniae is an example of a typical extracellular bacterial pathogen. Pathogenicity requires adherence to host cells, along with the ability to replicate and to escape clearance and/or phagocytosis. Under normal conditions in a healthy host, anatomic and ciliary clearance mechanisms prevent clinical infection. However, clearance may be inhibited by chronic (smoking, allergies, bronchitis) or acute (viral infection, allergies) factors, which can lead to infection. Alternatively, pneumococci may reach normally sterile areas, such as the blood, peritoneum, cerebrospinal fluid, or joint fluid, by hematogenous spread after mucosal invasion.

S. pneumoniae is considered the most frequent bacterial agent of pneumonia acquired in the community. Approximately 500,000 to 1,000,000 cases of pneumococcal pneumonia with 50,000 deaths are estimated to occur in the United States each year. In developing countries, approximately 1,000,000 deaths per year are estimated in children under 5 due to pneumococcal pneumonia (Facklam 1991). Although exact rates are difficult to determine, the World Health Organization (WHO) estimates that, worldwide, 1.6 million deaths were caused by pneumococcal disease in 2005, with 700,000 to 1 million of these occurring in children younger than 5 years (WHO 2007). Even in patients in developed countries, invasive pneumococcal disease carries a high mortality rate—an average of 10-20% in adults with pneumococcal pneumonia, with much higher rates in those with risk factors for disease (Rudan 2009).

Risk factors for invasive pneumococcal disease include extremes of age, alcoholism, HIV disease, end-stage renal disease, sickle cell disease, diabetes mellitus, dementia, malnutrition, malignancies, diseases affecting B lymphocyte function and immunosuppressive disorders. As classically described by previous generations of clinicians, *S. pneumoniae* causes a lobar pneumonia with by the sudden onset of fever with a single, hard-shaking chill, cough productive of rusty-colored mucopurulent sputum, and pleuritic chest pain. An increasing percentage of strains are becoming resistant to several antimicrobials, especially penicillin, as reported from both industrialized and developing countries. In addition to decreased

susceptibility to penicillin, resistance to chloramphenicol, erythromycin, sulfamethoxazole-trimethoprim, tetracycline, and other drugs has also been reported (Facklam 1991).

3.8.2 Beta- haemolytic streptococci:

Group A streptococcal (*Streptococcus pyogenes*) pharyngitis is one of the most frequent bacterial infections, especially in the pediatric age group. Peak incidence is in early school age (5-8 years of age), although all groups are susceptible. *S. pyogenes* pneumonia is also uncommon except during influenza epidemics. This pneumonia is often accompanied by the rapid development of large empyemas. β-haemolytic colonies of Gram-positive, catalase negative cocci can be presumptively identified as *S. pyogenes* by using the bacitracin disk or PYR tests (Facklam 1991). Serological grouping using anti-serum specific for group A carbohydrate allows the confirmatory identification of the isolate. Group B, C, F, G beta haemolytic streptococci have less pathogenicity in respiratory infections

3.8.3 *Staphylococcus aureus:*

Staphylococcus aureus is a common human pathogen, easily grown in the laboratory, that asymptomatically colonizes approximately 30% of Americans (Mainous 2006). Over the past several decades, *S. aureus* has gained increasing recognition for its role as a cause of both community acquired (CAP) and healthcare-associated pneumonia (HAP). Infections by *S. aureus* have increased in both the community and hospital settings over the past 20 years. Data from the National Nosocomial Infections Surveillance (NNIS) system of the Centers for Disease Control and Prevention from January 1990 through May 1999 show that *S. aureus* was the most common cause of nosocomial pneumonia. Additionally, the proportion of *S. aureus* infections caused by methicillin- resistant *S. aureus* (MRSA) had dramatically increased. This increase in the number of MRSA strains may be a consequence of selective use of antimicrobials, such as cephalosporins and vancomycin.

A recent investigation of culture-positive CAP and HAP, employing a database of 4,543 patients admitted to almost 60 U.S. hospitals in 2002-2003 found that *S. aureus* comprised >25% of cases of CAP and >40% of those with HAP (Kaye 1990). The epidemiology of MRSA in Europe is different than the United States, and varies

widely depending on the country, ranging from 0.6% in Sweden to an average of 41% in Belgium, Greece, Ireland, and the United Kingdom (Sader 2006). Among patients with VAP, MRSA is more likely to cause pneumonia late in the course of mechanical ventilation. MRSA pneumonia was associated with significantly higher mortality rates in several early studies (Rello 1994).

3.8.4 *Klebsiella pneumoniae*:

K. pneumoniae is a relatively common cause of pneumonia in patients suffering from alcoholism and with underlying diseases, such diabetes and chronic lung disease. In some parts of the world, *K. pneumoniae* is an important cause of community-acquired pneumonia in elderly persons. Studies conducted in Malaysia and Japan estimate the incidence rate in elderly persons to be 15-40%, which is equal to, if not greater than, that of *H. influenzae*. However, in the United States, these figures are different. Persons with alcoholism are the main population at risk, and they constitute 66% of people affected by this disease. Mortality rates are as high as 50% and approach 100% in persons with alcoholism and bacteremia. *Klebsiella* are also important in nosocomial infections among adult and pediatric populations. *Klebsiella* account for approximately 8% of all hospital acquired infections. In the United States, depending on the study reviewed, they comprise 3-7% of all nosocomial bacterial infections, placing them among the top 8 pathogens in hospitals.

According to the data reported to the Centers for Disease Control (CDC) by hospitals participating in the National Nosocomial Infections Study (NNIS) to describe the epidemiology of endemic *K. pneumoniae* infections, in the 8-year period from 1975 through 1982 the nosocomial *K. pneumoniae* infection rate was 16.7 infections per 10,000 patients discharged. The rate of infection at medical school-affiliated hospitals was significantly greater than at non-affiliated hospitals.

Klebsiella pneumonia has a high mortality rate of approximately 50% even with antimicrobial therapy. The mortality rate approaches 100% for persons with alcoholism and bacteremia. In neonatal units, outbreaks caused by ESBL producing strains present a more serious problem and may be associated with increased mortality (Obiamiwe Umeh, American Medical Association). Over the past 10 years, a

progressive increase in Carbapenem-Resistant *K. pneumoniae,* CRKP has been seen worldwide. (Berrie 2007).

Long-chain lipopolysacharide (LPS) protects strains from the action of serum complement, and polysaccharide capsules are thought to confer protection against phagocytosis. In common with other members of the Enterobacteriaceae, *Klebsiella* express type 1 fimbriae that exhibit mannose-sensitive haemagglutination. Type 3 fimbriae also cause haemagglutination of erythrocytes pretreated with tannin.

Clinical isolates of *Klebsiella* spp. characteristically produce a β-lactamase that renders them resistant to Ampicillin, Amoxicillin and other Penicillins, but combinations of these drugs with β-lactamase inhibitors such as Clavulanic acid are usually effective.

3.8.5 *Pseudomonas aeruginosa:*

Pseudomonas is motile, gram-negative rods, is an opportunistic pathogen that is a common cause of hospital-acquired infections, particularly infecting patients with predisposing factors, such as burn victim, immunocompromised hosts, or those with metabolic disorders. In cystic fibrosis (CF) patients, *P. aeruginosa* is believed to be a major contributory factor to chronic lung infections, which could form biofilm and adhere to human mucin in the lower respiratory tract. *P. aeruginosa* is a common nosocomial pathogen that can cause serious human infections and is the most prevalent respiratory pathogen among patients suffering from cystic fibrosis (CF) (Forbes 2002).

P. aeruginosa is responsible for 10–15 % of nosocomial infections worldwide (Blanc 1998). *P. aeruginosa* represents a phenomenon of antibiotic resistance, demonstrating practically all known enzymic and mutational mechanisms of bacterial resistance. These mechanisms are often present simultaneously, conferring combined resistance to many strains (McGowan 2006).

3.8.6 *Acinetobacter* spp.

Acinetobacter is a gram-negative coccobacillus that during the past three decades has emerged from an organism of questionable pathogenicity to an infectious agent of importance to hospitals worldwide (Fournier 2006). In humans, acinetobacter

can colonize skin, wounds, and the respiratory and gastrointestinal tracts. Acinetobacter was cited as the cause of 17% of cases of ventilator-associated pneumonias in a Guatemalan ICU, second only to *Pseudomonas*, which caused 19% of cases — years before becoming a concern in ICUs in the United States (Berg 2001). The most frequent clinical manifestations of acinetobacter infection are ventilator-associated pneumonia and bloodstream infections. Vascular catheters and the respiratory tract have been the most frequent sources of acinetobacter bacteremias, (Cisneros 1996).

3.8.7 *Enterobacter* spp.

E. aerogenes is important nosocomial pathogens responsible for various infections, including bacteremia, lower respiratory tract infections and other numerous infections. Most of the infections that are caused by *E. aerogenes* result from specific antibiotic treatments, venous catheter insertions, and surgical procedures. Specific risk factors for infection with nosocomial multidrug-resistant strains of *Enterobacter* species include the recent use of broad-spectrum cephalosporins or aminoglycosides and ICU care. The National Healthcare Safety Network (NHSN) reported on healthcare-associated infections (HAI) between 2006 and 2007. They found *Enterobacter* species to be the eighth most common cause of HAI (5% of all infections) and the fourth most common gram-negative cause of HAIs (Hidron 2008).

Previous reports from the National Nosocomial Infections Surveillance System (NNIS) demonstrated that *Enterobacter* species caused 11.2% of pneumonia cases in all types of ICUs, ranking third after *S. aureus* (18.1%) and *P. aeruginosa* (17%).

3.8.8 *Haemophilus influenzae:*

H. influenzae is a Gram-negative, pleomorphic coccobacillus with fastidious growth requirements. Nonencapsulated strains are normal inhabitants of the nasopharynx and rarely cause bacteremic diseases or pneumonia in children, although they are a major cause of otitis media, sinusitis, and upper respiratory mucous infections. These strains are also an important cause of lower respiratory tract infections in patients with cystic fibrosis. It is reported that 30% to 50% of all children

carry the bacillus asymptomatically in the nasopharynx, generally as avirulent, non-encapsulated organism (Munson 1989).

H. influenzae is considered the second most significant cause of bacterial pneumonia in several parts of the world. One of the most striking features of *H. influenzae* infections is the relationship between age and susceptibility. Invasive infections predominate during the age of relative humoral immunodeficiency (3 months to 3 years). The incidence of pneumonia is higher in children younger than 5 years of age, with a peak between 4 and 7 months of age. *H. influenzae* is a frequent cause of pneumonia in elderly patients and in patients with serious underlying diseases including chronic obstructive lung disease (Woodhead 1980).

More than 95% of the invasive infections are associated with type b encapsulated *H. influenzae*, which accounts for a third of all bacterial pneumonias among 4-month to 4-year old children. The incidence of this bacterium is reported to be 5 – 10 cases per 100,000 persons in USA of which 95% are caused by type b (Kilian M 1991).

Resistance to ampicillin in *Haemophilus* is primarily due to the production of β-lactamase and, therefore, can be easily and quickly detected by a β-lactamase production test. Of particular concern has been the finding of strains resistant to both ampicillin and chloramphenicol in different parts of the world.

3.8.9 *Klebsiella oxytoca:*

Klebsiella oxytoca may cause infections of the respiratory tract. These infections may be community-acquired or sometimes a person may develop the infection while he is a patient in a hospital. According to information from the March 1983 journal "Thorax," *Klebsiella oxytoca* may also cause respiratory infections such as pneumonia. Symptoms include coughing, chest pain, fever, shortness of breath and general weakness. *Klebsiella oxytoca* infections may be resistant to common antibiotics, and in that case long-term treatment with more powerful drugs is sometimes needed to cure the patient.

3.8.10 *Citrobacter freundii:*

As an opportunistic pathogen, *C. freundii* is responsible for a number of significant opportunistic infections. *C. freundii* represents approximately 29% of all opportunistic infections. They only affect patients with a weak immune system, signifying that they need an "opportunity" to infect the person. Therefore, in patients with a suppressed immune system, *Citrobacter* species are known to cause a wide variety of nosocomial infections of the respiratory tract, urinary tract, and the blood (Whalen 2007).

3.8.11 *Proteus mirabilis:*

P. mirabilis is a rod-shaped, Gram negative bacterium that is an opportunistic pathogen of humans. *P. mirabilis* are easily isolated from individuals in long-term care facilities and hospitals and from patients with underlying diseases or compromised immune systems.

3.8.12 *Moraxella catarrhalis:*

M. catarrhalis is an important cause of LRTI, particularly in adults with chronic obstructive pulmonary disease, (Hager 1987). In immunocompromised host, the bacterium can cause a variety of severe infections including pneumonia. In addition, hospital outbreaks of respiratory disease due to *M. catarrhalis* have been described, establishing the bacterium as a nosocomial pathogen.

3.8.13 *Mycoplasma pneumonia:*

Various investigators have determined this microorganism to be the cause of 13% to 27% of community-acquired pneumonias. It can also cause hospital-acquired pneumonias, and it has caused as many as 50% of pneumonias during epidemics in closed populations. The disease occurs in all age groups including toddlers and the elderly but peaks between ages 5 to 15 years (Mansel 1989).

3.9 Complications of LRTI:

3.9.1 Lung Abscess

It is a condition in which localized cavity is filled with pus resulting from tissue necrosis, with surrounding pneumonitis, localized necrotic lesion of the lung parenchyma containing purulent material. Aspirations of upper airway anaerobic organisms, inadequately treated pneumonia (especially *S. aureus, K. pneumoniae*), bronchial obstruction (tumour, foreign body), pulmonary infarction, septic emboli are the etiological and pathophysiological factors for Lung Abscess.

Acute or insidious with early symptoms like pneumonia, purulent sputum, may be blood streaked, putrid odor anaerobes, weight loss, anemia, clubbing chronic abscess, physical signs of consolidation are the signs and symptoms seen.

3.9.2 Bronchiectasis

Bronchiectasis is a structural derangement of the bronchial wall that is characterised by airway dilatation and bronchial wall thickening. As a result of this abnormality, chronic inflammation and airway colonisation are characteristic findings. Tuberculosis, pneumonia, Ig deficiencies, bronchopulmonary aspergillosis and cystic fibrosis (CF) are the main causes of bronchiectasis.

Bronchiectasis is more common among children in lower socioeconomic classes and in developing countries, presumably due to more frequent and recurrent respiratory infections, environmental airway irritants, poor immunization rates, and malnutrition.

Chronic cough, purulent sputum (but 10-20% have a dry cough), hemoptysis (can be massive), recurrent pneumonia, local crackles (inspiratory and expiratory), wheezes clubbing are the symptoms seen. The most frequent microorganisms isolated are *H. influenzae and Pseudomonas* spp. (Cabello H 1997). Other Gram positive bacteria, such as *S. pneumoniae* and *S. aureus*, may also colonize the lower airways of these patients. It has been demonstrated that patients with bronchiectasis colonized by *Pseudomonas* spp. exhibit more extensive lung lesions, suffer from more severe

impairment of lung function, and have a more intense inflammatory response in the lung (Evans SA 1996).

3.9.3 Empyema

It is a condition of pus in pleural space or an effusion with organisms seen on a gram stain or culture. Contiguous spread from lung infection (most commonly anaerobes), infection through chest wall (e.g. trauma, surgery) are the etiological cause of empyema. Fever, pleuritic chest pain investigations, thoracentesis and PMNs (lymphocytes in TB), ± visible organisms on Gram stain are the signs and symptoms seen.

3.9.4 Persistent Bacterial Bronchitis

Persistent bacterial infection of the conducting airways is a well recognized feature of the progressive bronchiectasis noted in patients with cystic fibrosis and is generally recognized to be an important component of the morbidity associated with other forms of bronchiectasis. The prevalence of persistent bacterial infection of the conducting airways appears to be increasing, probably due to the significant fall in the use of antibiotics to treat young children with acute respiratory tract symptoms (Arnold 2006). Recent reports have identified persistent bacterial bronchitis as the commonest cause of a persistent wet cough in childhood and as a major cause of ‘difficult asthma’(Marchant 2006).

A variety of pathogens, most notably *H. influenzae*, *S. pneumonia* and *M. catarhallis* appear ideally suited to colonise the respiratory tract when presented with impaired mucocillary clearance be it in the middle ear, sinuses or the lower airway. Once established it is likely that these organism can, through quorum sensing, organize themselves into colonies establishing their own biofilms within the mucus lining the airways. As a result a chronic endobronchial infection develops. Infllammatory cells, most notably neutrophils, vainly strive to eradicate the organisms from the airways. Products such as human neutrophil elastase and myeloperoxidases stimulate mucus production and causing collateral damage which further impairs mucocillary clearance. Over a period of time the organisms are likely to extend their areas of influence through release of buds containing organisms that seed new colonies.

3.10 Symptoms seen in LRTI patients

3.10.1 Cough and Fever

Cough, which continues throughout the day and night, often producing green, yellow, brown, or gray mucus (sputum) from the lungs and Fever, which may be high with some lower respiratory system infections, such as pneumonia are observed.

3.10.2 Sputum production

The tracheobronchial tree can produce up to three ounces of sputum, which is 90 cc or 3 ounces per day. Increased sputum production may result in external stimuli or from such internal causes as chronic bronchial infection or a lung abscess. Excess production of sputum that separates into layers may indicate bronchiectasis. Foul smelling sputum may result from an anaerobic infection, such as an abscess. Blood tinged or rust color sputum may result from trauma caused by coughing or from such underlying pathology as bronchitis, pulmonary infection, tuberculosis, and tumors. A color change from white to yellow or green indicates infection.

3.10.3 Haemoptysis

Hemoptysis is the expectoration of blood or blood-tinged sputum from the lungs or tracheobronchial tree. Haemoptysis may present as a life-threatening condition, with a mortality rate reaching 80% in the absence of adequate and prompt management. Associated pulmonary symptoms such as chronic cough with sputum production, change in cough, shortness of breath on exertion, chest pain (especially of a pleuritic nature), and wheezing are important in the evaluation of hemoptysis.

Infarction of lung tissue with hemoptysis can occur in numerous diseases. Pulmonary emboli often present with hemoptysis as a result of ischemic pulmonary parenchymal necrosis. Infections causing blood vessel invasion with infarction include primarily *S. aureus, P. aeruginosa.* (Holmes 2001)

3.10.4 Dyspnoea

Dyspnoea from activities suggests poor ventilation or profusion, or insufficient breathing mechanisms. Wheezing sounds result from small airway obstructions (for

example, from aspirated foreign body, a tumor, asthma, or congestive heart failure). Stridor results from tracheal compression or laryngoedema.

The disorder is a result of an imbalance between the respiratory drives that originate from the complex respiratory center and the responses of the cardiopulmonary systems. Congestive heart failure (CHF) is probably the most common cause of dyspnoea on exertion and of paroxysmal nocturnal dyspnoea (PND). The next most common causes of dyspnoea are diseases of the lungs that are characterized by airflow obstruction, such as bronchial asthma, chronic obstructive pulmonary disease (COPD), and a wide variety of less common obstructive diseases (Manning 1995).

3.10.5 Local hyperemia

It is seen in the common bacterial pneumonias such as *S. pneumoniae.* Vascular congestion is followed by red hepatization of the lung where alveoli are filled with blood-tinged fluid and bacteria to which neutrophils and fibrin are added.

3.10.6 Chest pain

Chest pain in disease of the respiratory system usually originates from involvement of the parietal pleura. Most conditions giving rise to pleuritic pain are acute and inflammatory on origin: either infective when there is usually associated pneumonia.

3.11 Diagnosis of LRTI

3.11.1 Clinical Diagnosis

The chest x-ray visualizes the appearance and status of the entire respiratory system. A CAT scan or MRI locates a specific area of the lung to be X-rayed and it shows in more detail that particular area. A bronchoscopy is used to directly examine the larger airways or the tracheo-broncho tree. Pulmonary function studies (PFT'S) evaluate ventilation (respiratory) function of the lungs and the chest wall and can help in diagnosing pulmonary disorders. (Melbye H 1992)

Acute Pneumonia

Clinical symptoms of pneumonia are not specific. Due to the poor predictive value of the symptoms, clinical and radiological examinations must be repeated to confirm the diagnosis and monitor progression. A CT-scan may be useful.

Nosocomial Pneumonia

Diagnosing these nosocomial infections becomes difficult when there are multiple concomitant pathologies. Nosocomial pneumonia acquired under mechanical ventilation should be suspected if there is fever or hypothermia, hyperleukocytosis or leukopenia, if secretions are purulent and there is a decline in respiratory gas values, with a new or extensive infiltrate on the chest X-ray. (American Thoracic Society 2001)

3.11.2 Microbiological Diagnosis

i) Non-Invasive Tests: Sputum Gram stain and culture

Gram stain provides a quick pointer for treatment (e.g. Gram positive diplococci suggest the presence of Pneumococci). After homogenization and microscopic examination the appropriate specimen is cultured in MacConkey agar, Blood agar and Chocolate agar.

ii) Invasive Tests

Invasive diagnostic techniques enable a better quality of sample to be obtained, as contamination by oropharyngeal flora is limited. They are recommended in cases of pneumonia in ventilated or immunocompromised patients. (Reimer 1998)

3.11.3 Other Laboratory Tests

- **Blood cultures**: high specificity for certain microorganisms, low sensitivity.
- **Serological testing** of intracellular microorganisms (*M. pneumoniae, C. pneumoniae, and L. pneumophila*) is essentially of epidemiological interest because of delayed results.
- **Rapid tests** for detecting viruses: influenza, RSV, adenovirus, etc.

The urinary pneumococcal antigen test: In adults, its sensitivity is around 80% in cases of bacteremic pneumonia, but only around 50% in non-bacteremic cases. It has a high positive predictive value. This test provides a rapid diagnosis, which is not rendered negative by a 7-day antibiotic treatment and the presence of antigen persists for several weeks. In children, its interpretation is more difficult, due to the frequency of pneumococcal infections/carriers at this age and the period of antigenuria. It does however have a good negative predictive value.

• **Molecular testing:** Based on PCR type amplification techniques and on real-time detection, these tests increase detection sensitivity (e.g. when compared with rapid tests) and reduce time-to-result to 3 or 4 hours (compared with more than 2 days with culture-based techniques). They are used for detecting "atypical" microorganisms (*M. pneumoniae, C. pneumoniae, and L. pneumophila*), RSV, *Bordetella pertussis*, influenza and other viruses, as well as emerging pathogens: SARS, avian influenza, etc. (Carrol 2002)

3.12 Antibiotic susceptibility in LRTI

Bacterial ALRI is easily treatable with antibiotics, whereas more severe cases of ALRI require hospitalization for intravenous antibiotics, oxygen therapy or inpatient supportive care. Although many children do not receive adequate care for ALRI, others with similar symptoms but without antibiotic-treatable infection receive unnecessary therapy. Over prescription of antibiotics by both qualified and unqualified medical practitioners is common in developing countries, and self medication through the purchase of antibiotics from drug vendors and pharmacies is also widespread. The overuse of antibiotics has increased resistance among common (Okeke 2005).

Eradication of the causative agents of respiratory tract infections is recognized as a requirement (Dagan 2001), however during the last few years, the increase in the rate of antibiotic resistance amongst the major microbial causes of the respiratory infections in the community has comprised the selection of empirical treatment for some respiratory tract infections (Gonzalo 2004). The consequences of increased drug resistance are far reaching since bacterial infection of the lower respiratory tract is a major cause of death due to infectious disease (Kumari 2007).

3.13 Antibiotic resistance

Drug resistance is condition in which infecting bacteria can resist the destructive effects of drugs such as antibiotics and sulfa drugs. Drug resistance is the inability of a drug to bring about an effect on a disease-causing agent that occurred previously in the presence of that same medication. In the United States, *S. pneumoniae*, a common cause of pneumonia, bronchitis, ear infections, and other conditions, was universally sensitive to penicillin prior to 1990. As of June 1999, however, penicillin was either no longer effective or was required in higher than previously effective doses to treat about 25–35 percent of all *S. pneumoniae* isolates.

ALRI-causing bacteria, such as *S. pneumoniae* and *H. influenzae* type b. The former was once universally sensitive to penicillin, but is now only <50% sensitive in many countries and <25% sensitive in some. Moreover, multiple drug resistance is increasing; for example, 25.5% of *S. pneumoniae* strains in South Africa, 53.2% in the Far East and 21.1% in Mexico are resistant to any three classes of drugs, excluding penicillin, from among the following: β-lactams, macrolides, tetracyclines, phenicols, folate-pathway inhibitors and quinolones. For *H. influenzae* type b, resistance rates to penicillin and amoxicillin of 30–50% have been reported in Indonesia, Singapore, Thailand, and Vietnam and ≤50% in hospital isolates in Guatemala (Okeke 2005).

3.13.1 Mechanisms of resistance

In supposedly well-regulated human medicine the major problem of the emergence of resistant bacteria is due to misuse and overuse of antibiotics by doctors as well as patients (WHO 2002). Also unsound practices in the pharmaceutical manufacturing industry can contribute towards the likelihood of creating antibiotic resistant strains.

The four most important antibiotic resistance mechanisms are alteration of the target site of the antibiotic, enzyme inactivation of the antibiotic, active transport of the antibiotic out of the bacterial cell, and decreased permeability of the bacterial cell wall to the antibiotic. Alteration of the target site is the mechanism for one of the most problematic antibiotic resistances worldwide, methicillin resistance among *S. aureus*. The most common mechanism by which bacteria are resistant to antibiotics is by producing enzymes that inactivate the drugs. For example, β-lactam antibiotics

(penicillins and cephalosporins) can be inactivated by enzymes known as β-lactamases. Active transport systems (efflux pumps) have been described for the removal of some antibiotics (such as tetracyclines, macrolides, and quinolones) from bacterial cells. The resistance that *P. aeruginosa* exhibits to a variety of penicillins and cephalosporins is mediated by an alteration in porin proteins.

In the case of MRSA, increased rates of MRSA infections are seen with glycopeptides, cephalosporins and especially quinolones (Muto 2003). High resistance rates are prevalent in Europe: 25-50% in hospitals in the UK and most of southern Europe, 2-5% in northern Europe.

3.13.2 Multidrug resistance

Multiple drug resistance is a condition enabling a disease- causing organism to resist distinct drugs or chemicals of a wide variety of structure and function targeted at eradicating the organism. Multidrug-Resistant Organisms (MDROs) are defined as microorganisms that are resistant to two or more classes of antimicrobial agents. • Three most common MDROs are: 1. Methicillin-Resistant Staph aureus (MRSA) 2. Vancomycin Resistant Enterococci: (VRE) 3. Extended Spectrum Beta-Lactamase producing Enterobacteriaceae (ESBLs). (CDC: Jane D)

The incidence of Gram-negative pathogens resistant to multiple antibiotics and multiple classes of antibiotics is increasing and the resultant deficit in effective therapeutic agents emphasizes the urgent need for novel agents and novel therapeutic approaches to the treatment of Gram-negative infectious disease. Multiple antibiotic resistances (MAR) occur as a result of the accumulation of multiple mutations and/or resistance genes (Livermore 2002), though single mutations can also promote multidrug resistance. Increasingly, multidrug resistance in Gram-negative bacteria results from the acquisition of multiple resistance genes on mobile DNA elements such as plasmids or transposons, often as part of an integron, a genetic element that is particularly adept at recruiting resistance genes for recent reviews of integrons. Integrons have been implicated in the multidrug resistance of a number of Gram-negative pathogens, including *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., *P. aeruginosa* spp., *Acinetobacter* spp., *Salmonella* spp., and *Citrobacter* spp. Infections due to multidrug resistant Gram-negative pathogens are associated with excess morbidity and mortality (Leverstein 2002).

CHAPTER - IV

4. MATERIALS AND METHODS

4.1 Study subjects and specimens

It was a prospective study comprising 214 cases of suspected patients of LRTI from 15th April, 2010- 14th July, 2010. This study was conducted to find out the bacteriological profile and their antibiotic sensitivity pattern in suspected patients of LRTI. The study population, were patients who attended the Bir Hospital, Kathmandu, Nepal, either as inpatient or outpatient with symptoms suggestive of RTIs. All sputum samples taken from patients clinically suspected as having LRTI as determined by the treating doctors were included in the study.

4.2 Selection of sample: Expectorated sputum

Inclusion criteria:

Patients were diagnosed by the clinician concerned depending upon the presence of two of the following symptoms:

1. Increased cough

2. Increased purulence and/or volume of expectorations

3. Increased severity of dyspnoea.

4. All cases who had evidence of pneumonia or bronchiectasis developed as sequelae of other disease, clinically or on chest radiography and if they had an admission diagnosis of (probable) bronchiolitis, (probable) pneumonia, or respiratory failure.

5. Sputum smears containing less than 10 squamous epithelial cells and more than 25 leucocytes of pus cells per low power field.

Exclusion criteria:

1. Those specimens not fulfilling the criteria of American Society for Microbiology.

Sample rejection: Specimen with more than 10 epithelial cells per low power field indicating saliva contamination; specimen not submitted in appropriate transport

container; improperly labeled specimen; insufficient volume; external contamination. If an unacceptable specimen was received, the physician or nursing station was notified and another specimen was requested before the specimen was discarded.

4.3 Sample collection, transport and processing

Sputum samples were collected from the patients in Wide-mouthed clean sterile containers after rinsing the mouth twice with plain water. In few out-patients second sample could not be collected because of non-compliance of patients. Patient was instructed to take a deep breath and cough up sputum directly into a wide-mouth sterile container avoiding saliva or postnasal discharge. Minimum volume taken was about 1 ml. The sputum samples were collected into well-labeled sterile, wide mouthed containers with screw cap tops as described by Kolawale (2009). On the labels were the name, age, sex, and time of collection. The laboratory request form was completed thereafter.

Handling and transport

Sample was transported as quickly as possible to the laboratory to reduce overgrowth by commensal oral flora. Both microscopic and cultural examinations were routinely performed on homogenized specimens.

Homogenisation of sputum

All sputum samples were processed in an exhaust protective cabinet. Sputum specimens which were very thick and mucoid were homogenized before processing. For this equal volume of Sputasol (dithiothreitol 1.4%) (Oxoid Ltd) was added to the sputum sample. The mixtures were shaken well then incubated at 37^0C for 30 minutes with periodic shaking. (Miller 1999)

4.4 Laboratory methods

4.4.1 Macroscopic examination

This included the visual examination of the sputum specimens for

Consistency: Sputum may be purulent, mucopurulent, mucoid or salivary

Purulent: Green-looking, mostly pus

Mucopurulent: Green-looking with pus and mucus

Mucoid: Mostly mucus

Mucosalivary: Mucus with a small amount of saliva

When the sputum sample received contained mostly saliva, it was reported as "unsuitable for microscopic investigation" and requested for another appropriate sample.

Color: The color of the sputum sample was noted.

Red sputum: It indicates that the sample is contaminated with blood.

Green sputum: Green colored sputum usually contains *P. aeruginosa* as possible pathogens.

Brown sputum: It indicates the air is polluted with CO_2 and CO.

4.4.2 Microscopic examinations

Specimens were examined as soon as possible after collection. Microscopic examination was done by Gram's staining. Gram stains of sputum were assessed for leukocytes, epithelial cells, and bacteria. According to the ASM criteria a reliable LRT specimen has more than 25 leucocytes and fewer than 10 epithelial cells per low power field.

Using a disposable loop, a loopful of homogenised sputum was spread over a glass slide and a Gram-stained film was prepared. The films were examined under low power magnification (X 20 objective) for squamous epithelial cells and under high-power magnification (X 50 objective) for leucocytes and potential pathogens.

4.4.3 Cultural examinations: Culture was done on the following media:

(i) 5% Blood agar (BA) and 5% Chocolate agar in 5% CO_2 (ii) Mac Conkey agar and (iii) Nutrient agar (NA). The inoculum on the plate was streaked out for discrete colonies with a sterile wire loop following standard procedures (Cheesbrough 2006).

Culture plates were incubated at 37°C for 24 hrs and observed for growth through formation of colonies. All the bacteria were isolated and identified using

morphological, microscopy and biochemical tests following standard procedures described by Cowan and Steel (1974) and Cheesbrough (2006).

To facilitate recognition of the presence of *S. pneumoniae,* a 5 µg optochin disk was placed directly on the primary streaking area of the 5% BA plate incubated in CO2 jar; similarly a 10 IU of bacitracin disc was placed on the chocolate agar plate to aid detection of beta-haemolytic streptococci. A plate bile test was performed on all specimens by adding a drop of 2% bile to an area where lysis of discrete alpha-hemolytic colonies could be observed on the BA plate. In addition, one to two individual alpha-hemolytic colonies were subcultured to 5% BA plates for performance of the optochin test as recommended, whether or not they grossly resembled typical pneumococcal colonies. All isolates from both sputum were finally identified as *S. pneumoniae* by optochin susceptibility with pure cultures and a zone of inhibition >15 mm. On BA plates streaking with *S. aureus* was done to facilitate growth of *H. influenzae* and incubated at 37°C in a CO_2 enriched atmospher for overnight for Satellitism Test. *H. influenzae* was also identified by its requirements for factors X and V using factor identification discs. Gram stain reporting was done according to Bartlett's grading system and culture isolates were identified according to standard techniques.

4.5 Antibiotic susceptibility testing:

Antibiotic sensitivity for the pathogenic organisms isolated in culture was done by Kirby-Bauer method according to CLSI standards using antibiotics containing discs of Hi media Laboratories Ltd. Culture was standardized according to the methods described by the Clinical and Laboratory Standard Institute (Wayne 2007). The 24 hrs culture of each organism was suspended into sterile bottles containing 5 ml nutrient broth and incubated for 2-4 hrs at 37°C. Normal saline was gradually added so as to compare its turbidity to McFarland Standard of 0.5 which corresponded to approximately 1.0×10^8 cfu/ml.

Antibiotic discs were placed on a plate containing a Mueller-Hinton agar that has been swabbed uniformly with a standardized broth suspension of a pure culture of the bacteria to be tested and they were then left at room temperature for 3-5 mins to allow diffusion of the antibiotics into the agar medium. The plates were then

incubated at 37°C for 24 hours. The commercial antibiotics discs and the concentrations used were ampicillin (10 µg), ciprofloxacin (5 µg), co-trimoxazole (25 µg), chloramphenicol (30 µg), erythromycin (15 µg), gentamicin (10 µg), levofloxacin (5 µg), cefotaxime (30 µg), cephalexin (30mcg), amikacin (30mcg), cloxacillin (5 µg). Zones of growth inhibition were then measured to the nearest millimeter and recorded. The mean of triplicate results was taken as the zone diameter. Isolates were classified as either resistant or intermediate sensitive or sensitive based on the standard intermediate chart updated according to the standard of the Clinical and Laboratory Standard Institute (2007). An isolate was considered multi-drug resistant if it was resistant to at least two of the antibiotics classes (Santos 2007). Interpretation of results was done using zone sizes. Some laboratory strains of known sensitivity of *Staphylococcus aureus* and *Pseudomonas aeruginosa* were used as quality control strains for the antimicrobial discs.

4.6 Identification of isolated organisms

Following microbiological techniques were performed for identification of isolates.

Study of colony morphology/cultural characteristics

Study of colony morphology involved the observation of following characteristics:

Shape, Size, Elevation, Margin, Pigmentation, Haemolysis, Opacity, Consistency

Biochemical tests

Following biochemical tests were performed for the identification of the isolates [Appendix IV]

Catalase test	Citrate-utilization test
Oxidase test	Sulphur Indole Motility Test
Coagulase test	Bile solubility test
Indole test	Oxidation- fermentation test
Triple-sugar iron test	Urease test

Some other specific tests: Satellitism test, Optochin sensitivity test, Bacitracin sensitivity test, X, V and XV factor discs test.

4.7 Purity plate

The purity plate was used to ensure that the inoculation used for the biochemical tests was pure culture and also to see whether the biochemical tests were proceeded in an aseptic condition or not. So while performing biochemical tests, the same inoculum was subcultured in respective media and incubated. Pure growth of organisms both in pre inoculation and post inoculation of the medium was the indication of aseptic condition.

4.8 Quality control: It was applied at different levels during the study.

1. Specimen collection and transport: Patients were given a leak proof container and requested him or her to cough deeply to produce a sputum specimen. While collecting, adequate safety precaution was taken to prevent the spread of infectious organisms. Samples were processed on the day of collection as soon as possible.

2. Microbiological techniques: Sputum specimens were examined in a biological safety cabinet wearing masks and gloves.

3. Culture media: Good performance and reproducibility was ensured by using freshly prepared media, sterility testing and performance testing. For this well characterized and stable strains of bacterial species were used as control organisms.

4. Stains and reagents: Whenever a new batch of stain was prepared, a control smear was stained to ensure correct staining reactions.

5. Equipment: Microscope, incubator, centrifuge, refrigerator, water bath, autoclave, anaerobic jars etc were checked regularly to ensure the correct functioning of equipment for the reliability of results.

6. Recording results: Results were recorded neatly and clearly.

4.9 Data management and analysis

Editing, coding and classification of the collected data were done through data process software. Main focus was on frequency and percentages. Chi-square (χ^2) test was done wherever applicable with a P value <0.05 regarded as significant. (GN Prabhakara 2006).

CHAPTER – V

5. RESULTS

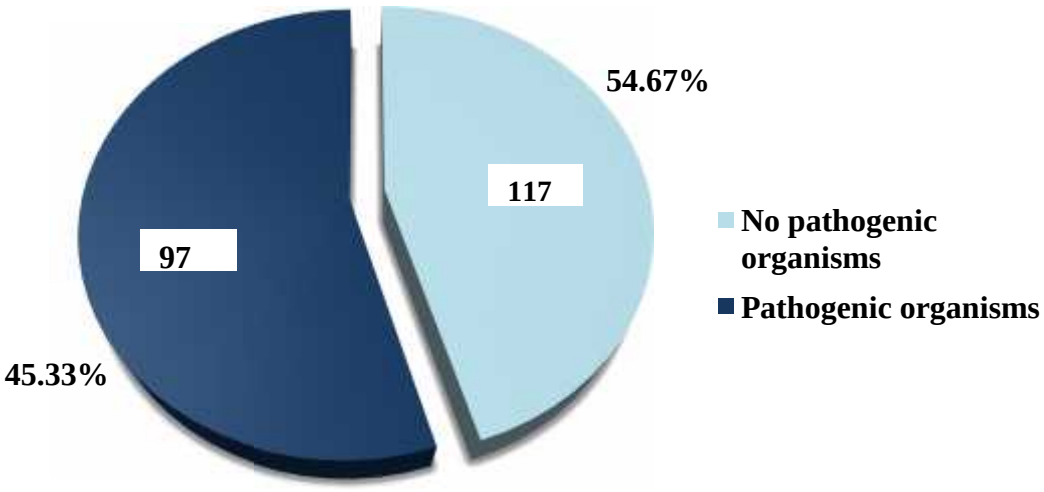
Out of total samples (222), 214 samples were further processed as they met our criteria while the remaining 8 samples did not meet our criteria and were rejected. This study comprised of 214 patients (93 as in-patients and 121 as out-patients). Of the samples analyzed, 12 different bacteria were isolated, giving a prevalence rate of 45.33%. Out of the total processed samples (214), growth of pathogenic organisms was obtained in 97/214 (45.33%) while in 117 samples (54.67%) no pathogenic organisms were isolated. (Figure 1)

Subsequent culture identified an organism consistent with that seen on Gram stain in 70% (68/97). The yield of a maximally informative result from all samples was therefore 32% (68/214).

Table 1: Percentage of sputum samples those met ASM criteria

Specimen	Accepted sample		Rejected sample	
	Number	%	Number	%
Sputum	214	96.4	8	3.6

Fig 1: Pattern of growth



5.1 Relationship between appearance of sputum and bacterial growth:

In 97 samples (45.33%) bacterial infection was found. This study demonstrates, sputum color is most useful to maximize the likelihood that a sputum specimen will yield useful information on microbiological analysis. In the present study more than half of the specimens with yellow, green, or rust colors demonstrated acceptable Gram stains, according to the criteria chosen. Conversely, white or clear specimens were acceptable less than 25% of the time. The association between color and microbiologic findings was even more striking. It was observed that purulent or mucopurulent sputum gave better isolation of pathogens than mucoid or mucosalivary sputum. Although a statistically significant relationship between sputum colour and a bacterial infection exists, it cannot be used to confirm the suspicion of a bacterial bronchitis or to base the decision on for or against antibiotic therapy. Yellowish or greenish colour of the sputum sample and bacterial infection showed a significant correlation. This study found a bacterial infection significantly more often in yellowish or greenish sputum samples. (Table 2)

Table 2: Sputum color and microbiological proof of bacterial infection

Color of the sputum	Bacterial infection	No bacterial infection	Totals
Yellowish or greenish sputum/cream colored Sputum sample	86(47.48%)	94(52.22%)	180(100%)
Colorless sputum sample	11(32.35%)	23(67.65%)	34(100%)
Totals	97(45.33%)	117(54.67%)	214

5.2 Bacteriological profile

Twelve different bacteria were isolated, giving a prevalence rate of 45.33%. 65.98% of the organisms isolated were Gram negative bacteria whereas 34.02% were Gram positive bacteria. The bacteria isolated from the samples included *Klebsiella pneumoniae* (24.74%), *Staphylococcus aureus* (18.56%), Beta-haemolytic streptococci (9.28%), *Pseudomonas aeruginosa* (9.28%), *Acinetobacter* spp. (9.28%),

Enterobacter spp. *(7.22%)*, *Streptococcus pneumoniae* (6.18 %), and *Haemophilus influenzae* (5.15%), *Klebsiella oxytoca* (4.12%), *Citrobacter freundii (3.1%)*, *E. coli* (2.06%) and *Proteus mirabilis* (1.03%) in order of ranking. Incidence of *Haemophilus influenzae* and *Streptococcus pneumoniae* was found low in our study. (Table 3)

Table 3: Distribution of microbial isolates from LRTI

Organisms	Number of cases	% amongst corresponding bacterial isolates	% amongst total isolates
Gram Positive Bacteria			
Staphylococcus aureus	18	54.55	18.56
β -haemolytic *streptococcus*	9	27.27	9.28
Streptococcus pneumoniae	6	18.18	6.18
Total	**33**	**100%**	
Gram Negative Bacteria			
Klebsiella pneumoniae	24	37.50	24.74
Pseudomonas aeruginosa	9	14.06	9.28
Acinetobacter spp.	9	14.06	9.28
Enterobacter spp.	7	10.94	7.22
Haemophilus influenzae	5	7.81	5.15
Klebsiella oxytoca	4	6.25	4.12
Citrobacter freundii	3	4.69	3.1
Escherichia coli	2	3.13	2.06
Proteus mirabilis	1	1	1.03
Total	**64**	**100%**	**100%**

5.3 Clinical history

Patients enrolled for the study were found to have the following clinical history: Cough, sputum with fever (16), Pneumonia (11), Chest pain with cough (8), Shortness of breath (SOB) (7), COPD (7), Cough for 3 months (5), Pneumonia with COPD (4), Blood mixed sputum (4), SOB with chest pain (3), Pneumonia with anaemia (3), Chest infection with symptomatology PTB (3), cough 7 days with post PTB (1), COPD with Acute Exacerbation (AE)(1), Pneumothorax (1), ICU with pneumonia (1), Lung abscess (1), Liver abscess (1). Clinical histories of 20 patients were not available in the request form.

5.4 Distribution of significant growth among cases examined: Growth of pathogens was obtained from 45.16% of sputum samples in case of in-patients and 45.45% in out-patients. (Table 4)

Table 4: Distribution of significant growth among cases examined

Patient	Cases examined		Significant growth	
	Number	%	Number	%
Outdoor patient	121	56.54	55	45.45
Indoor patient	93	43.46	42	45.16
Total	214	100%	97	

5.5 Distribution of bacterial isolates among the patient visiting to hospital:

It was found that gram positive bacteria and gram negative bacteria were isolated in higher number both from the out-patients. (Table 5)

Table 5: Distribution of bacterial isolates among the patient visiting to hospital:

Bacteria	Inpatient		Outpatient		Total no.
	Number	%	Number	%	
Gram positive	13	39.40	20	60.60	33
Gram negative	29	45.31	35	54.69	64

5.6 Distribution of organisms in outdoor and indoor patients

Gram negative bacilli (65.98%) outnumbered the growth of Gram positive organisms. *K. pneumoniae* (35.71%, 15/42) and *S. aureus* (26.19%, 11/42) were the predominant isolate amongst total number of in-patients (42) followed by *P. aeruginosa* (14.29%) and *Acinetobacter* spp (7.14%). Whereas *K. pneumoniae* (16.36%, 9/55) was responsible for causing most of the infections followed by β-haemolytic *streptococcus* (14.55%, 8/55), *S. aureus* (12.73%), *Acinetobacter* spp. (10.91%) and *S. pneumoniae*, *H. influenzae* and *Enterobacter* spp. (9.10% each) amongst total number of out-patients (55). 62.5% of *K. pneumoniae* were isolated from in-patients, whereas 61.11% of *S. aureus* were predominant in hospitalized patients. Single organism was isolated in most of the samples 92.78% (90/97) but in 7.22% cases (7/42) growth of two organisms was observed. (Table 6)

Table 6: Distribution of organisms in outdoor and indoor patients

Bacteria	No. of cases in inpatient	Percentage (among bacterial isolates)	No. of cases in outpatient	Percentage (among bacterial isolates)	Total no. of bacteria
K. pneumoniae	15	62.5	9	37.5	24
S. aureus	11	61.11	7	38.89	18
β -haemolytic *streptococcus*	1	11.11	8	88.89	9
P. aeruginosa	6	66.67	3	33.33	9
Acinetobacter spp.	3	33.33	6	66.67	9
Enterobacter spp.	2	28.57	5	71.43	7
S. pneumoniae	1	16.67	5	83.33	6
H. influenzae	0	0	5	100	5

5.7 Age and gender wise distribution of positive cases:

Sex-wise distribution showed 61 (62.89% of the positive cases) males as compare to 36 (37.11%) females. It was found that male patients were higher in number both in outdoor and indoor. Statistical analysis showed that there is significant relationship between gender and infection i.e. male are more prone to LRTI than female. (Table 7)

Table 7: Gender wise distribution of positive cases:

Type of visit	Male			Female	
	Number	%		Number	%
Inpatient	30	71.43		12	28.57
Outpatient	31	56.36		24	43.64
Total no. of male positive cases	61		Total no. of female positive cases	36	

5.7.1 Gender wise distribution of bacterial isolates in LRTI patients:

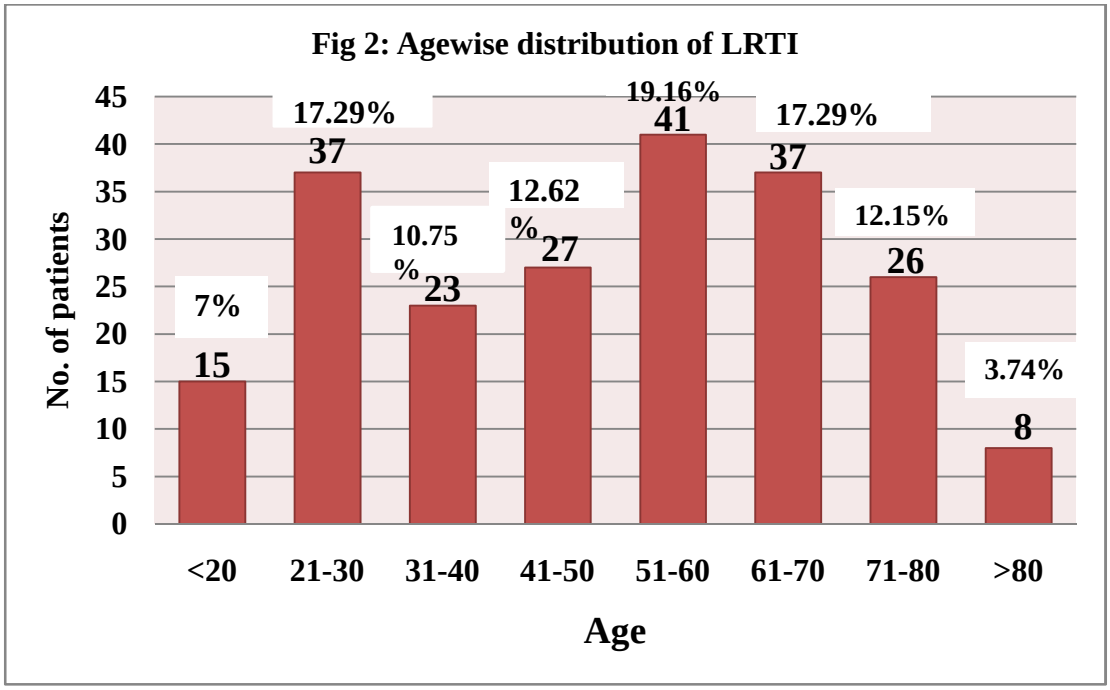
Gram negative organisms were isolated more in number than Gram positives from both male and female populations. But statistical analysis of this contingency table (Table 8) showed that there is no significant relationship between gender and organisms.

Table 8: Gender wise distribution of bacterial isolates in LRTI patients:

Bacteria	Male patients		Female patients	
	Number	%	Number	%
Gram positive	23	37.70	10	27.78
Gram negative	38	62.30	26	72.22
Total no. of patients	61	100%	36	100%

5.7.2 Age wise distribution of LRTI patients:

The maximum numbers of patients (52.34%) were above 50 years of age. In this study, LRTI was found to occur more frequently among the age group above 50. However the statistical analysis showed that there is no significant difference in age and LRTI. (Figure 2)



5.7.3 Age group distribution of LRTI pathogens:

Klebsiella pneumoniae was found predominant in patients of age group above 61. *Staphylococcus aureus* was found to be predominant in patients of age group above 50. Prevalence of pathogens was low among age group below 20. However, this difference was found to be statistically insignificant (P> 0.05). (Table 9)

Table 9: Age Group distribution of LRTI pathogens:

Organisms	Age group					
	<20	21-30	31-40	41-50	51-60	>61
Klebsiella pneumoniae	2	4	0	4	4	10
Staphylococcus aureus	2	3	3	1	5	4
β-haemolytic streptococci	1	1	2	2	0	3
Pseudomonas aeruginosa	0	0	1	1	3	4
Acinetobacter spp.	0	0	1	2	1	5
Other GNB	1	4	2	1	6	8
Streptococcus pneumoniae	0	2	0	0	3	1
Total	6	14	9	11	22	35

5.8 Antibiotic sensitivity pattern

5.8.1 Antibiotic sensitivity pattern of *Klebsiella pneumoniae*

K. pneumoniae showed 91.67% (22/24) sensitivity towards Amikacin, 70.83% (17/24) sensitivity towards Ciprofloxacin, 66.67% (16/24) towards Gentamicin and Cefotaxime. Antibiotics least effective were Cephalexin (33.33%, 8/24) and Ampicillin (8.33%, 2/24). (Table 10)

Table 10: Antibiotic sensitivity pattern of *Klebsiella pneumoniae* (n=24)

Name of antibiotics	Sensitive	Intermediate	Resistant	Sensitivity %
Ampicillin	2		22	8.33%
Ciprofloxacin	17		7	70.83%
Cephalexin	8		16	33.33%
Gentamicin	16		8	66.67%
Amikacin	22		2	91.67%
Cefotaxime	15	1	8	66.67%

5.8.2 Antibiotic sensitivity pattern of *Staphylococcus aureus*

S. aureus was more susceptible to Ciprofloxacin (72.22%, 13/18) and Cloxacillin (66.67%, 12/18). It was least susceptible to Ampicillin and Cotrimoxazole (33.33% each). (Table 11)

Table 11: Antibiotic sensitivity pattern of *Staphylococcus aureus* (n=18)

Name of antibiotics	Sensitive	Intermediate	Resistant	Sensitivity %
Ampicillin	5	1	12	33.33%
Ciprofloxacin	12	1	5	72.22%
Cephalexin	10	1	7	61.11%
Cloxacillin	12		6	66.67%
Erythromycin	9		9	50%
Cotrimoxazole	6		12	33.33%

5.8.3 Antibiotic sensitivity pattern of β-haemolytic streptococci

All β-haemolytic streptococci isolates were found sensitive to Ampicillin (100%, 9/9) and Ciprofloxacin (100%, 9/9). (Table 12)

Table 12: Antibiotic sensitivity pattern of β-haemolytic streptococci (n=9)

Name of antibiotics	Sensitive	Resistant	Sensitivity %
Ampicillin	9		100%
Ciprofloxacin	9		100%
Cephalexin	7	2	77.78%
Erythromycin	8	1	88.89%
Cotrimoxazole	6	3	66.67%
Cloxacillin	6	3	66.67%

5.8.4 Antibiotic sensitivity pattern of *Pseudomonas aeruginosa*

P. aeruginosa was sensitive towards Levofloxacin (100%, 9/9), Ciprofloxacin (80.89%, 8/9), Gentamicin (77.78%, 7/9). The isolates were least susceptible to Ampicillin (0%), Cefotaxime and Cephalexin (11.11% each, 1/9) and Amikacin (44.44%, 4/9). (Table 13)

Table 13: Antibiotic sensitivity pattern of *Pseudomonas aeruginosa* (n=9)

Name of antibiotics	Sensitive	Resistant	Sensitivity %
Gentamicin	7	2	77.78%
Amikacin	4	5	44.44%
Cephalexin	1	8	11.11%
Ciprofloxacin	8	1	80.89%
Cefotaxime	1	8	11.11%
Levofloxacin	9	0	100%

5.8.5 Antibiotic sensitivity pattern of *Acinetobacter calcoaceticus*

Most *A. calcoaceticus* were found to be resistant towards Ampicillin and Amikacin. Gentamicin and Ciprofloxacin was found to be 100% (9/9) effective towards *Acinetobacter*. (Table 14)

Table 14: Antibiotic sensitivity pattern of *Acinetobacter calcoaceticus* (n=9)

Name of antibiotics	Sensitive	Intermediate	Resistant	Sensitivity %
Ampicillin	3	1	5	44.44%
Ciprofloxacin	8	1	0	100%
Cephalexin	5	1	4	66.67%
Gentamicin	9		0	100%
Amikacin	3		6	33.33%
Cefotaxime	5		4	55.55%

5.8.6 Antibiotic sensitivity pattern of *Enterobacter* spp.

Enterobacter spp. was 100% (7/7) resistant to Ampicillin and Cephalexin. Cefotaxime, Ciprofloxacin and Amikacin were found to be effective in 85.71% (6/7) of isolates. (Table 15)

Table 15: Antibiotic sensitivity pattern of *Enterobacter* spp. (n=7)

Name of antibiotics	Sensitive	Intermediate	Resistant	Sensitivity %
Ampicillin	0		7	0%
Ciprofloxacin	6		1	85.71%
Cephalexin	0		7	0%
Gentamicin	4	2	1	85.71%
Amikacin	6		1	85.71%
Cefotaxime	6		1	85.71%

5.8.7 Antibiotic sensitivity pattern of *Streptococcus pneumoniae*

S. pneumoniae was found to be most suspectible to Ampicillin, Ciprofloxacin, Erythromycin and Cloxacillin (100% each, 6/6) followed by Cephalexin and Cotrimoxazole (66.67% each, 4/6). (Table 16)

Table 16: Antibiotic sensitivity pattern of *Streptococcus pneumoniae* (n=6)

Name of antibiotics	Sensitive	Resistant	Sensitivity %
Ampicillin	6	0	100%
Ciprofloxacin	6	0	100%
Cephalexin	4	2	66.67%
Erythromycin	6	0	100%
Cotrimoxazole	4	2	66.67%
Cloxacillin	6	0	100%

5.8.8 Antibiotic sensitivity pattern of *Haemophilus influenzae*

H. influenzae was susceptible to all the antibiotics tested against it. (Table 17)

Table 17: Antibiotic sensitivity pattern of *Haemophilus influenzae* (n=5)

Name of antibiotics	Sensitive	Resistant	Sensitivity %
Ampicillin	5	0	100%
Ciprofloxacin	5	0	100%
Cephalexin	5	0	100%
Chloramphenicol	5	0	100%
Gentamicin	5	0	100%
Cefotaxime	5	0	100%

5.8.9 Antibiotic sensitivity pattern of *Klebsiella oxytoca*

K. oxytoca showed 100% (4/4) sensitivity towards Ciprofloxacin, Amikacin and Cefotaxime while only 50% sensitivity (2/4) towards Cephalexin and Gentamicin. It was 100% (4/4) resistance to ampicillin. (Table 18)

Table 18: Antibiotic sensitivity pattern of *Klebsiella oxytoca* (n=4)

Name of antibiotics	Sensitive	Resistant	Sensitivity %
Ampicillin	0	4	0%
Ciprofloxacin	4	0	100%
Cephalexin	2	2	50%
Gentamicin	2	2	50%
Amikacin	4	0	100%
Cefotaxime	4	0	100%

5.8.10 Antibiotic sensitivity pattern of *Citrobacter freundii*

Ciprofloxacin and Cefotaxime were 100% (3/3) effective against *C. freundii* while Ampicillin and Cephalexin were only 33.33% (1/3) effective. Gentamicin and Amikacin were 66.67% (2/3) each effective against it. (Table 19)

Table 19: Antibiotic sensitivity pattern of *Citrobacter freundii* (n=3)

Name of antibiotics	Sensitive	Resistant	Sensitivity %
Ampicillin	1	2	33.33%
Ciprofloxacin	3	0	100%
Cephalexin	1	2	33.33%
Gentamicin	2	1	66.67%
Amikacin	2	1	66.67%
Cefotaxime	3	0	100%

5.8.11 Antibiotic sensitivity pattern of *Escherichia coli*

Amikacin (100%, 2/2) was most effective against *E. coli* followed by Gentamicin (50%, 1/2) and Cefotaxime (50%, 1/1). *E. coli* was found to be 100% (2/2) resistance to Ampicillin, Ciprofloxacin and Cephalexin. (Table 20)

Table 20: Antibiotic sensitivity pattern of *Escherichia coli* (n=2)

Name of antibiotics	Sensitive	Resistant	Sensitivity %
Ampicillin	0	2	0%
Ciprofloxacin	0	2	0%
Cephalexin	0	2	0%
Gentamicin	1	1	50%
Amikacin	2	0	100%
Cefotaxime	1	1	50%

5.8.12 Antibiotic sensitivity pattern of *Proteus mirabilis*

P. mirabilis showed 100% (1/1) sensitivity towards Ciprofloxacin, Gentamicin and Amikacin. It was 100% (1/1) resistant towards Ampicillin, Cephalexin, Cefotaxime. (Table 21)

Table 21: Antibiotic sensitivity pattern of *Proteus mirabilis* (n=1)

Name of antibiotics	Sensitive	Resistant	Sensitivity %
Ampicillin	0	1	0%
Ciprofloxacin	1	0	100%
Cephalexin	0	1	0%
Gentamicin	1	0	100%
Amikacin	1	0	100%
Cefotaxime	0	1	0%

5.8.13 Antibiotic sensitivity pattern of Gram negative bacteria

Gram negative bacteria were found most susceptible to Chloramphenicol (100%, 5/5), Levofloxacin (100%, 9/9) followed by Ciprofloxacin (82.81%, 53/64), Gentamicin (76.56%, 49/64), Amikacin (74.58%, 44/59) and Cefotaxime (64.06%, 41/64). They were least susceptible to Cephalexin (34.38%, 22/64) and Ampicillin (18.75%, 12/64). (Table 22)

Table 22: Antibiotic sensitivity pattern of Gram negative bacteria

Antibiotics	Sensitive		Resistant		Total
	No	%	No	%	
Ampicillin	12	18.75	52	81.25	64
Ciprofloxacin	53	82.81	11	17.19	64
Cephalexin	22	34.38	42	65.62	64
Amikacin	44	74.58	15	25.42	59
Gentamicin	49	76.56	15	23.44	64
Cefotaxime	41	64.06	23	35.94	64
Chloramphenicol	5	100	0	0	5
Levofloxacin	9	100	0	0	9

5.8.14 Antibiotic pattern of Gram positive bacteria: Gram positive bacteria were found most susceptible to Ciprofloxacin (84.85%, 28/33) followed by Cloxacillin (75%, 18/25), Erythromycin (69.70%, 23/33), Cephalexin (66.67%, 22/33) and Ampicillin (63.64%, 21/33). They were least susceptible to Co-trimoxozole (37.5%, 16/33). (Table 23)

Table 23: Antibiotic pattern of Gram positive bacteria

Antibiotics	Sensitive		Resistant		Total
	No	%	No	%	
Ampicillin	21	63.64	12	36.36	33
Ciprofloxacin	28	84.85	5	15.15	33
Cephalexin	22	66.67	11	33.33	33
Co-trimoxozole	16	48.48	17	51.52	33
Erythromycin	23	69.70	10	30.30	33
Cloxacillin	18	75	6	30.43	25

5.9 Prevalence of Multi Drug Resistance among the total isolates from LRTI

The data shows that *S. aureus* (72.22%, 13/18), *K. pneumonia* (58.33%, 14/24), *Pseudomonas aeruginosa* (55.56%, 5/9), *C. freundii (*100%, 3/3*), E. coli (*100%, 2/2*), S. pneumoniae* (16.67%, 1/6), β-haemolytic streptococci (22.22%, 2/9) and *H. influenzae* (20 %, 1/5) were resistant to at least 2 different antibiotic classes.

The analysis of the total MDR strains showed that the prevalence was higher in Gram negative bacteria than in Gram positive bacteria. (Table 24)

Table 24: Percentage of MDR among the total isolates

S.No.	Pathogens	No. (%) of bacteria	No. (%) of MDR
1	*Klebsiella pneumoniae*	24 (24.74%)	14 (58.33%)
2	*Staphylococcus aureus*	18 (18.56%)	13 (72.22%)
3	β- haemolytic streptococci	9 (9.28%)	2 (22.22%)
4	*Pseudomonas aeruginosa*	9 (9.28%)	5 (55.56%)
5	*Acinetobacter calcoaceticus*	9 (9.28%)	4 (44.44%)
6	*Enterobacter* spp.	7 (7.22%)	3 (42.86%)
7	*Streptococccus pneumoniae*	6 (6.18%)	1 (16.67%)
8	*Haemophilus influenzae*	5 (5.15%)	1 (20%)
9	*Klebsiella oxytoca*	4 (4.12%)	3 (75%)
10	*Citrobacter freundii*	3 (3.1%)	3 (100%)
11	*Escherichia coli*	2 (2.06%)	2 (100%)
12	*Proteus mirabilis*	1 (1.03%)	0 (0%)

CHAPTER - VI

6. DISCUSSIONS AND CONCLUSION

6.1 Discussions

It was a prospective study carried out at Bir Hospital, Kathmandu from 15th April, 2010-14th July, 2010. This study was undertaken to find out the bacteriological profile and the sensitivity pattern of the isolates.

In the epidemiological projections of WHO, World Bank and Harvard School of Public Health for all ages by the year 2020, the acute lower respiratory infections are the third cause of death in the world in 1990 and will rank in the fourth place in the order of causes of death estimated for 2020 (Murray 1996). According to the report of Ministry of Health, Government of Nepal, 3.4% of morbidity is caused by acute RTI. It is among top ten diseases of Nepal.

This study comprised of 214 patients (93 in-patients and 121 out-patients). All the sputum samples were subjected to gram staining, bacterial culture and antibiotic sensitivity for bacterial isolates as per standard techniques.

Growth of pathogenic organisms was obtained in 97/214 (45.33%). Culture positivity depends on nature of sputum, transportation time and the number of organism present in the sample. Arora N, 2001 had obtained growth in 72% cases, whereas Dalvi 1983 obtained growth in 57% of samples. The yield of sputum culture in this study was low (45.32%) but comparable to other studies (Bartlett JG 1998, 1995 and File 1996) which report a 50% yield with sputum cultures. Decreased sputum positivity may be due to prior use of antibiotics, inappropriate sputum production and non-productive cough.

Twelve different bacteria were isolated, giving a prevalence rate of 45.33%. This consisted of 62.89% from male patients and 37.11% from females. The bacteria isolated from the samples included *K. pneumoniae* (24.74%), *S. aureus* (18.56%), Beta-hemolytic *streptococcus* (9.28%), *P. aeruginosa* (9.28%), *Acinetobacter* spp. (9.28%), *Enterobacter* spp. (7.22%), *S. pneumoniae* (6.18 %), and *H. influenzae* (5.15%), *K. oxytoca* (4.12%), *C. freundii* (3.1%), *E. coli* (2.06%) and *P. mirabilis* (1.03%).

It is interesting to note that the majority of GNB were isolated from adults (31.25%) and elderly (64.06%) patients while the least frequent isolates were from pediatric (4.6%) patients. This may be attributed to nosocomial viral infections that are common in pediatric age groups and thus highlights the need for study on viral agents in the younger age group (Hall CB 1981).

In this study more than half of the specimens with yellow, green, or rust colors demonstrated acceptable, according to the criteria chosen. Conversely, white or clear specimens were acceptable less than 25% of the time. It was observed that purulent or mucopurulent sputum gave better isolation of pathogens than mucoid or mucosalivary sputum. As this study demonstrates, sputum color is most useful to maximize the likelihood that a sputum specimen will yield useful information on microbiological analysis.

Few prior studies have specifically examined the role of sputum color. In a study of in-patients at a hospital, yellow was the only sputum color that correlated with good or fair quality on Gram stain of the specimen (Flournoy 1993). In a recent study, Johnson et al. examined such 289 consecutive outpatient samples, finding that the bacterial yield from sputum colors green, yellow-green, yellow, and rust was higher than the yield from cream, white, or clear samples (Johnson 2008).

Limitations of this study relate largely to the lack of information collected about the individual patients from whom the samples were obtained. Disease processes that were responsible for their sputum production are unknown. In addition, no attempt was made to correlate the specific antibiotics being taken with the culture results.

Gram negative bacteria (65.98%) outnumbered the growth of Gram positive bacteria. It is estimated that more than 40% of all exacerbations are of bacterial origin (White 2003). Presence of Gram negative bacilli colonizing the oropharynx increases with severity of underlying respiratory alteration. The proportion of Gram-negative bacteria causing LRTIs was 65.98%, which was close to the 63.9% - 68.4% of a report from Germany (Kohlenberg 2008) and this percentage appears to be increasing.

Pathogens were obtained from 45.16% of sputum samples in case of in-patients and 45.45% in out-patients. *K. pneumoniae* (62.5%) was the most common pathogen isolated from in-patients, whereas *S. aureus* (61.11%) was the second predominant

organism in hospitalized patients. Mechanically ventilated patients are at particular risk of developing nosocomial pneumonia (Celis 1988). The endotracheal tube bypasses the upper-airway host defence system and allows direct entry of bacteria into the lower respiratory tract. The multiple defence functions of the lung are compromised as a result of impairment of the cough reflex, interference with mucociliary function, and stimulation of excessive mucus secretion. These inductions of mucus production can promote colonization by *P. aeruginosa* which can bind directly to mucus (Levine & Niederman 1991).

This study was very much related to the similar study carried by Egbagbe EE, 2006 and Christopher Aye Egbe, 2010 in which *K. pneumoniae* (52.5%) was the most common organisms followed by *S. aureus* (10%). Prevalence rate was 47.2% which is close to the prevalence of this study (45.32%). This isolation of *K. pneumoniae* as predominant organism also agreed with another study carried by A.O Okesola 2002-2005 and by Navaneeth, 2002. This study is in concordance with that of A.H Eldeev, 2006 where *K. pneumoniae* (11.27%) was most prevalent followed by *P. aeruginosa* (6.26%) among GNB and *S. aureus* (17.71%) as the most prevalent followed by β-haemolytic streptococci (12.34%) among GPB. Antibiotic sensitivity pattern was also seen to be related with this study. In the study done by Mohanasoundaram KM (2010), *K. pneumoniae* (23%) was found to be the most prevalent organisms followed by *P. aeruginosa* (12.7%) in hospitalized patients. Similar study carried by Hidron, 2008 found *K. pneumoniae* as the most common pathogens. Study done in South Asian Countries by HUI, et al. in 2006-2008, isolated *K. pneumoniae* as most frequent organism from patients of Acute Exacerbation (AECB) followed by *P. aeruginosa* and *Acinetobacter* spp.

But these results differ from the findings of Ozyilmaz from Turkey (2005), Liebowitz from S. Africa, where *H. influenzae* was the most prevalent single pathogen followed by *S. pneumoniae*. This study was different from the Ravi P Shankar's 2003 study in Western Nepal where they isolated *H. influenzae* as the common pathogens followed by *K. pneumoniae*, *S. aureus* and *S. pneumoniae*. U. shrestha 2005 showed *H. influenzae* and *K. pneumoniae* as the most predominant organisms (19.51% each). P. Gauchan, 2003 found *H. influenzae* as most common followed by *K. pneumoniae*. This study showed that males (62.89%) were found more at risk and infections was most prevalent in the age group >50 agreed with P.

Gauchan's study (61.3%). This difference in the incidence and types of bacterial pathogens between these studies could be due to differences in the socio-demographic characteristics, life style.

It is well known that *S. pneumoniae* is the commonest causative organism (35%) for community acquired pneumonia. Furthermore, it is common among the elderly and a high incidence is found during the winter seasons (Wilkinson 2004). However, in this study, there was only 6 isolates of *S. pneumoniae*. This study showed much lower isolation rate (6.18%) for *S. pneumoniae*, which was close to those obtained by Cosentini (1996) (10%). *H .influenzae* was also isolated in a few numbers in our study and other Indian studies, though it is a common pathogen in western countries (Ball 1995). In this prospective study, there were only 5 isolates of *H. influenzae.* It is difficult to offer a reasonable explanation for the inability to isolate many *S. pneumoniae* and *H. influenzae* in this study. Many patients cannot produce good sputum specimens and often have received antimicrobial treatment prior to collection of sputum samples. The yield of *S. pneumoniae* from the sputum cultures of patients with bacteremic pneumococcal pneumonia is only 40%–50%, according to the studies conducted a few decades ago (Barrett-connor 1971). It could be a result of underdiagnosis or that the methodology was not good enough to isolate the organism. Another useful explanation is that most patients who may be infected with *S. pneumoniae* and *H. influenzae* may have had some unsuccessful antibiotic treatment which will make the organism difficult to grow in the normal growth medium. This may be due to the sensitivity of this organism to antibiotics and most patients may have had antibiotics prior to hospital admission. This could be due to partially treated patients before or during the hospital stay, or these patients may be affected by viral, fungal or atypical pathogens. This may explain the reason for the inability to isolate *H. influenzae* and *S. pneumoniae* routinely because of their fastidious nature. There is a clear seasonality, with infections of *S. pneumoniae* peaking in the fall and winter months (Lynch 2010).

This study shows *K. pneumoniae* as the common isolate which is disimilar to other studies from Nigeria (Macfarlane 1999, Dosumu 2002). The most common clinical presentation in the patients in our study included history of cough with sputum production, fever, chest pain, pneumoniae and dyspnoea. This mode of

presentation in the studied patients is similar to those of an earlier study (Dosumu 2002).

K. pneumoniae is an important pathogen in hospital infections which showed that the age of elderly patients and hospital infection rate was positively correlated because the elderly with increasing age and decreasing immune function are prone to nosocomial infection. Because of intensive care unit (ICU) patients with long hospital stay the opportunities of cross-infection are high. More widespread use of antibiotics, hormones, and more invasive operation, and serious underlying diseases are the risk factors.

P. aeruginosa is responsible for 10–15 % of nosocomial infections worldwide (Blanc 1998). *P. aeruginosa* is a common nosocomial pathogen that can cause serious human infections and is the most prevalent respiratory pathogen among patients suffering from cystic fibrosis (CF) (Forbes 2002).

In North America, *S. aureus* is the most common pathogen causing pneumonia and was shown to account for 28% of isolates (Gotfried MH 2001). The high prevalence of *S. aureus* among patients suffering from LRTIs was reported by others (Torres 1989 and Kayser 1992). The study performed by Hawan (2000) illustrated that the highest rate of isolation was for *S. aureus* (34.33%) followed by *P. aeruginosa* (32.84%). The high prevalence of *S. aureus* could be explained on basis of increased resistance of *S. aureus* to the commonly used antibiotics. It could also be attributed to the fact that *S. aureus* is one of the normal floras of the respiratory system and colonizing strains may serve as endogenous reservoirs for overt clinical infections or may spread to other patients. *S. aureus* accounts for 2.5% of community-acquired pneumonia and 11% of hospital-acquired pneumonia.

β-haemolytic streptococci was isolated from both old and young patients. Most of them were female with the history of cough and fever. In 2 male patients of age >50 yrs, there was blood mixed sputum. According to Hinshaw, 1966, the most commonly occurring hemolytic streptococci in pneumonia belong to Group ‘A’ Lancefield. Haemolytic streptococci was found to contribute to 2% of LRTI in 206 adult patients in a study on etiology of adults in the community carried out by MacFarlane in 1993 preceded by *S. pneumoniae;* 30% and *H. influenzae;* 8%.

Acinetobacter is a gram-negative coccobacillus that during the past three decades has emerged from an organism of questionable pathogenicity to an infectious agent of importance to hospitals worldwide (Fournier 2006). Most alarming are the organism's ability to accumulate diverse mechanisms of resistance, the emergence of strains that are resistant to all commercially available antibiotics, (Lolans 2006) and the lack of new antimicrobial agents in development.

Enterobacter infections are most common in neonates and in elderly individuals, reflecting the increased prevalence of severe underlying diseases at these age extremes. Previous reports from the National Nosocomial Infections Surveillance System (NNIS) demonstrated that *Enterobacter* species caused 11.2% of pneumonia cases in all types of ICUs, ranking third after *S. aureus* (18.1%) and *P. aeruginosa* (17%).

E. coli was diagnosed in the older patients (>60) who were hospitalized. *E. coli* are often present in the lower respiratory tract, especially in surgical or otherwise debilitated patient who are being treated with the antibiotics to which they are resistant as a result of gastro intestinal or urinary tract infections of elderly patients, with the spread to lung secondary to bacteremia (Berk 1985).

Respiratory syncytial virus can also cause symptomatic lower respiratory tract disease, especially in elderly patients. Nonviral agents that have been implicated include *M. pneumoniae*, *Chlamydia pneumoniae* and *Bordetella pertussis*. There was no diagnostic testing for atypical organisms, like Legionella or Mycoplasma, done for any of the patients in the series. *M. pneumoniae* and *C. pneumoniae* serology is the method of choice for its identification but it is expensive. Culture is rarely performed because it requires specialized media, prolonged incubation. Yeast cells accounted for 10% of the total isolates. These yeast cells may be the alternate forms of dimorphic moulds. This however cannot be ascertained since the culture was incubated at 37^0C.

The difference in the results may be attributed to those organisms responsible for LRTI that vary from one place to another depending on socioeconomic conditions, which are considered important predisposing etiological factors. Also, the age of patient, the habits of the population as well as the season, during which the study is carried out, may influence the incidence of different organisms (Abd-Elrehim 1988).

The maximum numbers of patients (52.34%) were above 50 years of age. *K. pneumoniae* was found predominant in patients of age group above 61. *S. aureus* was found to be predominant in patients of age group above 50. The maximum numbers of cases showing LRTI were >50 years of age in this study, which can be explained by the fact that chronic bronchitis has highest prevalence in fifth and sixth decade (Jindal SK 1993). Older individuals are more likely to be diagnosed with COPD. It has been estimated that 10% of the population aged 55-85 have COPD (National Heart, Lung, and Blood Institute: Data Fact Sheet 2001).

Sex-wise distribution showed 61 (62.89% of the positive cases) males as compare to 36 (37.11%) females. Predominance of male over female patients as shown in the study can be explained by the fact that in our country males are exposed more to outside environment because of their more mobility as compare to females. Moreover smoking habits are more pronounced in males that constitute one of the predisposing factors for the development of COPD (Sethi S 2000).

Children younger than 2 years carry the highest burden of *S. pneumoniae* disease worldwide. Adults older than 55-65 years are the next most commonly affected age group worldwide. But in this study the number of patients of this age group visiting to our hospital was low. This may be the factor responsible for low yield of *S. pneumoniae*. In a review of six studies, the recovery of *S. pneumoniae* from expectorated sputum processed by standard laboratory techniques had only a 40-55% yield when compared with more invasive specimen collection techniques (Bartlett 1989).

The National Nosocomial Infections Study consistently reports that aerobic GNB cause more than 60% of nosocomial pneumonias (CDC 1984). Baron (1994) found that *K. pneumoniae* was susceptible to aminoglycosides, quinolones and third generation cephalosporins. This study also showed good activities for aminoglycosides, quinolones and for third generation cephalosporins. El-Daly (1990) reported 100% activity for gentamicin. Kamal (1999) found that amikacin and ciprofloxacin were the most potent antimicrobials against *Klebsiella* spp. *K. pneumoniae* was the most prevalent bacteria with a susceptibility of 91.67 % (Amikacin), 70.83% (Ciprofloxacin), 66.67% (Gentamicin and Cefotaxime), 33.33% (Cephalexin) and 8.33% (Ampicillin). When *K. pneumoniae* was isolated, the

sensitivity pattern was better with amikacin where results were over 80%. Isolates were however resistant to ampicillin, and cephalexin. The clinical presentation of studied groups consisted of history of cough with sputum production, fever, chest pain and dyspnoea; these were noticed in 60% of cases.

S. aureus was the second most prevalent bacteria with a susceptibility of 72.22% (Ciprofloxacin), 66.67% (Cloxacillin), 61.11% (Cephalexin), 50% (Erythromycin), 33.33% (Ampicillin and Cotrimoxazole).

The susceptibility profile of *P. aeruginosa* was, 80.89 % (Ciprofloxacin), 77.78% (Gentamicin), 44.44% (Amikacin), 11.11% (Cephalexin and Cefotaxime) and 0% (Ampicillin). Out of the 9 positive cases, 8 were resistant to cefotaxime and cephalexin. They were only sensitive to potent antibiotics such as Gentamicin and Levofloxacin. Chah et al. (2003) isolated *P. aeruginosa* resistance to cephalexin (80.0%), cotrimoxazole (80.0%), ampicillin (73%) and gentamycin (70.0%).

The high rate of resistance to ampicillin, cefotaxime, cephalexin, amikacin observed in this study may reflect the fact that these are the most commonly prescribed antibiotics in the hospital and also the most easily available for community without prescription. *S. pneumoniae* had a susceptibility profile of 100% (erythromycin), 66.67% (cotrimoxazole and cephalexin), 100% (ciprofloxacin), 100% (ampicillin), and 100% (cloxacillin). The susceptibility profile displayed by the other organisms followed similar patterns.

Quinolones were found to be most effective antibiotic against both GNB and GPB. *S. pneumoniae* isolates showed sensitivity to penicillins, macrolides and quinolones. In this study, most of the isolates were sensitive to the quinolones (ciprofloxacin), but poorly sensitive to ampicillin, cotrimoxazole and erythromycin and these are in agreement to the study conducted in Algeria, where susceptibility to cotrimoxazole was particularly low (Ramdani-Bougessa 2003), but contradicts the reports of Larsson et al. (2000) in Vietnam. High antimicrobial resistances to ampicillin and penicillin have also been reported in France, Germany and Japan (Schito 2000).

An isolate was considered multi-drug resistant if it was resistant to at least two of the antibiotics tested (Santo 2007). Most of the isolates displayed multi-drug

resistance with *S. aureus, P. aeruginosa, K. pneumoniae and K. oxytoca* showing the highest number of multi-drug resistance to most of the antibiotics except the fluoroquinolones. Although, multi-drug resistant strains of organisms were identified, Ciprofloxacin and Gentamicin are recommended as antibiotics of choice against the pathogens. These findings have clinical and epidemiological significance. Differences in the prevalence of antimicrobial susceptibility may be due to several factors, including different patterns of antimicrobial usages, which lead to selective pressure, as well as the distribution of specific serotypes and the spread of resistant clones within certain areas.

The data shows that *S. aureus* (72.22%), *K. pneumoniae* (58.33%), *Pseudomonas aeruginosa* (55.56%), *C. freundii* (100%), *E. coli (100%), S. pneumoniae* (16.67%), β- haemolytic streptococci (22.22%) and *H. influenzae* (20 %) were resistant to at least 2 different antibiotics classes. In a similar study done by Jaya Sharma in Nepal (2004), 80% of *K. pneumoniae* and 63.63% of *S. aureus* were found to be MDR. In another study in Vietnam, 90% of *S. aureus,* 68% *of H. influenzae* were resistant to at least one antibiotic (Larsson 2000). These organisms were reported to be Beta-lactamase producers. The incidence of bacterial resistance mediated by Beta lactamases has been reported in several African countries including Nigeria and South Africa (Zeba 2005). Beta-lactam-resistant strains of the common pathogens as well as macrolide and fluoroquinolone resistant strains are being isolated with increasing frequency in many countries. The range of species isolated has increased over the years, possibly as a result of changing medical interventions. Studies in Lagos and Ibadan have reported that between 70 - 90% of strains of enterobacteriaceae including *E. coli, Klebsiella* sp. and *Proteus* spp. were resistant to many of the commonly available antibiotics (Okesola 2009).

Several researchers have argued that the prevalence of resistance to a particular antibiotic does not always reflect the antibiotic consumption in a given locality. In addition to the antibiotic stress, horizontal gene transfer is also considered as a factor in the occurrence of antibiotic resistance in clinical isolates (Brown 2005).

6.2 Conclusion

Overall comparison of results of different studies on the frequency of the various bacterial etiologic ARI agents are difficult in view of the differences in methodological approaches, laboratory procedures, and the characteristics of the populations analyzed (e.g., age, health, and economic status). So, for the management of these cases, there is need for more studies involving large population for proper management of these cases. Changes in the biological characteristics of ARI bacterial agents can be detected and defined only by keeping continuous local bacteriological surveillance. These efforts would make a major contribution not only to improvements in accurate etiologic diagnosis but also in a more rational use and development of effective therapeutic and prophylactic approaches for a group of infections with remarkably high rates of morbidity and mortality.

The treatment of patients with bacterial LRTIs is, therefore, becoming more complicated. In particular, the emergence of resistance to commonly prescribed antimicrobial agents by respiratory tract pathogens has compounded the problem. These results highlight the need for systematic interventions to ensure more consistent application of recommended guidelines for antimicrobial use. This study demonstrated that antimicrobial resistance of pathogens isolated from lower respiratory tracts has become a serious problem. This situation appears to be worsening annually and creates selective pressure on physicians to use certain antibiotics.

CHAPTER – VII

7. SUMMARY AND RECOMMENDATION

7.1 Summary

1) LRTI is among the most commonly encountered infectious diseases causing significant morbidity and mortality. It was a prospective study carried out at Bir Hospital, Kathmandu from 15th April, 2010-14th July, 2010 among the patients suspected of LRTI.

2) Twelve different bacteria were isolated, giving a prevalence rate of 45.33%. 65.98% of the organisms isolated were GNB whereas 34.02% were GPB.

3) Subsequent culture identified an organism consistent with that seen on Gram stain in 70% (68/97) cases. Yellowish or greenish colour of the sputum sample and bacterial infection showed a significant correlation.

4) The bacteria isolated from the samples included *K. pneumoniae* (24.74%), *S. aureus* (18.56%), β-haemolytic streptococci (9.28%), *P. aeruginosa* (9.28%), *Acinetobacter* spp. (9.28%), *Enterobacter* spp. (7.22%), *S. pneumoniae* (6.18 %), and *H. influenzae* (5.15%), *K. oxytoca* (4.12%), *C. freundii* (3.1%), *E. coli* (2.06%) and *P. mirabilis* (1.03%) in order of ranking. Incidence of *H. influenzae* and *S. pneumoniae* was found low in our study.

5) The data shows that *S. aureus;* 72.22% (13/18), *K. pneumoniae;* 58.33% (14/24), *Pseudomonas aeruginosa*; 55.56% (5/9), *C. freundii;* 100% (3/3), *E. coli;* 100% (2/2), *S. pneumoniae;* 16.67% (1/6), β-haemolytic streptococci; 22.22% (2/9) and *H. influenzae;* 20% (1/5) were MDR.

6) GNBs were found most susceptible to Chloramphenicol (100%), Levofloxacin (100%) followed by Ciprofloxacin (82.81%), Gentamicin (76.56%), Amikacin (74.58%) and Cefotaxime (64.06%). They were least susceptible to Cotrimoxazole (37.5%), Cephalexin (34.38%) and Ampicillin (18.75%).

7) GPBs were found most susceptible to Ciprofloxacin (84.85%) followed by Cloxacillin (75%), Erythromycin (69.70%), Cephalexin (66.67%) and Ampicillin (63.64%). They were least susceptible to Cotrimoxazole (37.5%).

7.2 Recommendations

1. A larger sample size is needed for better analysis. There is need for more studies involving large population for proper management of these cases. Further research should be focused on viral infection that may trigger the bacterial/fungal infections. Incidence of infection can be studied throughout the year to obtain the seasonal frequency of the opportunistic infections.
2. *K. pneumoniae* and *S. aureus*, are the frequently isolated pathogens in this study which are the most common agents involved in nosocomial infections as well. To prevent this, antibiotic and disinfectant policy should be set up in each hospital so as to prevent the infection.
3. The increase in the rates of antibiotic resistance amongst the major pathogens has compromised the selection of empirical treatment for some respiratory tract pathogens with traditional agents. A definitive bacteriological diagnosis and susceptibility testing would, therefore, be required for effective management of RTI.
4. It is important to periodically monitor the prevalence and antimicrobial sensitivity pattern before empirical therapy is initiated in hospitals. The use of antibiotics in haphazard way is also thought to promote the emergence and spread of antibiotic-resistant organisms. A reduction in the use of antibiotics in this context forms part of a national strategy to encourage the ‘prudent use’ of these drugs in order to tackle the growth of multidrug resistance organisms.
5. The identification of polymicrobial infection is very important for treatment strategies and to avoid a false impression of clinically resistant strains.

CHAPTER – VIII

8. REFERENCES

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