CHAPTER I

1 INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a major cause of chronic morbidity and mortality and represents a substantial economic and social burden throughout the world (GOLD, 2007). COPD affects over 5% of adults. It is the fourth leading cause of death worldwide, and is the only major cause of death that is still rising (Pauwels *et al*., 2004). Its prevalence is increasing steadily, and it has been predicted that COPD will be the third leading cause of death worldwide by 2020 (Murray *et al.*, 1997).

The guidelines published by the American Thoracic Society (ATS) define COPD as 'a disease state characterized by the presence of airflow obstruction due to chronic bronchitis or emphysema, the airflow obstruction is generally progressive, may be accompanied by airway reactivity, and may be partially reversible' (1995). COPD exacerbation is defined as: an event in the natural course of the disease characterized by a change in the patient's baseline dyspnea, cough, and/or sputum that is beyond normal day-to-day variations, is acute in onset, and may warrant a change in regular medication in a patient with underlying COPD (GOLD, 2007).

The most common causes of an exacerbation are infection of the tracheobronchial tree and air pollution, but the cause of about one-third of severe exacerbations cannot be identified (White *et al*., 2005).

The infectious agents in COPD exacerbations can be viral or bacterial (Anthonisen *et al.*, 1987). Male gender, advanced age, cigarette smoking, occupational exposure, and low socioeconomic status are well-known independent risk factors for COPD. An increasing COPD prevalence in developing countries is expected due to cigarette smoking and environmental pollution (Ehlrich *et al*., 2005). Furthermore, the use of wood and other biomass fuels by > 50% of population is an additional burden (Smith, 2003).

Over the last decade, studies using new molecular diagnostic techniques have established that respiratory viruses are a major cause of exacerbations of both asthma and COPD. The most prevalent viruses detected during exacerbations are the

rhinoviruses. Despite the burden of disease associated with exacerbations, little is known about the mechanisms of virus-induced exacerbations of airway diseases (Malia, 2006).

An increase in sputum volume and purulence points to a bacterial cause, as do prior history of chronic sputum production identified (Blasi *et al*., 2002). The true prevalence of these organisms is not known. Bacteria can be isolated from 40–60% of sputum samples of patients experiencing AECB. The three predominant bacterial species isolated are non-typeable *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Streptococcus pneumoniae*. Other less frequently isolated potential pathogens are Gram-negative enterobacteria, *Haemophilus parainfluenzae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Gram negative enterobacteria and *Pseudomonas aeruginosa* are more frequently isolated in patients with severe underlying disease (Anzeuto, 2005). Viral infections are present in approximately 30% of exacerbations, *Mycoplasma pneumoniae* in 1–10% and *Chlamydophila pneumoniae* in 4–5% (serologically identified) (Annemarie *et al.*, 2007).

According to recent recommendations of the European Respiratory Society, antibiotic treatment should be used in patients with severe acute exacerbation of COPD requiring mechanical ventilation (Ewig *et al.*, 2000).

In the study conducted by Pandey (1984) the prevalence of chronic bronchitis in Nepal according to time spent near fire place was found to be 13.7%.

Shree Birendra Hospital is a tertiary care hospital. In this hospital military personnel and their relatives come for their health check up. This study was conducted to study the microbiological profile in exacerbations of chronic obstructive pulmonary disease (COPD) and the antibiotic susceptibility pattern of the bacteria isolated, as there are scanty of reports from Nepal.

CHAPTER II

2 OBJECTIVES

2.1 GENERAL OBJECTIVE

To analyze the bacteriological profile in the exacerbation of chronic obstructive pulmonary disease.

2.2 SPECIFIC OBJECTIVE

1. To identify the bacterial pathogens from the sputum sample of patients with chronic obstructive pulmonary disease.
2. To determine the proportion of the fungal pathogens from the sputum sample of patients with chronic obstructive pulmonary disease.
3. To assess the appropriate antibiotic susceptibility pattern for the isolated bacterial pathogens.

CHAPTER III

3 LITERATURE REVIEW

3.1 Epidemiology of chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is an inflammatory disorder of the lung that is characterized by irreversible or partially reversible airflow limitation (Rabe *et al.,* 2007).Chronic obstructive pulmonary disease (COPD) is a major and growing cause of morbidity and mortality in countries at all levels of economic development (Mannino *et al*., 2007; Buist *et al*., 2008) with smoking being recognized as its most important causative factor (Buist *et al*., 2008).

According to the WHO estimates, 80 million people in the world have moderate to severe COPD. More than 3 million people died of COPD in 2005, which corresponds to 5% of all adult deaths globally and it is estimated that by 2020 it will become the third leading cause of death worldwide (GOLD, 2007).This chronic disease is however, barely even acknowledged in the health statistics of many countries. Many patients remain undiagnosed, experience high levels of symptoms, their quality of life is often poor and they usually die prematurely of it or its complications (Menezes *et al.*, 2005; Halbert *et al.*, 2006; Menezes *et al.*, 2008, Ko *et al.*, 2008).

The costs of COPD to health services and society are substantial. Consultation rates in primary care are high and exacerbations of COPD are one of the most common causes of hospital admission. In developed countries, exacerbations of COPD account for the greatest burden on the health care system. In the European Union, the total direct costs of respiratory disease are estimated to be about 6% of the total health care budget, with COPD accounting for 56% (€38.6 billion) of this cost. In the United States in 2002, the direct costs of COPD were $18 billion and the indirect costs totaled $14.1 billion (Rafael, 2009).

Chronic Obstructive Pulmonary Disease (COPD) is a preventable and treatable disease with some significant extra pulmonary effects that may contribute to the severity in individual patients. Its pulmonary component is characterized by airflow

limitation that is not fully reversible. The airflow limitation is usually progressive and associated with an abnormal inflammatory response of the lung to noxious particles or gases.

The total number of moderate to severe COPD cases in the 12 countries of Asia pacific region, as projected by the model, is 56.6 million with an overall prevalence rate of 6.3%. The COPD prevalence rates for the individual countries range from 3.5% (Hong Kong and Singapore) to 6.7% (Vietnam). This study concluded that the COPD prevalence rates projected by the model reflect the high prevalence of the risk factors for the disease in Asia. The combined prevalence of 6.3% for these countries is considerably higher than the overall rate of 3.8% as extrapolated from WHO data for this region. These estimates highlight the need for further epidemiological studies to support appropriate allocation of resources for the prevention and management of COPD (Respirology, 2003).

The chronic airflow limitation characteristic of COPD is caused by a mixture of small airway disease (obstructive bronchiolitis) and parenchymal destruction (emphysema), the relative contributions of which vary from person to person. Airflow limitation is best measured by spirometry, as this is the most widely available, reproducible test of lung function (GOLD, 2007).

Because COPD often develops in long-time smokers in middle age, patients often have a variety of other diseases related to either smoking or aging (Soriano *et al*., 2005). COPD itself also has significant extra pulmonary (systemic) effects that lead to co morbid conditions. The findings by Finkelstein supported the conclusion that COPD is an independent risk factor for cardiovascular disease (Finkelstein *et al.*, 2009).

3.2 Spirometric classification of severity and stages of chronic obstructive pulmonary disease (GOLD, 2007)

A simple spirometric classification of disease severity into four stages is recommended by GOLD. Spirometry is essential for diagnosis and provides a useful description of the severity of pathological changes in COPD. Specific spirometric cut-points (e.g., post-bronchodilator FEV_1/FVC ratio < 0.70 or FEV_1 < 80, 50, or 30%

predicted) are used for purposes of simplicity. A study in a random population sample found that the post-bronchodilator FEV_1/FVC exceeded 0.70 in all age groups, supporting the use of this fixed ratio (Soriano *et al*., 2005). However, because the process of aging does affect lung volumes, the use of this fixed ratio may result in over diagnosis of COPD in the elderly, especially of mild disease. The characteristic symptoms of COPD are chronic and progressive dyspnea, cough, and sputum production which may be preceded by the development of airflow limitation by many years. This pattern offers a unique opportunity to identify smokers and others at risk for COPD, and intervene when the disease is not yet a major health problem. Conversely, significant airflow limitation may develop without chronic cough and sputum production (GOLD, 2007).

Stage I: Mild COPD - Characterized by mild airflow limitation ($FEV_1/FVC < 0.70$; FEV_1 >80% predicted). Symptoms of chronic cough and sputum production may be present, but not always. At this stage, the individual is usually unaware that his or her lung function is abnormal.

Stage II: Moderate COPD - Characterized by worsening airflow limitation ($FEV_1/FVC < 0.70$; $50\% \leq FEV_1 < 80\%$ predicted), with shortness of breath typically developing on exertion and cough and sputum production sometimes also present. This is the stage at which patients typically seek medical attention because of chronic respiratory symptoms or an exacerbation of their disease.

Stage III: Severe COPD - Characterized by further worsening of airflow limitation ($FEV_1/FVC < 0.70$; $30\% \leq FEV_1 < 50\%$ predicted), greater shortness of breath, reduced exercise capacity, fatigue, and repeated exacerbations that almost always have an impact on patients' quality of life.

Stage IV: Very Severe COPD - Characterized by severe airflow limitation ($FEV_1/FVC < 0.70$; $FEV_1 < 30\%$ predicted or $FEV_1 < 50\%$ predicted plus the presence of respiratory failure). Respiratory failure is defined as an arterial partial pressure of O_2 (PaO_2) less than 8.0 k Pa (60 mm Hg), with or without arterial partial pressure of CO_2 ($PaCO_2$) greater than 6.7 k Pa (50 mm Hg) while breathing air at sea level. Respiratory failure may also lead to effects on the heart such as cor pulmonale (right heart failure). Clinical signs of cor pulmonale include elevation of the jugular

venous pressure and pitting ankle edema. Patients may have the FEV_1 > 30% predicted, whenever these complications are present. At this stage, quality of life is very appreciably impaired and exacerbations may be life threatening.

In a study by Tsimogiami, of the 94 patients enrolled, sputum from 36 yielded bacterial pathogens. These patients were characterized by a higher frequency of purulent sputum, lower FEV_1, BMI and PaO_2 and more frequent use of inhaled steroids ($P < 0.05$) (Tsimogianni, 2009).

While asthma can usually be distinguished from COPD, in some individuals with chronic respiratory symptoms and fixed airflow limitation it remains difficult to differentiate the two diseases (Fairall *et al*., 2005). In many developing countries both pulmonary tuberculosis and COPD are common. In countries where tuberculosis is very common, respiratory abnormalities may be too readily attributed to this disease. Conversely, where the rate of tuberculosis is greatly diminished, the possible diagnosis of this disease is sometimes overlooked. Therefore, in all subjects with symptoms of COPD, a possible diagnosis of tuberculosis should be considered especially in area where this disease is prevalent (GOLD, 2007).

3.3 Risk factors

3.3.1 Smoking

Smoking is the detrimental risk factor in the development and progression of COPD. Emphysema, a major component of COPD, is thought to be due to an excess of protease, causing destruction of elastin and collagen matrix supporting the lung structure. Tobacco smoking causes an influx of neutrophils into the lungs and a subsequent release of elastase and proteases. Oxidants inhaled from tobacco smoke and released from the activated inflammatory cells play a role in the development of emphysema by impairing endogenous antiproteases (Kant and Gupta, 2008).

While smoking rate is declining among men in some countries, it is rising among women worldwide. By the year 2025, the number of women smoker is expected to triple. Women born in United States have the highest level of smoking (32%) compared to South European (20%) and Asian born women (21%). Growing

participation of women in active professional life has increased the number of smokers. Industry marketing and advertising strategies are particularly targeted to women and young girls. “Feminine” brands emphasizing low tar length and slimness have successfully played on traditional fear of weight gain. This has been attributed to the fact that in Scotland rate of COPD have almost doubled amongst women in past 10 years (ASH, 2006).

China has the largest production and consumption of tobacco worldwide. Approximately 67% of men and 4% of women > 15 years of age in China are smokers, and the total of > 320 million Chinese smokers represents about one third of all smokers worldwide. The incidence of smoking-related diseases has yet to peak, while it is estimated that currently 1 million Chinese die annually from these diseases (Zhang, 2003).

Cigarette smokers, have higher prevalence of lung function abnormalities and respiratory symptoms, a greater annual rate of decline in FEV_1 and higher death rate from COPD as compared to non smokers. The decline in FEV_1 in smokers was proved in a longitudinal study in UK (Fletcher *et al*., 1976). Pipe and cigar smokers have higher COPD mortality and morbidity rates than non smokers, although it was lower than that of cigarette smokers (US Surgeon General).

Bidi are small brown cigarettes, often flavored, consisting of tobacco hand-rolled in tendu or temburni leaf and secured with a string at one end; they are the preferred means of smoking in subcontinent India, especially in the rural areas. In India alone, bidi consumption is 900 billion sticks per year. The amount of tobacco in bidis is twice to that used in cigarette (WHO, 1993). Although the risk of smoking bidis relative to smoking cigarettes has not been fully studied, existing data suggest that bidis are at least as harmful as cigarettes. The population prevalences of chronic bronchitis were 8.2% in bidi users compared with 5.9% in cigarette users in adults > 35 years of age. In one cohort study of tobacco users in Mumbai, India, the relative risks of all-cause mortality were 1.8 for bidi smokers compared with 1.4 for cigarette smokers. Much less is known of the extent of harmful outcomes from the hookah or water pipe, which is used to smoke tobacco in several Asian countries such as China, India, and Pakistan, and is causing global concern as it has become a fashionable or

exotic form of tobacco smoking among the young in both the East and West (Wan *et al*., 2008).

Active tobacco smoking was associated with a high risk of *H. influenzae* isolation (Miravelttes, 1999).

Environmental tobacco smoke (ETS) constitutes a common problem in many countries. Passively inhaled tobacco smoke contains several known and probable human carcinogens, as well as irritants and toxic substances (Jakkola, 2000).

Passive smoke to cigarette may also contribute to respiratory symptoms and COPD by increasing the lungs total burden of inhaled particulars and gases (Buist *et al*, 1985; Dayal *et al*, 1994). The Canadian Human Time-Activity Pattern Survey has shown that exposure to ETS is common among children particularly in their home (Leech *et al*, 1999). Recent studies have shown significant effects from ETS on the occurrence of chronic respiratory symptoms in adults, but only in a limited number of studies has exposure to ETS actually been associated with COPD in adults and with small impairment of lung function (Kant, 2008).

Results from animal studies showed that fetal lung development was adversely influenced by maternal smoking. This suggested that prenatal exposure to substances inhaled by the smoking mother may be a risk factor for COPD (Collins *et al*, 1985). Maternal smoking is related to lung function deficit in neonates (Hanrahan *et al*, 1992). Postnatal exposure to tobacco smoke conveys increased risks of lower respiratory tract infections and reduced lung function (Davis R, 1989). Smoking during pregnancy affects the lung growth and development in uterus and possibly the priming of immune function (Morgan W J, 1998; Holt P J, 1987). Infants of smoking parents have more respiratory illness than infants of non smoking parents (Colley *et al*, 1970).

The *S. pneumoniae* carriage rate was higher among children exposed to smoking than among nonexposed children (76% vs. 60%). Carriage rates of *S. pneumoniae* were higher among mothers who smoked than among mothers exposed to smoking and among nonexposed mothers (32%, 15%, and 12%, respectively). There were no

differences in *H. influenzae* carriage rates between children and mothers from smoking and nonsmoking families (Greenberg *et al*., 2005).

3.3.2 Work exposure or occupational dust

For many years researchers have known that chronic exposures to fumes, chemical substances and dusts in the work place are one of the main risk factors for the development of COPD. The most important are grain, isocyanates, calcium, cola and other mineral dusts, heavy metals, adhesives and welding fumes. Rapid population growth and urbanization has led to lack of services such as supply of food, water and housing. With this, occupational health hazards are also increasing (Kant and Gupta, 2008).

When the exposures are sufficiently intense or prolonged, occupational dust and chemicals (vapors, irritants and fumes) can cause COPD independently of cigarette smoking. It increases the risk of disease in presence of concurrent cigarette smoking (Kauffmann *et al*, 1979). There is now growing trend that airway obstruction (COPD) is caused by other exposures rather than tobacco smoke alone, and that occupational exposures, particularly dusts, are important amongst such cases. The widespread habit of tobacco smoking, in industrial population has delayed the recognition of the other factors contributing the disease (Kant and Gupta, 2008).

Farmers, grain workers, consumption or cement exposed workers, construction or cement exposed workers' foundry workers, wood workers and workers exposed to excess heat including furnace worker has been identified as the increased risk groups. Longitudinal studies of the effects of occupational exposures have been performed in coal miners (Love *et al*, 1982) hard rock miners (Hnizdo *et al.*, 1990) tunnel workers (Holman *et al.*, 1987) concrete manufacturing workers (Meijer *et al.*, 2001) and in a subject of nonmining industrial workers in Paris (Kauffmann *et al.*, 1982). Grain dust exposure also has been established as a risk factor in COPD, both in smoker and non smokers (Zejda *et al.*, 1993).

Development of disease is influenced by the amount of exposure and toxicity of the dust, and the disease is characterized by long latency periods; therefore, even in countries in which exposures have been recognized and controlled, the disease rates are only gradually declining. Rate trends in developing countries are mostly unknown but the magnitude of problem is substantial (Chen *et al*, 2003).

3.3.3 Air pollution

3.3.3.1 Outdoor pollution

Air pollution is other important risk factors for COPD. Chronic exposure to elevated air pollution seems to correlate with chronic bronchitis and lung function impairment. The population of rapidly expanding mega cities of Asia, Africa, and Latin America are increasingly exposed to levels of ambient air pollution that rival and often exceed those experienced in industrialized countries in the first half of the 20^{th} century (Krzyzwanoski *et al.*, 1999).

The factors accounting for the deteriorating urban air quality are growing industrialization and increasing vehicular traffic. Industrial emissions, automobile exhaust and the burning of fossil fuels leads to respiratory damage, heart disease and lung disease (Kant and Gupta, 2008).

Conventional outdoor pollutants include fossil fuel smoke, sulphur dioxide, nitrogen di oxides and ozone. Air pollution has worsened due to traffic congestion, poor housing, poor sanitation, and drainage and garbage accumulation. A study reported related photochemical oxidants and multiple primary air pollutants such as sulfur dioxide particles and hydrocarbons to chronic respiratory symptoms and pulmonary function abnormalities in both smokers and non smokers (Rowak *et al.*, 1980).

3.3.3.2 Indoor pollution

Household energy and indoor air pollution pose a substantial vulnerability to the health of rural women and children. The highest concentration of indoor air pollutants emerges from burning of biofuels such as wood, agriculture crops and dung cake, which are extensively used by rural households. It has been estimated that approximately half the global population and up to 90 percent of rural households in

developing countries still rely on biomass fuels (world resource institute, 1999). Typically cooked indoors in open fires or poorly functioning stoves, which leads to levels of air pollution that are amongst the highest ever measured. Therefore indoor air pollution with biofuels is an issue that requires to be addressed through gender, energy, environment and health policy. Some of highest concentrations of pollutants come due to the use of biofuel for cooking in rural indoor environment (Sarkar, 2004). In developed countries transformation has been come with a shift from biofuel to petroleum products (kerosene, LPG) and electricity. In developing countries even where cleaner more sophisticated fuels are available, households often continue to use biomass (Smith, 1987).Although the portion of global energy derived from biofuel has fallen substantially which is evidence that biofuel is increasing among the poor (Kanta,2008).

In addition to passive smoking which is well known for its harmful action on respiratory health, a number of compounds and mixtures have been identified as relevant air pollutants indicator (Vieggi *et al*, 1999). These may be derived from heating, combustion, photochemical reactions furniture, building materials, biological organisms and fibers. The smoke from combustion of solid such as wood, dried dung and crop residue used for cooking and heating is the significant cause of indoor air pollution. Over-crowding and inadequate housing conditions contribute to indoor air pollution and related diseases. Respiratory symptoms have been related to the use of several domestic biofuels, such as kerosene and other fuels. It is accountable for large number of COPD in the rural inhabitants in general and women in particular (Smith, 2000). Main health effects from indoor air pollution are respiratory symptoms, lung function reduction and decline, bronchial hyper responsiveness and respiratory infections some of which are also characteristics of COPD (Kant and Gupta, 2008sss).

3.3.4 Nutritional effect on COPD

There is usually a sequence in the emergence of chronic disease as the diet in developing countries becomes westernized. Bruney *et al* in a study showed that changes in dietary habits, such as increasing salt intake, decreasing intake of fruits and vegetables, and changing fatty acid consumption of the diet, were suggested to contribute to the rise in COPD mortality and morbidity (Kant and Gupta, 2008).

The fact that not all patients with COPD are smokers but only 20% of the smokers develop COPD (Medison *et al*, 1998) has led to the alternative theories as to why some people appear to be more vulnerable to effects of cigarette smoke. Various studies have described the presence of nutritional abnormalities in patient with COPD. The most obvious clinical expression of these nutritional abnormalities is unexplained weight loss. Malnutrition contributes to respiratory muscle weakness resulting in increased frequency of hospitalization, Cor pulmonale and increased mortality. Nutrition depletion, as indicated by weight loss and loss of lean body mass a common complication of advanced COPD (particularly, but not limited to the emphysematous type). Low body weight or recent weight loss and in particular depleted lean body mass in patients with COPD have shown to be independent predictor mortality, poor outcomes after acute exacerbations, hospital admission rates, and need for mechanical ventilation. The factors thought to contribute to nutrition depletion in these patients include elevated resting and activity related energy expenditure, reduced dietary intake relative to energy expenditure, accelerated negative nitrogen balance, particularly during acute exacerbations of COPD, medication effects, and perhaps most importantly an elevated systemic inflammatory response (Kant *et al*., 2008).

3.3.5 Alcohol

Global alcohol consumption has increased in recent decades, with most or all of this increase occurring in developing countries. Reports on the association between alcohol consumption and the prevalence of COPD vary. Heavy alcohol consumption was associated with respiratory symptoms and reduced lung function in a study by Lebowitz (Lebowitz *et al*, 1991) even when controlled for smoking. Smoking, however judged to be a far more important risk factor. The effect of passive smoking would have to be also considered in many heavy drinker, smoker or not, who consumed his or her favorite beverage in heavy tobacco-polluted bars or restaurants.

3.3.6 Socioeconomic status

Socioeconomic status may be assessed by using a series of indicators, which include income, education, type of work, housing conditions, crowding index (family size with respect to house size). A low socioeconomic level is a risk factor for the development of emphysema and chronic bronchitis (Vieggi *et al.*, 1988). Strachan

(Strachan *et al.*, 1995) analyzing the 1979-1983 mortality rates in Great Britain, showed that, for both bronchitis-emphysema-asthma and trachea-bronchus-lung cancer, the standardized mortality ratio increased with a decreasing socioeconomic status. An association between the prevalence of chronic bronchitis and low socioeconomic status, even after adjusting for smoking and other risk factors was detected in Brazil in 1999. in Norway, Bakke *et al*. (Bakke *et al*, 1995) found adjusted odds ratios of spirometric airflow limitation of 5.2 and 1.8 for subjects who had completed only primary and secondary education, respectively compared to university graduates. The socioeconomic status in early life might be a relevant risk factor COPD as shown by a longitudinal analysis of the British Medical Research Council's national survey of health and development of the 1976 birth cohort (Britten *et al*, 1987). At the age of 36 years, in both sexes, the presence of respiratory symptoms and the peak expiratory flow were independently associated with current indices of social circumstances and with poor environment at the age of 2 years.

3.3.7 Gender

Studies from developed countries, (Mannino *et al*., 2002) show that the prevalence of the disease is now almost equal in men and women, probably reflecting the changing patterns of tobacco smoking. Some studies have suggested that women are more susceptible to the effects of tobacco smoke than men (Anthonisen *et al.*, 1994; Xu *et al*., 1994).

Recent years have also witnessed a major shift in the sex profile of the disease. Although earlier studies showed that men accounted for the majority of COPD-related deaths (Murphy *et al.*, 1962), the prevalence and mortality rate of COPD in women have more than doubled during the past 20 years in industrialized countries while they stabilized in men (Mannino *et al.*, 2000). In 2001, more women died from COPD than men in the United States, a trend also observed in other countries (Rennard *et al.*, 2002; Bryanton *et al.*, 2001)). The increase in prevalence and mortality for COPD among women compared with men is usually attributed to the delayed rise of smoking prevalence in women (Urlik *et al.*, 2003). Another possibility is that the susceptibility to COPD risk factors, mainly tobacco, varies between sexes. This hypothesis has been difficult to prove because conflicting conclusions have been reached by different studies. For a given tobacco consumption, airflow obstruction is worse in women

(Chen *et al.*, 1991; Prescott *et al.*, 1997). Although this may be due to under-reporting in women, it nevertheless supports the idea that they are at greater risk to develop COPD than men. Studies investigating sex differences in the vulnerability to the deleterious effects of tobacco either reported greater susceptibility to tobacco in women (Prescott *et al.*, 1997; Cornett *et al.*, 2003; Langhammer *et al.*, 2003) or failed to detect any sex disparities (Burrows *et al.*, 1977; Van pelt *et al.*, 1994; Volmer *et al.*, 2000). Female smokers show greater bronchial responsiveness (Leynaert *et al.*, 1997), lower baseline forced expiratory volume in 1s (FEV_1) and faster decline in FEV_1, although this is not always the rule.

3.3.8 Genetic Factors

It is believed that many genetic factors may influence an individual likelihood of COPD developing. Studies have demonstrated an increased risk of COPD within families with COPD probands. Some of this risk may be due to shared environmental factors, but several studies (Silverman *et al*., 1996) in diverse populations have also suggested a shared genetic risk. To date, α_1-antitrypsin (α_1-AT) deficiency, a major circulating serine protease inhibitor, is the only genetic factor that is definitely linked to the development of emphysema or COPD, independent of tobacco exposure. Affected individuals have a 40-fold increase in the risk of the development of COPD compared to unaffected people. This rare hereditary deficiency is a recessive trait most commonly seen in individuals of Northern European origin and is extremely rare among Asians. Limited data have failed to link COPD with α_1-AT deficiency in Chinese either by determining the levels in the serum (Chen, 1999) or by genotyping and electrophoretic phenotyping (Huo *et al.*, 1990). Although variants of α_1-AT deficiency have been reported such as variant Siiyama in Japan (Siama, 2001), and METokyo an Mpirare among Chinese, they have not been linked to COPD (Huo *et al*., 1990).

A number of candidate genes in whites have been implicated in an increased risk for COPD, including ABH nonsecretor status (Cohen *et al*., 1980) microsomal epoxide hydrolase level (Smith *et al*., 1997) glutathione S-transferase level (Harrison *et al*., 1997), α_1-antichymotrypsin level (Faber *et al*., 1993) the complement component GcG level (Schelnberg *et al.*, 1998) cytokine tumor necrosis factor-α level (Huang et *al*., 1997) and microsatellite instability (Siafakas *et al*., 1997). The results are often

inconsistent but could be related to the potential pathogenic mechanisms of COPD. In Asians, studies on putative candidate genes found in whites have not yielded consistent results in Chinese (Liu *et al.*, 2007), Thais (Chierkaul *et al*., 2005), Koreans (Yim *et al*., 2000) or Japanese (Homma *et al*., 2006).

3.3.9 Infection

A history of severe childhood respiratory infection has been associated with reduced lung function and increased respiratory symptoms in adulthood (Silverman *et al*., 2000). However, susceptibility to viral infections may be related to another factor, such as birth weight, that it self is related to COPD (MacNee, 2003).

3.4 Pathophysiology of chronic obstructive pulmonary disease

The pulmonary manifestation of COPD, such as decrease in FEV_1 compared to predicted FEV_1 and a reduction in the percentage of FEV_1/FVC (forced vital capacity), could be due to at least two different pathological manifestations, each with a distinct structural expression chronic bronchitis and emphysema (Daheshia, 2005).

Chronic bronchitis is defined by the presence of a mucus-producing cough most days of the month, 3 months a year for 2 successive years. Thus, the disease affects the airways causing stimulation of mucus production and an increase in the thickness of the airway wall, which is caused by hypertrophy/hyperplasia of the epithelium and/or by inflammatory cells invading the bronchial tubes. The thickened wall, hyper secretion, and an increase in airway mucus along with epithelial debris could lead to narrowing of the airway diameter and obstruction of the air passage (Pauwels *et al*., 2001).

Emphysema, evidenced by parenchymal damage, on the other hand, displays destruction of the alveolar septae, leading to enlargement of distal air spaces, a decrease in alveolar elastic recoil, decreased traction support of small airway lumens, and impaired exhalation (Snider, 1989). Because capillary-rich alveolar walls are also destroyed in areas of emphysema, these enlarged redundant air spaces have very high ventilation/perfusion ratios, creating physiologic dead spaces (Maxfield, 2004). One physiological outcome of these structural changes is gas trapping due to the decreased

elastic tethering forces required to maintain airway patency during the expiratory phase. In emphysema, because of increased residual volume, inspired gas is always mixed with less oxygenated air, and pulmonary oxygenation is decreased.

Although COPD is primarily a pulmonary illness, the disease has also systemic manifestations, like most complex disorders, it affects far more than a single organ system. For example, at the late stage of the disease muscle weakness and weight loss (Berry *et al.*, 2004), cardiovascular disease (Gan *et al.*,2004) eoporosis (Gan *et al.*, 2004), heart enlargement, and depression (Fehrenbach, 2004) have been associated with COPD by substantially limiting the daily activities of the patients, leading to immobility, social isolation, and a poor quality of life. Nature of pulmonary inflammatory responses in COPD is described here (Dahsehia, 2005).

3.4.1 Neutrophils/macrophages

COPD is characterized by chronic inflammation, thus the inhaled toxin could induce pulmonary inflammatory reactions (Barnes *et al*, 2002). For example, exposure of the lung to cigarette smoke causes an influx of inflammatory cells (Stringer *et al*, 2004), and the bronchoalveolar lavage fluid of smokers contains increased numbers of neutrophils and macrophages (Saetta, 1999). Indeed, an increase in the number of pulmonary neutrophils and macrophages has been reported in COPD patients and associated with an increase in inflammatory mediators and adhesion molecules. Additionally, it has been reported that the inflammatory response in the lungs of COPD patients increases with the severity of the disease and that the inflammatory response persists even after the smoking ceases (Hogg *et al.*, 2004). For example, Turato *et al*. (2002) reported that as compared with smokers with mild or no COPD, smokers with severe COPD had an increased number of leucocytes in the small airways, which showed a positive correlation with the radiological score of emphysema and a negative correlation with the values of FEV_1.

Cosio *et al*. (2004) reported significantly higher numbers of neutrophils and macrophages in the bronchoalveolar lavage fluid of smokers and COPD patients compared to nonsmokers. In addition, Baraldo *et al*. (2004) showed that smokers with COPD had an increased number of neutrophils in the airway smooth muscle compared with nonsmokers. Smokers with normal lung function also had a

neutrophilic infiltration in the airway smooth muscle, but to a lesser extent. When all the subjects were analyzed as one group, neutrophilic infiltration was inversely related to FEV_1. Additionally, Keatings *et al*. (1996) found a significant increase in neutrophils and increased concentrations of tumor necrosis factor alpha (TNFα) and interleukin (IL)-8 in the patients with COPD compared with the smoking and nonsmoking control subjects.

The role of macrophages in the pathogenesis of COPD has been highlighted by Cosio *et al*. (Cosio *et al*., 2004) who reported that alveolar macrophages from smokers and COPD patients showed increased release of IL-8 and TNFα, and by Finkelstein *et al*. (Finkelstein *et al*., 1995) who described that the extent of lung destruction during the disease was directly related to the number of alveolar macrophages per cubic millimeter.

Proteinase/antiproteinase imbalance (Turino, 2002) and oxidant/antioxidant disparity (Mac Nee *et al*., 1999) have been advanced to account for the pathology of COPD. Both neutrophils and macrophages have the capacity to release an array of bioreactive oxidant (Rahman *et al*.,1999) and proteinases (Daheshia *et al*., 2005) that can induce pulmonary inflammation, parenchymal destruction, and structural changes associated with COPD. For example, Finlay *et al*. (1997) detected elevated levels of matrix metalloproteinase (MMP)-l and -9 in macrophages from emphysematous patients vs. those from control subjects; and MMP-9 complexes were secreted by emphysematous patients but not by control subjects. Furthermore, Lim *et al*. (Lim *et al*., 2000) reported that cultured airway macrophages from smokers released greater amounts of MMP-9 at baseline and in response to IL-1 beta (β) and lipopolysaccharide than did those of nonsmokers. And Segura-Valdez *et al*. (2000), who also analyzed the lungs of COPD patients, reported an increase in the levels of MMP-8 and -9 associated with neutrophils. Additionally, Molet *et al*. (2005) reported an increase in MMP- 12 in the lungs of COPD patients. The authors reported that macrophages in BALF samples and in bronchial biopsies expressed a higher amount of MMP-12 than in normal subjects.

Thus, macrophages and neutrophils could readily be involved in COPD, at least partly through release of free radical/oxidative stress molecules and proteinases. Interestingly, Kim and Nadel (Kim *et al*., 2004) have suggested that the increased

necrosis of neutrophils in the airway lumen of COPD patients could lead to discharge of elastase and reactive oxygen species causing mucus hypersecretion.

3.4.2 Airway epithelium

Airway epithelium has also been suggested as an initiator of COPD by generating multiple mediators. For example, Puljic and Pahl (2004) described release of several chemokines by lung epithelial cells due to cigarette smoke, and these inductions were steroid resistant. It has also been shown that exposure of human airway epithelial cells to cigarette smoke induces activation of P38 and nuclear factor kappa beta and releases IL-6 and IL-8 (Beisswenger *et al*.,2004). Additionally, Fuke *et al*. (2004) reported that airway epithelium from smoker with COPD produced higher amount of chemokines such as IL-8, macrophage inflammatory protein-1 alpha, and monocyte chemoattractant protein-1 (MCP-1) when compared with smokers without airflow limitation or never smokers.

3.4.3 T lymphocytes

An increase in T-cells has also been reported in the airways and lung parenchyma of COPD patients with a predominance of CD_8 cells (Saetta *et al*.,1999). For example, Hogg *et al*. (2004) reported the importance of inflammation in small airways as a determinant of the progression and severity of the disease and found an increase in the absolute number of CD_8 cells as the disease progressed. Interestingly, the increase in CD_8 cells was not limited to pulmonary tissues, as there was an increase in the number of these cells in the paratracheal lymph nodes from smokers with COPD (Saetta *et al*.,2003). Although the nature of antigen specificity of these T-cells is not clear, Grumelli *et al*. (2003) reported that T-cells isolated from lung biopsies of COPD patients displayed activation phenotype. The invading lymphocytes associated with COPD suggested that cytotoxic CD_8 cells in the pulmonary parenchyma may contribute to the parenchymal destruction found in COPD by release of granzymes and perforins. Furthermore, the CD_8 lymphocytes could activate the death signal in airway epithelial cells by releasing TNFα. Interestingly, Miotto *et al*. (2003) reported that CD_8 in the central airways of smokers with chronic bronchitis are a source of IL-4 and IL-13, leading to mucus hypersecretion, which occurs in chronic bronchitis.

Thus, a cellular mechanism can be conceived where toxin exposure activates the alveolar macrophages and airway epithelium, leading to recruitment of more inflammatory cells that have the potential to destroy lung parenchyma and induce pulmonary change to damage the pulmonary architecture. Additionally, infiltrating cells could themselves be a new source of chemo tactic factors, which could sustain the inflammatory reactions in the lung, leading to a chronic and progressive disease (Daheshia, 2005).

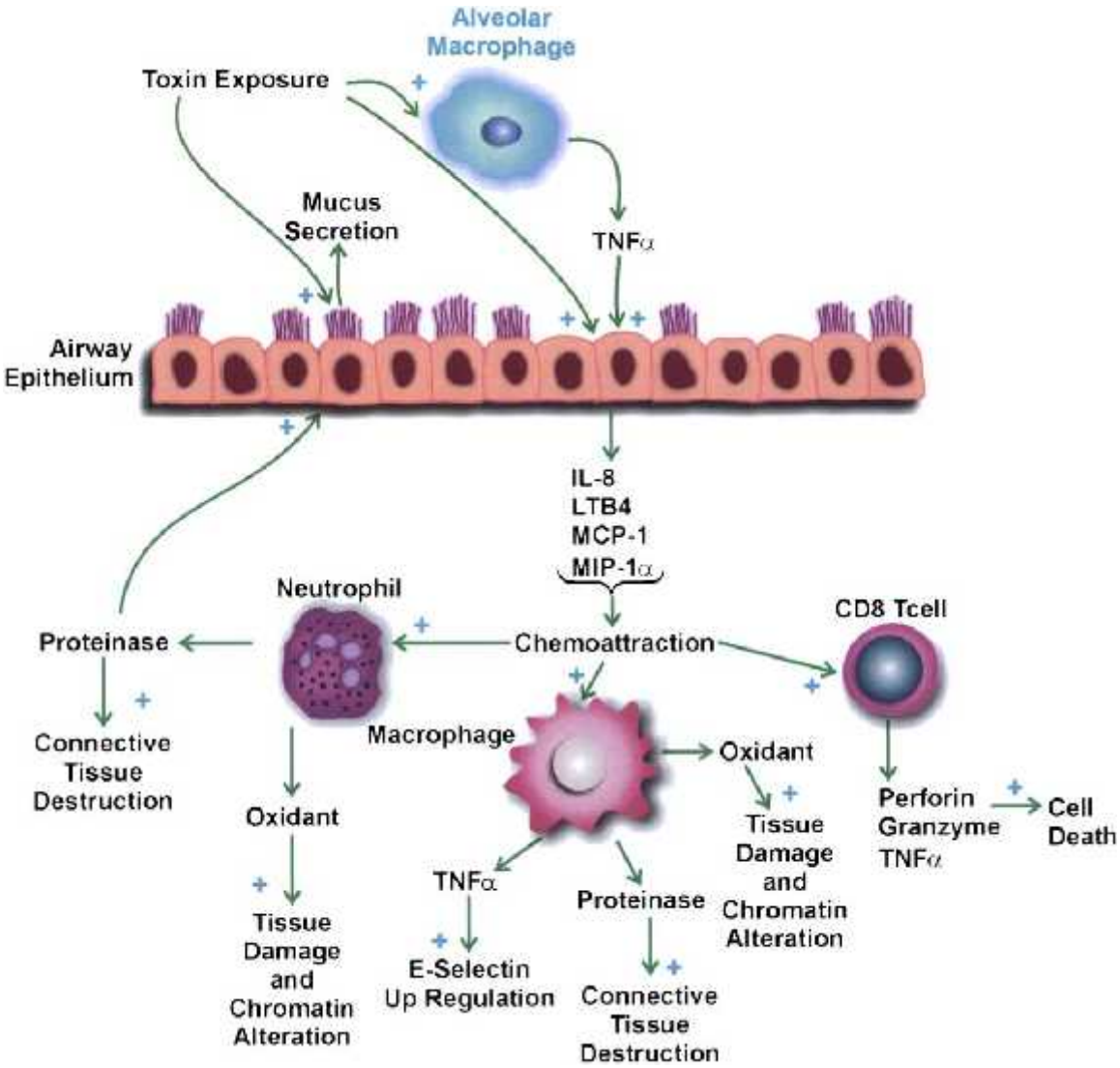

Fig: The molecular events and cellular involvements during the pathogenesis of COPD. The toxin exposure of the lung could activate the alveolar macrophages and also the airway epithelium to generate chemotactic factors that, once released, induce a cascade of events leading to infiltration of the lung with hematopoietic cells that, in turn, directly or in association with aerosol stimulate the release of several destructive factors.

3.5 Chronic obstructive pulmonary disease exacerbations

Exacerbation of COPD is defined as a sustained worsening of the patient's condition, from the stable and beyond normal day-to-day variations, that is acute in onset and necessitates a change in regular medication (Rodriguez *et al*., 2000) For example, a change in the patient's baseline dyspnea, cough, or sputum beyond day-to-day variability that is sufficient to warrant change in disease management, such as hospitalization, could be considered as an exacerbation. It is assumed that most of the morbidity, mortality, and healthcare costs of patients with COPD are related to the exacerbation of COPD, with more than 1 million emergency department visits per year (Rodriguez *et al*., 2000)

Although the etiology of COPD exacerbations has not been completely clarified, there is strong evidence for bacterial involvement in almost half of the exacerbations (Sethi *et al*., 2000). In addition, viral infection plays a substantial role in these processes and could account for one third of these exacerbations (Rodhge *et al*., 2003). It also has been reported that viral infection may impair host defense mechanisms, which could lead to increased colonization or infection with bacteria (Wongsurakiat *et al*., 2004). Bacterial, viral, and atypical pathogens, either alone or in concert, have been implicated in inducing the majority of acute exacerbations (Sethi, 2004).

The inflammatory component of exacerbation maybe of relevance because inhaled steroids (fluticasone propionate) were effective in preventing exacerbation of COPD (Spencer *et al*., 2004). Although there are few data available regarding cellular events during COPD exacerbation, it has been reported that the changes in inflammatory cells during exacerbation of COPD are the same as those observed during exacerbation of asthma (Saetta *et al*., 1994). For example, Saetta *et al*. (1994) reported that subjects with chronic bronchitis during exacerbations had around 30-fold more

eosinophils in their bronchial biopsies than did those examined under baseline conditions. To a lesser extent, the numbers of neutrophils, T lymphocytes, and TNF α positive cells were also increased during exacerbations. In another study Saetta *et al*. (1996) showed that the degree of eosinophilia was similar in bronchial biopsies of asthmatic patients and patients with exacerbation of chronic bronchitis. In support and extent of these studies, Zhu *et al*. (2001) reported that following an exacerbation, regulated on activation, normal T-cell expressed and probably secreted expression was up regulated and strongly expressed in the surface epithelium and sub epithelium, which could account for increased numbers of eosinophils. However, both groups of investigators reported that IL-5 expression was not augmented during exacerbation vs. control. Thus, the quality of inflammatory response could be somehow specific to COPD exacerbation.

Bacteria are isolated in 23–56% of the patients with severe acute exacerbation of COPD (Ewig *et al*., 2000). The predominant bacteria recovered from the lower airways of patients with COPD exacerbations are *H. influenzae, S. pneumoniae*, and *M. catarrhalis* (Anthonisen *et al*., 1987). So-called atypical pathogens, such as *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* (Anthonisen *et al*., 1987), have been identified in patients with COPD exacerbations, but because of diagnostic limitations the true prevalence of these organisms is not known.

In a study, bacteria isolated were *Haemophilus influenzae* (22%), *Pseudomonas aeruginosa* (15%), *S pneumoniae* (10%), *Moraxella catarrhalis* (9%), other Gram-negative bacteria (7%), and non-potentially pathogenic microorganisms (non-PPMs; 36%). *P aeruginosa* and *H influenzae* were isolated more frequently among the patients with FEV1 < 50% than among those with FEV_1 > 50% ($p < 0.05$). All patients with *P aeruginosa* in sputum had FEV_1 < 1,700 mL. FEV_1 < 50% was associated with a very high risk of *P aeruginosa* or *H influenzae* isolation: the odds ratios (ORs) are 6.62 (95% confidence interval, 1.2 to 123.6) and 6.85 (95% CI, 1.6 to 52.6), respectively (Miravettles *et al* 1999). A positive bacterial culture was obtained from 59% of purulent sputum samples (Demosthenes, 2007).

In a study conducted by Pudasaini (2004), 75% growth positive rate was found: *Staphylococcus aureus* (15.3%) followed by, *Klebsiella pneumoniae* (10.5%), *Streptococcus pneumoniae* (9.6%), *Moraxella catarrahalis* (4.8%). *Escherrichia coli*

(3.8%), AFB (2.8%) and *Pseudomonas aeruginosa* (1.9%). Fungal pathogen was also isolated in this study, in which, *Candida spp*. was most predominant (18.2%) one followed by *Aspergillus spp*. and *Mucor spp*.

In a study of LRTI including COPD conducted by Tamang et al. in Manipal teaching hospital, Pokhara, *H. influenzae* (28.6%), *S. pneumoniae* (21.16%) and *M. catarrhalis* (6.90%) was isolated.

A total of 193 patients with acute exacerbation were included. In 121 (62.6%) of them a microbial etiology could be identified, most frequently *Haemophilus influenzae* (32 strains), *Streptococcus pneumoniae* (22 strains) and *P. aeruginosa* (12 strains) (Lode *et al*., 2007).

In a study conducted by Patel *et al*, fifteen of the 29 patients (51.7%) were colonised by a possible pathogen: *Haemophilus influenzae* (53.3%), *Streptococcus pneumoniae* (33.3%), *Haemophilus parainfluenzae* (20%), *Branhamella catarrhalis* (20%), *Pseudomonas aeruginosa* (20%). The presence of lower airway bacterial colonisation in the stable state was related to exacerbation frequency (Patel *et al*, 2002).

Of the 498 samples analyzed, 246 (49.4%) were positive and 468 isolates were obtained. The most commonly isolated bacteria was *Streptococcus pneumoniae* (163 cases, 34.8%), followed by *Moraxella catarrhalis* (112, 23.9%), and *Haemophilus influenzae* (59, 12.6%) (Carles *et al*., 2006).

Similarly in the study by Lode *et al*., a total of 193 patients with acute exacerbation were included. In 121 (62.6%) of them a microbial etiology could be identified, most frequently *Haemophilus influenzae* (32 strains), *Streptococcus pneumoniae* (22 strains) and *P. aeruginosa* (12 strains) (Lode *et al*., 2007).

Older studies detected viruses in only 10 to 20% of exacerbations (Smith *et al*., 1980); however, as is the case in asthma patients, more recent studies using PCR have revealed that viruses have a more prominent role in the etiology of exacerbations. In a report (Seemungal *et al*., 2001) from the East London COPD cohort, a respiratory virus was identified in 39% of patients with exacerbations who were treated as outpatients, with rhinoviruses accounting for 58% of the viruses present. Two studies

(Rohde *et al*., 2003; Tan *et al*., 2003) in COPD patients with more severe exacerbations requiring hospital admission detected a respiratory virus in 56% and 64% of patients respectively. In COPD patients with very severe exacerbations requiring intubations and mechanical ventilation, viral infection was identified in 47% (Qui *et al*., 2003). Most studies have focused exclusively on bacterial or viral infection, but a recent study (Papi *et al*., 2006) that carried out sampling for both in sputum samples found evidence of co infection in 25% of patients with exacerbations. Together, these studies suggest that as many as 40 to 60% of acute exacerbations of COPD are associated with respiratory virus infection.

In chronic obstructive pulmonary disease, high levels of airway and systemic inflammatory markers are associated with a faster decrease in lung function. A study shows that patients colonized by *Pneumocystis jiroveci* have higher proinflammatory cytokine levels than do noncolonized patients. This suggests that *Pneumocystis* may play a role in disease progression (Enrique *et al*., 2007). A disseminated *Scedosporium apiospermum* infection was diagnosed in a woman with severe asthma being treated with corticosteroids (Munoz *et al*., 2000). In a study by Redo *et al*. (1998), 8 COPD patients with invasive pulmonary aspergillosis were found.

3.6 Symptoms of chronic obstructive pulmonary disease

COPD symptoms can range from mild to severe, depending upon how advanced the disease. COPD, or chronic obstructive pulmonary disease, is a lung disease characterized by a blockage or narrowing of the airways. It is an irreversible process that is usually brought on by airway irritants, such as smoking, second hand smoke, air pollution or occupational exposure.

3.6.1 Chronic cough

This type of cough is long term and doesn't seem to go away. A cough is a defense mechanism developed by the body in an attempt to clear the airways of mucus, inhaled toxic substances, foreign objects or other types of irritants. A productive cough clears mucus from the lungs, while a non-productive cough does not readily produce mucus. A cough is one of the most common symptoms of COPD.

3.6.2 Increased sputum production

Sputum, or mucus, is a substance produced from the lungs that is usually expelled by coughing or clearing of the throat. Copious amounts of sputum can be associated with inflammation or infection of the respiratory tract and may be indicative of COPD. The color and consistency of sputum that the body produces can be related to the type of COPD that the patient may have, and it is usually requested to describe. Sputum culture is helpful for isolation of bacteria causing exacerbation.

3.6.3 Dyspnea

Also known as shortness of breath, dyspnea is a result of air hunger that causes difficult or labored breathing. It is primarily due to a lack of oxygen in the bloodstream and is directly related to disturbances in the lungs such as with COPD.

3.6.4 Wheezing

Often described as a whistling sound heard during inhalation or exhalation, wheezing is caused by a narrowing or blockage of the airways. Oftentimes, wheezing can be so prevalent that you can hear it without the assistance of a stethoscope.

3.6.5 Chest tightness

Tightness in the chest can be described as a feeling of pressure within the chest walls that makes automated breathing difficult. Sometimes, this tightness makes deep breathing painful causing respiration to be short and shallow. Chest tightness can be due to infection of the lungs and is often associated with COPD.

3.6.6 Clubbing of the fingers

Clubbing is a sign of long-term oxygen deprivation and is associated with a wide number of diseases, COPD included. Initially, it manifests itself as sponginess of the nail bed along with loss of the nail bed angle, causing the nail bed to curve downward.

3.6.7 Haemoptysis

A symptom of lung and heart problems, hemoptysis is defined as the coughing up of blood from the lungs that is frothy and mixed with mucus. In COPD, the most common cause is infection in the lungs. It is important to note that the amount of blood that is coughed up does not always reflect the seriousness of the cause.

3.6.8 Cyanosis

Cyanosis is described as a bluish discoloration of the skin and is a late sign of chronic oxygen deprivation in the blood. Common places for cyanosis to appear are the lips, tongue, nail beds and earlobes.

3.7 Laboratory findings of chronic obstructive pulmonary disease

3.7.1 Chest radiography

Because emphysema is defined in anatomic terms, posteroanterior and lateral chest roentgenograms provide evidence of its presences. Hyperinflation is indicated by a low, flat diaphragm, an increased retrosternal air space, and a long, narrow heart shadow. Studies correlating lung structure and the chest radiograph show that emphysema is consistently diagnosed when the disease is severe but that diagnosis is not accurate when the disease is mild or even moderate. Right ventricular hypertrophy does not result in cardiomegaly in COPD. Comparison with previous chest radiographs may show the enlargement (Celli, 1998).

3.7.2 Computed tomography

Computed tomography, especially high-resolution CT scan (collimation of 1 to 2 mm), has much greater sensitivity and specificity than standard chest radiography. However, because this rarely alters therapy, CT has no place in the routine care of patients with COPD (Celli, 1998).

3.7.3 Pulmonary function tests

The diagnosis and classification of COPD rest on objective the demonstration of airways obstruction by spirometric testing. The forced expiratory volume in one

second (FEV1) and vital capacity are obtained from maximal forced and relaxed expirations into a spirometer. The results are obtained with predicted values based on age, sex, height and ethnic group. Peak expiratory flow (PEF) monitored via a small portable meter can be usefully recorded by patients in home or at work in order to assess asthma control on an objective basis. A normal FEV_1 and PEF are poor in COPD, and PEF in particular may underestimate the degree of airflow obstruction in these patients. Reversibility testing to salbutamol and ipratropium bromide is necessary to detect patients with substantial increase in FEV!, which is the best predictor of long term prognosis. Significant reversibility is defined at a 15.0% and at least 200ml increase in FEV_1. Evidence of a similar objective response to a course of oral prednisone (30mg daily for two weeks) should also be performed in all patients with COPD. Lung volumes show an increase in total lung capacity (TLC) and residual volume (RV) due to gas trapping; the carbon monoxide transfer factor and coefficient are markedly reduced in patients with a severe emphysema component (Haslett *et al*, 1997).

3.7.4 Arterial blood gases

Arterial blood gases reveal hypoxemia without hypercapnia in the early stages. As the disease progresses, hypoxemia become more severe, and hypercapnia supervenes. Hypercapnia is observed with increasing frequency as the FEV1 falls below 1 litre. Blood gas abnormalities worsen during acute exacerbation and may worsen during exercise and sleep. Erythrocytosis is infrequently observed in patients living at sea level who have PaO_2 levels of less than 55mm Hg; the frequency of erythrocytosis increases as PaO_2 levels fall below 55 mm Hg (Celli, 1998).

The normal arterial PaO_2 is over 12 k Pa at the age of 20 and falls to around 11 k Pa at 60 above this age a further fall in PaO_2 of up to 1.3 kPa may occur on lying down because of closure of small airways in the dependent regions of the lungs (Haslett *et al*, 1997).

3.7.5 Sputum

The sputum in chronic bronchitis is very variable (Crofton and Douglas, 2002) and at the early stage, it is often mucoid and forth. With an exacerbation, sputum usually

becomes purulent with an influx of neutrophils (Celli, 1998). Later it may be mucopurulent almost continuously. There may be considerable variation in volume and type of sputum from time to time.

In some patients when systemically examined, it may be found that many of the pus cells are eosinophils (McHardy *et al*, 1980; Turnbull *et al*, 1977). The neuraminic acid content of sputum is increased in more viscous sputum; purulence of tends to be more frequent in patients with higher neuraminic acid content (Keal *et al*, 1972). The gram stain usually shows a mixture of organisms. The most frequent pathogens cultured from the sputum are *S pneumoniae, H influenzae and M catarrhalis*. However, cultures and even Gram stains are rarely necessary before antimicrobial therapy is instituted in the outpatient setting (Celli, 1998).

3.7.6 Haematological findings

The white cell count may be raised in acute exacerbations, especially if there is bronchopneumonia. Occasionally there are eosinophils, most often when the picture is dominated by paroxysmal wheeze, but sometimes eosiniphilia is an unexpected finding. Such patients are more likely to have reversible airways obstruction and to respond to corticosteroid therapy, in which case perhaps they should be regarded as athmatics. Perhaps surprisingly, polycythaemia is closely related to the degree of chronic hyoxaemia between exacerbations (Hume *et al*, 1968) but there is also evidence that it may be related to the amount of carboxyhaemoglobin induced by smoking and that reduction in smoking can reduce the red cell volume (Smith *et al*, 1978). Improvement of exercise tolerance after exchange transfusion with dextran 40 has been demonstrated in a few polycythemiac patients (Harrison *et al*, 1971).

3.8 Management of chronic obstructive pulmonary disease

Outpatient management of patients with stable COPD should be directed at improving quality of life by preventing acute exacerbations, relieving symptoms and slowing the progressive deterioration of lung function. The clinical course of COPD is characterized by chronic disability, with intermittent acute exacerbations that occur more often during the winter months. When exacerbations occur, they typically manifest as increased sputum production, more purulent sputum and worsening of

dyspnea (Anthonisen *et al*, 1987). Although infectious etiologies account for most exacerbations, exposure to allergens, pollutants or inhaled irritants may also play a role (Gump *et al.*, 1976).

3.8.1 Smoking cessation

Smoking cessation remains the most important intervention in modifying the course of the disease (Anthonisen *et al*., 1994) and is cost effective (Tings *et al.*, 1995; Parrott *et al.*, 1998). Nicotine dependency is a chronic relapsing condition which may require repeated interventions (Roijin, 2006).

Even brief counseling is effective in producing quit rates of around 5% (Parrott *et al*). Several effective smoking cessation pharmacotherapies now exist. Nicotine replacement therapy in any form (nicotine gum, inhaler, nasal spray, transdermal patch, sublingual tablet, or lozenge) is effective in increasing long term quit rates. Several studies have shown the effectiveness of the antidepressant bupropion with counseling and support in producing increased long term quit rates at 1 year of 30% (Jorenby *et al*, 1999).

3.8.2 Atmospheric pollution

Although the traditional atmospheric air pollutants that resulted from the burning of fossil fuels, such as black smoke and sulfur dioxide, have decreased considerably in developed countries, recent evidence has implicated other pollutants derived increasingly from vehicle traffic, such as ozone and particulate air pollution, in the exacerbation and increased mortality in patients with COPD (Schwartz *et al*,1990; Dockerly *et al*, 1993). A biologically plausible hypothesis has been proposed to account for the harmful effects of particulate air pollution at such low levels that implicates the reactivity of the particles as a result of their ultra fine size and their oxidative properties, which create airway inflammation (Seaton *et al*, 1995). As a result it seems justified, if practical, for patients with COPD to stay indoors and certainly to sleep with closed windows during episodes of high air pollution (Roijin, 2006).

3.8.3 Bronchodilators

Prevention and relief of symptoms by regular use of bronchodilators remains central to the management of COPD (Urlik *et al.*, 1995).There is now compelling evidence, at least in more severe COPD, that a major benefit of bronchodilator therapy is to improve lung emptying during expiration. This reduces dynamic hyperinflation at rest and during exercise and so improves exercise performance (Belman *et al.*, 1996). The degree to which this occurs is not readily predictable from the improvement in FEV_1 after an acute bronchodilator trial.

Assessment of the effectiveness of bronchodilators is therefore best done by asking simple questions about changes in their symptoms. The choice between b agonists, anticholinergic drugs, theophylline or combination therapy depends on individual symptomatic responses. Combining bronchodilators may improve efficacy and decrease side effects compared with increasing the dose of a single bronchodilator (Chest, 1994).

Long acting inhaled β agonists such as salmeterol and formoterol have duration of action of 12 hours and significantly improve symptoms, exercise capacity, and health status in patients with COPD (Urlick, 1995; Boyd *et al*., 1997; Cazzola *et al*., 1995). A new long acting once daily anticholinergic agent, tiotropium, produces benefits of equivalent or greater size (Calverley, 2000) and is likely to be a useful addition to treatment for COPD. Both long acting β agonists and long acting anticholinergic agents reduce exacerbation rates in COPD, (Rannard *et al*., 2001; Casaburi *et al*., 2003) raising important questions as to what determines these events. There is no evidence of tachyphylaxis with these long acting bronchodilators and they are well tolerated. To date there are no data about the combination of different classes of long acting drugs, although short acting anticholinergic agents can be usefully combined with long acting β agonists (Van *et al*., 2000).

High doses of nebulised bronchodilators are still widely prescribed in severe COPD, but the BTS guidelines on nebuliser treatment (Thorax 1997) recommends that the appropriateness of their use should be assessed by a respiratory specialist. It is recommended that the response to high dose bronchodilators via a spacer device should be assessed before trying long term nebulised treatment.

Theophyllines remain somewhat controversial in the management of stable COPD. Their mode of action as a non-selective phosphodiesterase inhibitor is still controversial but they have been shown to produce bronchodilatation in COPD (Murciano *et al*., 1989; Mckay *et al*., 1993) with a variable effect on exercise tolerance and symptoms (Mullay *et al*., 1993; murciano *et al*., 1984; Cooper *et al*., 1987). The narrow therapeutic index of theophyllines limits their use. They have a slow onset of action and are used as maintenance treatment rather than for rapid relief of symptoms. Newer more specific phosphodiesterase inhibitors, particularly of phosphodiesterase 4 (PDE4) inhibitors, have been shown to improve lung function in COPD and may also reduce exacerbation rates (Edelson *et al*., 2001). However, the results of further studies are awaited before these drugs can be confidently recommended.

Given that long acting bronchodilators taken once/twice daily produce the same or better relief of symptoms than regular short acting bronchodilators, it is sensible to introduce these drugs earlier in the management plan in patients with COPD when they require regular treatment for symptom relief. Theophyllines can be considered in patients who remain symptomatic despite long acting bronchodilators, but clear evidence of improvement in symptomatology should be obtained before continuing these drugs, given the more complex management involved in administering them safely (MacNee, 2003).

3.8.4 Inhaled corticosteroids

Whether inhaled corticosteroids have an anti-inflammatory effect in patients with COPD remains controversial. The variable effects of corticosteroids on airway inflammation may reflect the heterogeneity of the disease and also the reproducibility sof markers of inflammation (Keatings *et al*.1997). What is clear is that these drugs do not modify the natural history of COPD, as measured by the rate of decline in FEV_1. Four large randomized controlled trials EUROSCOP (Pauwels *et al*., 1999) Copenhagen City Lung study, (Vestbo *et al*., 1999) ISOLDE, and Lung Health Study2 all found that inhaled corticosteroids had non-significant effects on the decline in FEV_1 in patients with mild and moderately severe COPD. One of these studies (Burge *et al*., 2000) in patients with more severe COPD showed a reduction in exacerbations (from 1.33 to 0.99 per year, a reduction of 25%), which supports results in an earlier

smaller study which showed a reduction in severity of exacerbations with inhaled corticosteroid (Paggiaro *et al*., 1998). From these studies it is concluded that inhaled corticosteroids should be recommended to patients who have a demonstrable FEV_1 response to a trial of corticosteroids or in those with moderate to severe disease (FEV1 <50% predicted) with repeated acute exacerbations (a reasonable number would be two or more exacerbations per year) requiring treatment with antibiotics or oral corticosteroids. The precise dose required to reduce exacerbations in patients with moderate to severe COPD is not known but, on present evidence, a higher dose of inhaled corticosteroids should be given to achieve such an effect (GOLD, 2007).

The effects of a combination of inhaled corticosteroids and long acting β agonists are being studied at present. Data currently in press indicate that combining a long acting β agonist and an inhaled corticosteroid produces significantly greater improvement in symptoms and pulmonary function than either alone, with equivalent reductions in exacerbation frequency. Full analysis of these data will be needed before a firm recommendation about optimal treatment can be made (MacNee, 2003).

3.8.5 Vaccines

Vaccination can reduce severe complications and mortality from influenza in older patients, including those with COPD, and is recommended to be given once in the autumn or twice in autumn and winter each year (Nichol *et al*., 1994; Hac *et al*., 1998). Pneumococcal vaccine has been used in patients with COPD and can reduce complications of pneumonia in elderly patients, but there are insufficient data to support its general use in patients with COPD (Simbekoff *et al*., 1986).

3.8.6 Antibiotics therapy

Several large scale control studies have shown that prophylactic or continuous antibiotics have no effect on the frequency of exacerbations in COPD, nor is there any effect of antibiotic prophylaxis during winter periods (Mac Nee, 2003).

Antibiotic therapy has been shown to have a small but important effect on clinical recovery and outcome in patients with acute exacerbations of chronic bronchitis and emphysema. Therefore, antibiotic administration should be considered at the

beginning of treatment for exacerbations of COPD. A recent meta-analysis of nine clinical trials demonstrated the benefit of antibiotic therapy in the management of COPD. Therapy for moderate acute exacerbations of chronic bronchitis and emphysema should be directed at *S. pneumoniae*, *H. influenzae* and *M. catarrhalis*, which are the most common pathogens, with *C. pneumoniae* and *Mycoplasma pneumoniae* occurring less often. Initial outpatient management may include orally administered doxycycline (Vibramycin), trimethoprim-sulfamethoxazole (Bactrim DS, Septra DS) or amoxicillin-clavulanate potassium (Augmentin). Patients who are older than 65 years of age or have more frequent exacerbations (four or more episodes per year) may need augmented penicillin or a fluoroquinolone. Hospitalized patients should receive intravenous treatment with antipseudomonal penicillin, a third-generation cephalosporin, a newer macrolide or a fluoroquinolone, as determined by local bacterial resistance patterns. In more severe exacerbations, infections with gram-negative bacteria (especially *Klebsiella* and *Pseudomonas* species) are more common. Thus, treatment should include a third-generation cephalosporin or augmented penicillin, plus a fluoroquinolone or an aminoglycoside for synergy (Hunter *et al*., 2001).

Antibiotic resistance poses an increasing problem, especially in infections caused by *S. pneumoniae*, beta-lactamase producing *H. influenzae* and *M. catarrhalis.* Consequently, physicians often are forced to use broader spectrum antibiotics for empiric therapy. Cultures of respiratory samples are useful for guiding antibiotic therapy in patients who require mechanical ventilation (GOLD, 2007).

3.8.7 Mucolytic drugs

Mucolytic drugs (ambroxol, erdosteine, carbocysteine, iodinated glycerol) have recently undergone a meta-analysis by the Cochrane collaborative group and have been shown to produce a statistically significant reduction in the number of exacerbations of chronic bronchitis compared with placebo. These studies are of relatively short duration, over a period of 2–6 months, in patients with mild COPD (FEV1 >50% predicted) and therefore the general use of mucolytic drugs in COPD is not yet recommended (MacNee, 2003).

3.8.8 Antioxidant agents

Oxidant related lung damage is an important mechanism contributing to lung damage in COPD. Antagonizing these effects is an attractive treatment strategy, and the antioxidant and mucolytic drug N-acetylcysteine has been shown to reduce the frequency of COPD exacerbations in most studies (Grandejan, 2000). Results of a long term randomised control trial to both assess the effect of N-acetylcysteine on the decline in FEV_1 in COPD patients and in reduction of exacerbations are awaited. However, at present this drug is not licensed for use in COPD, at least in the UK (MacNee, 2003).

3.8.9 Rehabilitation

A large number of clinical trials have now shown that pulmonary rehabilitation is beneficial in COPD (ATS, 1999). Benefits include an increased exercise capacity, reduction in the sensation of breathlessness, improvement in health status, and reduction in the number of hospital admissions, all of which have been shown in randomised control trials. If exercise training is maintained, these benefits can be sustained. Whether repeated rehabilitation courses enable patients to maintain benefits gained in the initial course is still a matter of debate. Rehabilitation programs are effective in inpatients, outpatients and in those treated at home (Griffths, 2000). Availability and cost may determine the setting which is used, an outpatient setting being the least expensive. There is also evidence which indicates that rehabilitation may reduce the length of hospital stay (Griffths, 2000).

3.8.10 Oxygen therapy

The indications for domiciliary oxygen therapy have changed in the last 5 years and there are now good data to suggest that less hypoxaemic patients do not benefit from domiciliary oxygen, (Gorecka *et al*, 2000) or do those showing isolated nocturnal desaturation with more preserved daytime gas tensions. This probably reflects the slow rate of deterioration in pulmonary haemodynamics recently observed in these patients. In contrast, breathing oxygen during exercise improves endurance time by reducing dynamic hyperinflation even in those who do not desaturate (MacNee, 2003).

3.8.11 Lung volume reduction surgery

Surgical interventions in COPD include lung transplantation and lung volume reduction procedures. Recent advances in immune suppression and an improved understanding of the timing of interventions and the selection of appropriate recipients have made transplantation a realistic option (MacNee, 2003).

The initially surprising observation that removing lung can increase exercise capacity (Cooper *et al*, 1999) has been repeatedly confirmed and reflects a combination of reduced dynamic hyperinflation, improved diaphragm function, and improved pulmonary elastic recoil. The effects on symptoms can persist for several years thereafter. The effectiveness of treatment has been confirmed in two randomized controlled trials with up to 12 month follow up. The effects on symptoms can persist for several years thereafter. However, the large National Emphysema Treatment Trials in the US have shown that the patients with FEV1 or carbon monoxide transfer factor (T*LCO*) of <20% predicted or a homogeneous distribution of emphysema on the CT scan have a higher mortality with surgical than with conservative medical treatment (2002).

CHAPTER IV

4 METHODOLOGIES

4.1 Materials

Various materials used in this study are enlisted in the appendix II.

4.2 Methods

This study was conducted at Shree Birendra Hospital, Chhauni, Kathmandu. In this study 191 sputum samples were collected from the suspected patients suffering from COPD and processed following the standard laboratory techniques in the Hospital.

4.2.1 Study design

A hospital based cross sectional study was done.

4.2.2 Population and sample size

Populations for the study were the patients attending Respiratory medical OPD and inpatients admitted to Shree Birendra Hospital, Chhauni, Kathmandu. All the samples according to the criteria of the study were included in the study.

4.2.3 Inclusion criteria

Patients previously diagnosed as COPD with symptoms of showing an acute exacerbation of COPD, who were above 40 years of age and not taking antibiotics from previous 72 hours were only included in the study. After the sample collection, before culturing, a wet preparation was examined microscopically. Sputum smear containing less than 10 squamous epithelial cells and more than 25 neutrophils per low power field confirmed the reliability of the specimen indicating that it was not contaminated with saliva. Sample not meeting these criteria was rejected.

4.2.4 Exclusion criteria

Any patient not having COPD or does not fit into the criteria for acute exacerbation were excluded from the study.

4.2.5 Site of study

Site for the collection of data was Shree Birendra Hospital, Chhauni, Kathmandu the sampling was done in the patients fitting inclusion criteria.

4.2.6 Time frame

The collection of the data and laboratory work was conducted for six months from November 2009 to February 2010.

4.3 Collection of the data

The data regarding the patients was collected directly by interview by using semi-structured questionnaire; Clinical History of the patient's involved in the study. The history of all the patients including age, weight, sex, spirometric value, smoking habit, fuel used for cooking purpose, previous history of hospitalization, symptoms was recorded in the data collection form. This study was carried out to isolate bacteria and fungi from COPD patients and to study antibiotic sensitivity pattern of the isolated bacteria.

4.4 Collection of samples

Sputum sample was collected in a wide mouthed, sterile bottle and brought directly to the SBH laboratory.

4.5 Processing of samples

Immediately after sputum sample were received in the laboratory, they were provided with unique laboratory identification numbers and further preceded.

4.5.1 Macroscopic examination of sample

In the laboratory, the macroscopic appearance of the sputum sample was examined first. The collected sputum was classified as follows-

1. Purulent-green looking, mostly pus,
2. Mucopurulent-yellowish green looking with pus and mucus,
3. Mucoid-mostly mucus,
4. Mucosalivary-mucus with small amount of saliva.

When the sputum sample received contains mostly saliva with froth, it is reported as 'unsuitable for microscopic examination' and discarded.

4.5.2 Identification with staining reaction

Gram-staining was performed to observe if the sample maintains the inclusion criteria and for presumptive identification of the isolates, the procedure of which is mentioned in Appendix IV.

4.5.3 Culture of sample

The sputum samples were inoculated into the Blood agar, Chocolate agar and MacConkey agar plates. For sputum, in Blood agar plate a 5 µg Optochin disc and in a Chocolate agar plate 10 U Bacitracin disc were added to screen out Streptococcus pneumoniae and Haemophilus influenzae respectively. The chocolate agar and blood agar plates were incubated at 37^0C for overnight in 5-10% CO_2 environment whereas the MacConkey agar plate was incubated at 37^0C in an aerobic condition. Sabouraud dextrose agar plate was also inoculated with the sputum and incubated for 5 days.

4.5.4 Identification of the isolates

4.5.4.1 Identification of Gram positive isolates

The identification of the bacterial isolates was done by standard diagnostic procedures. Bacitracin-Optochin sensitivity test, bile solubility test was done for *Streptococcus* spp., and coagulase test for *Staphylococcus* spp. were performed additionally in addition to various biochemical tests.

4.5.4.2 Identification of Gram negative isolates

The identification of various gram negative isolates was done by using standard microbiological techniques as described in Bergey's Manual of systemic bacteriology which comprises of studying the colonial morphology, staining reactions and various biochemical properties. Isolated colonies from the pure culture were identified by performing the standard conventional biochemical tests (appendix V).

4.6 Purity test

The inoculums used for the biochemical tests were pure culture. The purity was used to ensure that the inoculums used for the biochemical tests was pure culture. So, before performing biochemical test, the same inoculums was sub cultured in respective medium in order to confirm the purity of the inoculums.

4.7 Sensitivity tests for isolated organisms

Antibiotics selection criteria

For Gram negatives isolates: Since the study conducted was hospital based, primarily the antibiotics were chosen on the basis of the use in the hospital.

For Gram positive isolates: Similarly as for Gram negative isolates, antibiotics generally used in Hospital were tested.

However, some antibiotics were used for the research purposes on the basis of available literature.

Antibiotics used were penicillin, amoxicillin/clavaulinic acid, ciprofloxacin, tetracycline, erythromycin, sulfomethaxazole, cotrimoxazole, ceftazidime, ciprofloxacin, ampicillin, gentamicin, amikacin, cephalexin, penicillin/tazobactam, methicillin, cefexime, chloramphenicol, tetracycline.

After identification of isolated organism, the sensitivity test in vitro was performed for the clinically significant organism. This test was performed by following Kirby-Bauer disc diffuse technique. In this technique the antimicrobial agent diffuse from the disc into the medium. Following overnight incubation, the culture was examined

for areas of no growth around the disc. Bacterial strains sensitive to the antimicrobial are inhibited at a distance from the disc where as resistant strain grows up to the edge of the disc.

Isolated colony of organism was transferred to a test tube containing 4-5 ml nutrient broth and incubated at 37ºC for 24 hours. After 24 hours, it was swabbed on Mueller-Hinton Agar plates with the help of sterile cotton swab and left for 15 minutes at room temperature for drying. For Streptococcal isolates Blood agar were used for antimicrobial susceptibility tests. With the help of flamed forceps, selected antibiotic discs were then placed on the inoculated plate, no closer than 15 mm from the edge of the plate and 24 mm apart from each other and also from centre to centre. Then the plate was allowed for 30 minutes at room temperature for diffusion and incubated at 37ºC for 24 hours. After incubation, the zone of inhibition of test organism was measured and observed into sensitive, intermediate, and resistant categories by referring in an interpretative chart table supplied by the disc manufacturers.

The preparation and composition of Mueller-Hinton Agar medium is mentioned in the Appendix III.

The detail about antibiotic discs used and its interpretative chart are mentioned in the Appendix VI.

4.8 Quality control of the test

Quality control is considered as one of the important factor for the correct result interpretation. During this study, quality control was applied in various areas.

a. During sample collection, patient was verbally instructed to bring the sputum without contaminating it with the saliva.
b. During sample processing, the entire tests were carried out appropriately in aseptic condition.
c. While using ready made dehydrated media, the manufacturer’s instruction for preparation, sterilization and storage were followed to prevent the alteration of the nutritional, selective. Inhibitory and biochemical properties of the media.

d. The performance of newly prepared media was tested using control species of bacteria (i.e. known organism giving positive and negative reactions).
e. For strain and reagents, whenever a new batch of them were prepared, a control smear was stained to ensure correct staining reaction.
f. Control strains of E. coli (ATCC25922), Staphylococcu aureus (ATCC 25923) and *Pseudomanas aeruginosa* (ATCC 27853) were used for the standardization of the Kirby-Bauer test to adjust the diameter of the inhibition zone.

4.9 Data Analysis

All the results were entered in the worksheet of Statistical Package of Social Sciences (SPSS 14.0). Chi-square test was used to determine the association of independent variables like age, sex, color of sputum, number of exacerbation, spirometric value, previous history of hospitalization, smoking habit etc to culture positive cases.

CHAPTER V

5 RESULTS

Sputum samples were collected from patient having acute exacerbation of COPD. Inpatients as well as outpatients were included in the study. Of the total 191 samples collected only 158 (82.7%) met the inclusion criteria, which were further processed. Culture was done for the isolation and appropriate biochemical test was done for the identification of the isolate. Finally antibiotic susceptibility test was performed. The sample was collected after completely feeling the questionnaire.

5.1 Bacteriological pattern of the result

5.1.1 Growth pattern of bacteria

Out of the total 158 valid samples 70 gave the significant growth of pathogen and 88 were culture negative.

Table 1: Growth pattern of bacteria

S. No.	Growth	No. of Samples	Percentage of Samples
1	Culture positive	70	44.3
2	No Growth	88	55.7
Total		158	100.0

5.1.2 Pattern of different species of bacteria isolated

Of the total 70 pathogen isolated *S. pneumoniae* (28.6%) was the most predominant one and *S. pyogenes (*5.7%) the least.

Table 2: **Distribution of pathogens in COPD**

Organisms	Number	Percent
Gram Negative		
Klebsiella pneumoniae	15	21.4
Moraxella catarrhalis	13	18.6
Pseudomonas aeroginosa	12	17.1
Gram Positive		
Streptoccus pneumoniae	20	28.6
Staphylococcus aureus	6	8.6
Streptococcus pyogenes	4	5.7
Total	70	100

5.1.3 Sex wise distribution of culture positive cases

Out of 158 patients requested for sputum culture the number of female was 84 (53.2%). Female patients had requested more culture than male patients. Statistically there was no relation between sex of the patient and culture positive cases, i.e. the result was insignificant.

Table 3 : Sex wise distribution of culture positive cases

Sex	**Total cases(N=158)**		**Total culture positive cases (N=70)**		**p value**
	No.	**Percent**	**No.**	**Percent**	
Male	74	46.8	32	45.7	>0.05
Female	84	53.2	38	54.3	

5.1.4 Age wise distribution of culture positive cases

In this study, the age of the patient ranged from 41 years to 83 years. The number of patients was almost equal in all age groups except those belonging to the age group of

41-50. The highest percentage of infection (32.9%) was found in the age group in the range of 61-70 and more than 70. As the age of the patient increased, culture positive rate increased. Statistically, there was association between age group of patients and culture positive result.

Table 4: Age wise distribution of culture positive cases

Age group	**Total cases(N=158)**		**Total culture positive cases (N=70)**		**p value**
	No.	**Percent**	**No.**	**Percent**	
41-50	14	8.9	6	8.6	<0.001
51-60	51	32.3	18	25.7	
61-70	60	38.0	23	32.9	
>70	33	20.9	23	32.9	

5.1.5 Distribution of culture positive cases according to spirometric value

The patient's spirometric value was also noted and was classified into 5 groups. Maximum number of patient included in the study has spirometric value in the range of 60-70% and half of the culture positive cases were from this group. The spirometric value and the culture positive cases were found statistically significant.

Table 5: Distribution of culture positive cases according to spirometric value

Spirometric value	**Total cases(N=158)**		**Total culture positive cases (N=70)**		**p value**
	No.	**Percent**	**No.**	**Percent**	
Less than 50 %	1	0.6	1	1.4	<0.001
50-60 %	21	13.3	16	22.9	
60-70 %	56	35.4	33	47.1	
70-80 %	47	29.7	13	18.6	
More than 80 %	33	20.9	7	10.0	

5.1.6 Geographical wise distribution of culture positive cases

The patients living in the Kathmandu valley were classified as those of valley and those not living in valley as out of valley. The number of patient in both groups was almost equal. The data was statistically insignificant, i.e., there was no relation between the geographical location and culture positive cases.

Table 6 : Geographical wise distribution of culture positive cases

Locality	**Total cases (N=158)**		**Total culture positive cases (N=70)**		**p value**
	No.	**Percent**	**No.**	**Percent**	
Valley	77	48.7	36	51.4	>0.05
Out of valley	81	51.3	34	48.6	

5.1.7 Distribution according to the number of family members

The patients were categorized according to their family size. Patients having number of members up to five were 101 (63.9%) and more than five were 57 (36.1%).There was no significant difference between the family size and culture positive cases.

Table 7: Distribution according to the number of family members

No. of members	**Total cases (N=158)**		**Total culture positive cases (N=70)**		**p value**
	No.	**Percent**	**No.**	**Percent**	
1-5	101	63.9	44	62.9	>0.05
>5	57	36.1	26	37.1	

5.1.8 Distribution according to the smoking habit of the patient

Patients were classified into smoker, ex-smoker and non-smoker. Ex-smokers predominated the other two. Statistically, no significant difference was found between smoking habit and culture positive cases.

Table 8: Distribution according to the smoking habit of the patient

No. of members	Total cases (N=158)		Total culture positive cases (N=70)		p value
	No.	Percent	No.	Percent	
Smoker	27	17.1	13	18.6	>0.05
Ex-smoker	109	69.0	50	71.4	
Non-smoker	22	13.9	7	10	

5.1.9 Distribution according to the presence of other smokers in the house

To find the effect of passive smoking data was also collected in terms of other smokers in house. No significant difference was found between presence of other smokers in house and culture positive cases.

Table 9: Distribution according to the presence of other smokers in the house

Presence of other smokers	Total cases (N=158)		Total culture positive cases (N=70)		p value
	No.	Percent	No.	Percent	
Yes	63	39.9	26	37.1	>0.05
No	95	60.1	44	62.9	

5.1.10 Distribution according to the number of exacerbations

Patients were also classified according to the number of exacerbations they had in previous year. Most predominant one were those having one exacerbation i.e., 69 (43.7%) of total and 52 (32.9%) had no exacerbations. Number of exacerbations and culture positive cases were found to be statistically significant.

Table 10: Distribution according to the number of exacerbations

No. of exacerbations	Total cases(N=158)		Total culture positive cases (N=70)		p value
	No.	Percent	No.	Percent	
0	52	32.9	7	10	<0.001
1	69	43.7	38	54.3	
2	33	20.9	23	32.9	
3	4	2.5	2	2.9	

5.1.11 Distribution according to the color of sputum

Sputum was classified into four types according to its color. Sixty patients (38.0%) had yellow colored sputum followed by patient having green color sputum. The color of sputum and culture positiveness was found to be statistically significant.

Table 11: Distribution according to the color of sputum

Color of the sputum	Total cases(N=158)		Total culture positive cases (N=70)		p value
	No.	Percent	No.	Percent	
Clear	37	23.4	6	8.6	<0.001
Yellow	60	38.0	23	32.9	
Green	58	36.7	40	57.1	
Other	3	1.9	1	1.4	

5.1.12 Distribution according to the type of cooking fuels

Patient was classified into three groups on the basis of fuel they use for cooking purpose. In general patient included in this study used, firewood (61.4%), gas (38.0%) and kerosene (0.6%). The data was not significant statistically.

Table 12: Distribution according to the type of cooking fuels

Type of fuel	Total cases (N=158)		Total culture positive cases (N=70)		p value
	No.	Percent	No.	Percent	
Firewood	97	61.4	45	64.3	>0.05
Gas	60	38.0	25	35.7	
Kerosene	1	0.6	0	0	

5.1.13 Distribution according to the previous history of hospitalization

There were 106 (67.1%) previously hospitalized patient and 52 (32.9%) previously not hospitalized patient. There was no significant difference in the patients' previous history of hospitalization and culture positive cases.

Table 13: Distribution according to the previous history of hospitalization

Previously hospitalized	Total cases (N=158)		Total culture positive cases (N=70)		p value
	No.	Percent	No.	Percent	
Yes	106	67.1	51	72.1	>0.05
No	52	32.9	19	27.9	

5.2 Fungal pattern of the result

Of the total 158 sample, only 28 were growth positive for fungi and the remaining 130 did not show the growth of fungi.

Table14: Fungal pattern of the result:

S. No.	Growth	No. of Samples	Percentage of Samples
1	Growth positive	28	17.7
2	No Growth	130	82.3
Total		158	100.0

5.3 Antibiotic Susceptibility Pattern of bacterial isolates

Among the antibiotics evaluated, *S. pneumoniae,* was found highly susceptible to tetracycline and sulfomethaxazole (85%). The least susceptibility was towards penicillin which was 60%. *S. aureus* were found to be 100% susceptible to methicillin. The *S. pyogenes* isolated were found to be 100% susceptible to penicillin, erythromycin, tetracycline and 100% resistant to cotrimoxazole.

Table 15: Antibiotic susceptibility pattern of bacterial isolates

S.N.	Organisms isolated	Antibiotics used	Antibiotic Susceptibility Pattern			
			Susceptible		Resistant	
			No.	%	No.	%
1.	*Streptococcus pneumoniae* N=20	Penicillin	12	60	8	40
		Amoxycillin/clavaulinic acid	15	75	5	25
		Ciprofloxacin	16	80	4	20
		Tetracycline	17	85	3	15
		Erythromycin	14	70	6	30
		Sulfomethaxazole	17	85	3	15
2.	*Klebsiella pneumoniae* N=15	Cotrimoxazole	10	66.7	5	33.3
		Ceftazidime	8	53.3	7	46.7
		Ciprofloxacin	9	75.0	6	25.0
		Ampicillin	4	26.7	11	72.8
		Gentamicin	9	75.0	6	25.0
		Amikacin	10	66.7	5	33.3
3.	*Moraxella catarrhalis* N=13	Ampicillin	9	69.2	4	30.8
		Ciprofloxacin	11	84.6	2	15.4
		Amoxycillin-clavulanic acid	12	92.3	1	7.7
		Co-trimoxazole	9	69.2	4	30.8
		Cephalexin	9	69.2	4	30.8
		Ceftazidime	12	92.3	1	7.7

4.	*Pseudomonas aeruginosa* N=12	Amikacin	6	50.0	6	50.0
		Tazobactam	9	75.0	3	25.0
		Ciprofloxacin	5	41.7	7	58.3
		Gentamicin	3	25.0	9	75.0
		Cephalexin	2	16.7	10	83.3
		Cotrimoxazole	2	16.7	10	83.3
5.	*Staphylococcus aureus* N=6	Methicillin	6	100	0	0.0
		Erythromycin	6	100	0	0.0
		Co-trimoxazole	3	50.0	3	50.0
		Ciprofloxacin	2	33.3	4	66.7
		Cephalexin	2	33.3	4	66.7
		Amoxycillin-clauvalinic acid	4	66.7	2	33.3
6.	*Streptococcus pyogenes* N=4	Penicillin	4	100	0	0.0
		Cotrimoxazole	0	0.0	4	100
		Cefexime	3	75	1	25
		Erythromycin	4	100	0	0.0
		Tetracycline	4	100	0	0.0
		Chloramphenicol	4	100	0	0.0

Fig.1. Antibiotic susceptibility test of S. pneumoniae

Fig.2. Pure culture of *S. aureus* isolated on MSA

CHAPTER VI

6 DISCUSSIONS AND CONCLUSION

6.1 Discussion

The role of bacterial pathogen in acute exacerbation of COPD is controversial. In previous studies, the rates of isolation of bacterial pathogens were same during both acute exacerbation and stable condition. So, for a time, not so much emphasis was given for the role of pathogen in the progression of COPD. But nowadays, the isolation of a new strain of bacterial strain supports the causative role of bacteria in exacerbation of COPD (Sethy *et al.*, 2002).

Of the total sputum requested for culture, 82.7% were only accepted after microscopy. Sputum becomes contaminated with oropharyngeal flora during collection and due to lack of instruction patients sometimes are not able to differentiate between sputum and saliva. So, before sample collection, patients should be verbally instructed and sputum microscopy should be done before culture.

Our study has shown a pathogen prevalence rate of 44.3%, which shows that the half of exacerbation are caused by bacteria. So clinician while prescribing the drugs should take microbiology laboratory findings under consideration. Our result is quite similar to the findings of Growengen *et al.* (2003) which was 50.0% and Patel *et al.* (2002) 51.7%. In studies by Ewig *et al.* (2000) and Arora *et al.* (2001), the pathogen prevalence rate was found to be 72.0%, which is quite higher than our study. Growth positive rate, types and frequency of pathogens have been found to be different in different studies. In a study by Zhao *et al* (1999), the pathogen prevalence rate was found to be 26.6% and the major pathogens isolated were *Staphylococcus aureus, Micrococcus spp* and *Streptoccus pnumoniae.*

The number of gram positive and gram negative organism isolated were almost equal. This shows that both types of organisms are responsible for the exacerbation.

Organisms isolated in our study are, *Streptococcus pneumoniae, Klebsiella pneumoniae, Moraxella catarrhalis, Pseudomonas aeruginosa, Staphyoloccus aureus*

and *Streptoccus pyogenes*. This shows *S. pneumoniae* to be the predominant one, the organism may harmlessly inhabit the upper respiratory tract but may also gain access to the lungs by aspiration where it may establish the exacerbation. The patients with exacerbation of COPD are most of old age as well some are immunocompromised. Similar to our study, in a study by Arora *et al*. the predominant organism isolated was *Streptococcus pneumoniae* (25.86%). Other organism isolated were, *P aeruginosa* 12%, *Klebsiella* spp 10.3%, *Moraxella catarrhalis* 3.4% and *S. aureus* 1.7%. Similar to our study, *H. influenzae* was not isolated in this case. The highest numbers of *S. pneumoniae* were isolated by Barsan *et al*. In their cohort study 25% of their isolates were *S. pneumonia*e. Chodosh *et al*. in their study had 22.24% of their 24 isolates as *S. pneumoniae* (1992).

A variety of microorganisms have been shown to be associated with the acute exacerbation of chronic bronchitis. However various studies have shown the predominant organism to be *Haemophilus influenzae*. The role of *H. influenzae* in the initiation and perpetuation of the vicious cycle damage was shown by Cole and Wilson *et al.* (1989).

Our results differ from these studies as we could not isolate any *H. influenzae* from these patients even though chocolate agar with bacitracin disk, a selective medium for *H. influenzae* was plated with sputum in all cases. It could have occurred because of prior antibiotic use or seasonal variations in cause or due to the fluctuation of temperature in incubator.

Though *S. pyogenes* is not detected as pathogen responsible for exacerbation of COPD in most of researches, our research isolated *S. pyogenes* from sputum sample. In a research conducted by Jha *et al*. (2006) 0.81% of *S. pyogenes* was isolated from lower respiratory tract infection.

Though Ball *et al*. showed that *Pseudomonas aeruginosa*, *Staphylococcus aureus* and opportunistic gram negatives accounted for about 15% of all bacterial exacerbations; our study has shown a higher percentage (1995). Though Morrison *et al* have shown that oropharyngeal carriage of pseudomonads in otherwise healthy members of the community is low, being about 5%, it increase with increasing age, hospitalization, underlying chronic illness and debilitating disease (1984). A large number of patients

in our study population were the regular patients of the hospital; hence the high percentage of *Pseudomonas aeruginosa* in our study population could be because of increased oropharyngeal carriage by these patients. It has been shown that organisms persist in sputum after apparently effective therapy of acute exacerbation of chronic bronchitis and the same isolates were responsible for subsequent relapses (Calder *et al*., 1979). A higher incidence of *Pseudomonas aeruginosa* could also partly be explained due to co-existent bronchiectasis in some of these patients. So special attention in case of inpatients should be given and during their sputum culture care should be taken not to miss pseudomonads, which are also responsible for many hospital acquired infection. *Staphylococcus* spp. and gram negative organisms like *Pseudomonas* and *Klebsiella* spp. have been known to colonize bronchiectatic lung particularly in patients in whom antibiotics have suppressed the common invader or colonizer (Lees *et al*., 1959). Spencer *et al*. in their cohort demonstrated that prior antibiotic use increased the isolation of coliform bacilli from sputum (Woodhead *et al*., 1991).

The importance of Pseudomonas in acute exacerbations in severe COPD has been confirmed by another study in a group of patients with severe exacerbations of COPD requiring mechanical ventilation. This study revealed an unexpectedly high rate of Gram-negative organism and Pseudomonas/Stenotrophomonas spp. isolation in respiratory samples from these patients; these pathogens accounted for 44% of all PPMs identified, whereas H. influenzae was found in 33% and S. pneumoniae constituted only 11% of PPMs isolated (Soler *et al*., 1998).

In our study the numbers of female were almost equal to the number of male. Though our study did not find any significant difference in the disease prevalence due to sex, there are many studies which showed women are more susceptible to the disease. As female in case of Nepal are exposed to indoor air pollution while cooking, they are more prone to the disease. This insignificance should not be taken as the reason to neglect the increasing prevalence of COPD in women in many other countries.

In a study by Pandey, it is mentioned that approximately half the world's households especially in developing countries use biomass fuels for cooking and heating purposes often in stoves with no chimneys and in poorly ventilated houses. The pollutants are carbon monoxide, Sulphar and nitrogen oxides, polyaromatic hydrocarbons such as

benzo(α)pyrene, aldehydes such as formaldehyde (WHO 1984,Chen et. al, 1990). So in our study we tried to find the relation between the fuel used for cooking purpose and the organism isolation. As the data in our study is not normally distributed, this might be the reason behind insignificant result.

The severity of COPD increases with age which was also proved by our study. Patient of age more than 60 years gave maximum culture positive cases. The same result pattern was also obtained by the study by Ho *et al.* (2001) where he has mentioned that older age (75 years or more) is one of the important risk factor of Levofloxacin-resistant *S. pneumoniae*. Another possible explanation may be that elder people are more likely to be immunocompromised and are supposed to have large quantity of antibiotics in their previous lifetime that may develop resistance in the body. The study by Pudasaini (2004), also found significant difference in the age and culture positive cases. So, regular healths check up of elderly patients' having COPD even not at the time of exacerbation is recommended.

Though previous researchers have identified the degree of airways obstruction and current smoking as risk factors for bacterial colonization in COPD, we did not observe these relationships in our study. Study made by Patel *et al* (2002) also supports this fact where he also did not find an association between smoking and prevalence of pathogens. Similarly, in the study by Pudasaini (2004) in a study performed in NMC teaching hospital no association was found between these two factors. The reason behind insignificant result may be the data, as the number of smokers, ex-smokers and non-smokers are not equal. The numbers of ex-smokers were more in number. In contrast to our result, in a study by Miravittles *et al.* (1999), found active smoking to be independently and significantly associated with the isolation of bacteria.

Our study found significant difference in the number of exacerbations; patients had last year and the culture positive cases. As bacteria are responsible for exacerbation of the disease, so is exacerbation to culture positive case. So, patients with exacerbation, after sputum culture should be treated with appropriate antibiotics.

Similarly we also found the relation between color of sputum and culture positive cases. Current knowledge indicates that the presence of green (purulent) as opposed to

white (mucoid) sputum is one of the best and easiest methods of predicting a high bacterial load in respiratory tract secretions and the need for antibiotic therapy (Stockley *et al*., 2000). The production of green sputum is a surrogate marker for exaggerated bronchial inflammation associated with the presence of bacterial pathogens in increasing concentrations (Gompertz *et al*., 2001).

Eller and colleagues (June 1998) in which they showed that there was a correlation between deterioration of lung function (as measured with FEV_1 levels) and the bacteria isolated from patients with infective exacerbations of COPD, in that a significantly higher rate of *Enterobacteriaceae* and *Pseudomonas aeruginosa* were isolated from patients with more severe disease. Similar to this study, we also found the correlation between spirometric value and culture positive cases.

Though Kathmandu valley has higher level of pollution than the other places, geographical distribution of the patient was insignificantly associated with culture positive cases.

The previous history of hospitalization was not significantly associated with culture positive cases. In contrast to our result, study by Pudasaini (2004) found association between previous hospitalization and culture positive cases.

This study also isolated fungal pathogens from 28 (17.7%) cases. Similar to our study, in the study by Pudasaini (2004) also isolated fungi from the COPD cases, in which *Candida spp., Aspergillus spp.* and *Mucor spp*. were isolated. Objective of our study was to only isolate the fungal pathogen so identification was not done. Fungi, though less in proportion to bacteria are responsible for exacerbation.

Antibiotic resistance among pathogenic microorganisms is a matter of worldwide concern. Selective pressure by antimicrobial drugs is by far the most important driving force for the development of such resistance. Antibiotics are among the most commonly prescribed drugs in hospitals and in developed countries around 30% of the hospitalized patients are treated with (Van der *et al*, 2001) these drugs. In a study by (Shanker *et al*, 2003) documented that 29.5% of the patients were prescribed antibiotics. This is very similar to the reports from developed countries (Van der *et al*, 2001).

In our study, we tried to find the antibiotic susceptibility pattern of the isolates to different antibiotics. *S. pneumoniae*, showed maximum susceptibility i.e. 85% was found towards tetracycline and sulfomethaxazole. *Klebsiella pneumoniae* was found to be 75% susceptible to ciprofloxacin and gentamicin and 66.7% susceptible to cefotaxime and amikacin.

M. catarrhalis was found to be 92.3% susceptible to amoxicillin-clavulnic acid and ceftriaxome, 84.6% sensitivity to ciprofloxacin. Similar type of result was obtained by the study of Tamang *et al.* (2005) in which amoxicillin/clavulanic acid and ceftriaxone (96%) was the most sensitive followed by ciprofloxacillin (92%).

P. aeruginosa was found to be 75% susceptible to penicillin/tazobactam and highly resistant to cephalexin and cotrimoxazole. 100*% S. aureus* were found to be susceptible to methicillin. As a result no MRSA were isolated. The susceptibility towards ciprofloxacin and cephalexin was less whereas it was high for erythromycin. The *S. pyogenes* isolated were found to be 100% susceptible to penicillin, erythromycin, tetracycline and 100% resistant to cotrimoxazole.

6.2. Conclusion

44.3% gave significant growth of the bacteria whereas 17.7% gave significant growth of fungi. *Streptococcus pneumoniae* was the most predominant bacteria which were found to be most susceptible to sulfomethaxazole and tetracycline and out of six *Staphylococcus aureus*, no MRSA were isolated. Of the different parameters studied, age, color of sputum, number of exacerbations and spirometric value were found to be statistically significant with culture positive cases whereas geographical distribution of the patient, sex, smoking habit, number of family members, fuel used for cooking purpose were found to be insignificant with culture positive cases.

CHAPTER-VII

7 SUMMARY AND RECOMMENDATIONS

7.1 Summary

1. 17.3% of the sample received was rejected after microscopy.
2. 70 of the sample showed significant growth of PPMs. In total 44.3% was culture positive.
3. *S. pneumoniae* was the most predominant one 28.6% followed by *K. pneumoniae* (21.4%), *P. aeruginosa* (17.1%), *M. catarrhalis* (18.6%), *S. aureus* (8.6%) and *S. pyogenes* (5.7%).
4. Fungi were also isolated from the sample. 28 (17.7%) samples gave positive growth for fungi.
5. The prevalence of pathogen was found to be higher among patients of age more than 60 years.
6. Age of the patient and culture positive cases was found to be significantly related.
7. The color of the sputum was significantly related to the culture positive cases.
8. As the number of exacerbation determines the disease severity so it does to the isolation of pathogen.

7.2 Recommendations

1. Half of the samples collected were culture positive, so microbiology laboratory findings should be considered important basis for disease identification and treatment.
2. Gram staining should be performed compulsorily to check the reliability of sputum sample.
3. *Streptococcus pneumoniae* was the most predominant organism isolated. Appropriate laboratory environment (e.g. temperature) should be maintained for its isolation.
4. Maximum culture positive cases were from the patient of age more than 61 years. So special precaution should be taken with the sputum of elderly patients.
5. Antibiotics should not be prescribed without assessing the susceptibility pattern in laboratory.
6. Fungi were also isolated, so while giving treatment, not only bacteria but also fungal pathogen should be under consideration.

CHAPTER VII

7. REFERENCES

American Medical Association (1994) Guidelines for the diagnosis and treatment of nicotine dependence: how to help patients stop smoking. Washington, DC: American Medical Association

American Thoracic Society (1995) Standards for the diagnosis and care of patient's with COPD. Am J Respir Crit Care Med 14:152-177

American Thoracic Society. Pulmonary rehabilitation (1999) Am J Respir Crit Care Med 159:1666–82

Anthonisen NR, Manfreda J, Warren CP, Hershfield ES, Harding GK, Nelson NA (1987) Antibiotic therapy in exacerbations of chronic obstructive pulmonary disease. Ann Intern Med 106:196-204

Anthonisen NR, Connett JE, Kiley JP (1994) Effects of smoking intervention and the use of an inhaled anticholinergic bronchodilator on the rate of decline of FEV_1. The Lung Health Study. JAMA 272:1497–505

Anzueto A, Sethi S, Martinez FJ (2007) Exacerbations of chronic obstructive pulmonary disease. Proc Am Thorac Soc 4:295-301

Arora N, Daya MK, Mahajan R, Prakash SK, Gupta N (2001) Microbial pattern of acute infective exacerbation of chronic obstructive pulmonary disease in a hospital based study. Indian J Chest Dis Allied Sci 43:157-62

Ball P, Harris JM, Lowson D (1995) Acute infective exacerbation of chronic bronchitis for clinical and epidemiological parameters. QJ Med 25:755-79

Baraldo S, Turato G, Badin C, Bazzan E, Beghe B, Zuin R, *et al*. (2004) Neutrophilic infiltration within the airway smooth muscle in patients with COPD. Thorax 59:308–12

Barnes PJ (2002). New treatments for COPD. Nat Rev Drug Discov 1:437–46

Basran GS, Joseph J, Abbas AMA, *et al.* (1990) Treatment of acute exacerbation of chronic obstructive pulmonary disease: A comparison of amoxicillin and ciprofloxacin. J Antimicrob Chemother 26:19-24

Beisswenger C, Platz J, Seifart C, Vogelmeier C, Bals R (2004). Exposure of differentiated airway epithelial cells to volatile smoke in vitro. Respiration 71:402–9

Berry JK, Baum C (2004) Reversal of chronic obstructive pulmonary disease-associated weight loss: are there pharmacological treatment options? Drugs 64:1041–52

Blasi F, Damato S, Cosentini R, Tarsia P, Raccanelli R, Centanni S, *et al.* (2002) Chlamydia pneumoniae and chronic bronchitis: association with severity and bacterial clearance following treatment. Thorax 57:672-6

Bouros D, Pneumatikos I (2007) The Clinician through the Looking Glass: Sputum Microbiology in COPD Exacerbations. Respiration 74:13–14s

Britton J, Knox A (1999) Helping people to stop smoking: the new smoking cessation guidelines. Thorax 54:1–2

Bryanton V, Chen Y, Johanson H, *et al*. (2001) Respiratory disease in Canada. Statistiques Canada http://www.phac-aspc.gc.ca/publicat/rdc-mrc01/index.html

Buist AS,Vollmer WM, McBurnie MA (2008) Worldwide burden of COPD in high- and lowincome countries. Part I. The Burden of Obstructive Lung Disease (BOLD) Initiative. Int J Tuberc Lung Dis 12:703-708

Burge PS, Calverley PM, Jones PW, *et al.* (2000) Randomised, double blind, placebo controlled study of fluticasone propionate in patients with moderate to severe chronic obstructive pulmonary disease: the ISOLDE trial. BMJ 320:1297–303

Burrows B, Knudson RJ, Cline MG, Lebowitz MD (1977) Quantitative relationships between cigarette smoking and ventilatory function. Am Rev Respir Dis 115:195-205

Calder MA, Schonell Me (1971) Pneumococcal typing and problem of endogenous reinfection in chronic bronchitis. Lancet 1:1156-59

Calderón JE, Rivero L, Respaldiza N, Morilla R, Montes MA, Friaza V, Lobato FM, Varela JM, Medrano FJ, and Horra CDL (2007) Systemic Inflammation in Patients with Chronic Obstructive Pulmonary Disease Who Are Colonized with *Pneumocystis jiroveci.* Clinial Infectious Disease 45(2):e17–e19

Chakraborty P (2001) A Text Book of Microbiology, 1st ed. New Central Book Agency (P) Ltd, Calcutta

Cheesbrough M (1984) Medical Laboratory Manual for Tropical Countries Microbiology 1st ELBS ed. Cambridge: University Press

Cheesbrough M (2000) District Laboratory Practice in Tropical countries, Cambridge University Press

Chen Y (1999) Genetics and pulmonary medicine: 10. Genetic epidemiology of pulmonary function. Thorax 4:818-824

Chen Y, Horne SL, Dosman JA (1991) Increased susceptibility to lung dysfunction in female smokers. Am Rev Respir Dis 143:1224-30

Chierakul, N, Wongwisutikul, P, Vejbaesya, S, *et al.* (2005) Tumor necrosis factor-alpha gene promoter polymorphism is not associated with smoking-related COPD in Thailand. Respirology 10:36-39

Chodosh S (1992) Bronchitis and asthma. In : Gorbach SL, Barlett JG, Blacklow NR, ed Infectious diseases. Philadelphia : W.B. Saunders 14:476-485

CLSI (2007) Performance standards for antibiotics disk susceptibility tests. CLSI 27

Cohen BH, Bias WB, Chase GA *et al.* (1980) Is ABH nonsecretor status a risk factor for obstructive lung disease? Am J Epidemiol 111:285-291

Cole P, Wilson R (1989) Host microbial relationships in respiratory infection. Chest 95:2175-2215

Colle JG, Marmion BP, Fraser AG, Simmons A (2006) Mackie and Mc Cartney Practical Medical Microbiology, 14th ed: 62-64.Churcill livingstone indian reprint

Collier L, Balows A and Sussman M (1998) Topley and Wilson's Microbiology and Microbial infections (9th ed). London: Oxford University Press3

Connett JE, Murray RP, Buist AS, *et al*. (2003) Changes in smoking status affect women more than men: Results of the Lung Health Study. Am J Epidemiol 157:973-979

Cook DG, Strachan DP (1999) Summary of effects of parental smoking on respiratory health of children and its implications for research. Thorax 54:357-366

Cosio BG, Tsaprouni L, Ito K, Jazrawi E, Adcock IM, Barnes PJ (2004) Theophylline restores histone deacetylase activity and steroid responses in COPD macrophages. J Exp Med 200:689–95

Crofton J and Douglas A (2000) Respir Dis, Blackwell Science 616-695

Daheshia M (2005) Therapeutic inhibition of matrix metalloproteinases for the treatment of chronic obstructive pulmonary disease (COPD). Curr Med Res Opin 21:587-94

Daheshia M (2005) Pathogenesis of chronic obstructive pulmonary disease (COPD) Clinical and Applied Immunology Reviews 5:339–351

Davis R (1989) The epidemiology of cigarette smoking and its impact on chronic obstructive pulmonary disease. Am Rev Respir Dis 140:S82-S84

Eller J, Ede A, Schaberg T, *et al*. (1998) Infective exacerbations of chronic bronchitis: relation between bacteriologic etiology and lung function. Chest 113:1542–1548

Ewig S, Soler N, Gonzalez J, *et al*. (2000) Evaluation of antimicrobial treatment in mechanically ventilated patients with severe chronic obstructive pulmonary disease exacerbations. Crit Care Med 28:692–697

Faber, JP, Poller, W, Olek, K, *et al.* (1993) The molecular basis of α_1-antichymotrypsin deficiency in a heterozygote with liver and lung disease. J Hepatol 18:313-321

Fehrenbach C (2004). NICE guidelines for chronic obstructive pulmonary disease a review. Nurs Times 100:48–51

Finkelstein R, Fraser RS, Ghezzo H, Cosio MG (1995) Alveolar inflammation and its relation to emphysema in smokers. Am J Respir Crit Care Med 152:1666–72

Finkelstein J, Steven EC, Scharf M *et al.* (2009) Chronic obstructive pulmonary disease as an independent risk factor for cardiovascular morbidity. International Journal of COPD 4:337–349

Finlay GA, Russell KJ, McMahon KJ, D'arcy EM, Masterson JB, Fitzgerald MX, *et al*. (1997) Elevated levels of matrix metalloproteinases in bronchoalveolar lavage fluid of emphysematous patients. Thorax 52:502–6

Fiore MC, Smith SS, Jorenby DE, *et al*. (1994) The effectiveness of the nicotine patch for smoking cessation. A meta-analysis. JAMA 271:1940–7

Forbes BA, Sahm DF and Weissfeld AS (2002) Bailey and Scott's Diagnostic Microbiology, 11th ed. Mosby Inc, USA

Franciosi LG, Page CV, Celli BR, Cazzola M, Walker MJ, Danhof M, Rabe KF and Pasqua OED (2006) Markers of exacerbation severity in chronic obstructive pulmonary disease. Respiratory Research 7:74 *http://respiratory-research.com/content/7/1/74*

Fuke S, Betsuyaku T, Nasuhara Y, Morikawa T, Katoh H, Nishimura M (2004) Chemokines in bronchiolar epithelium in the development of chronic obstructive pulmonary disease. Am J Respir Cell Mol Biol 31:405

Gan WQ, Man SF, Senthilselvan A, Sin DD (2004) Association between chronic obstructive pulmonary disease and systemic inflammation: a systematic review and a meta-analysis. Thorax 59:574–80

Global Initiative for Chronic Obstructive Lung Disease (GOLD) (2007) Global Strategy for Diagnosis, Management and Prevention of COPD - Updated 2007. *Available from* http://www.goldcopd.org

Global Initiative for Chronic Obstructive Lung Disease (GOLD) (2001). Workshop summary. Am J Respir Crit Care Med 163:1256–76

Gompertz S, O9Brien C, Bayley DL, Hill SL, Stockley RA (2001) Changes in bronchial inflammation during acute exacerbations of chronic bronchitis. Eur Respir J 17:1112–1119

Gorecka D, Gorzelak K, Sliwinski P, *et al*. (1997) Effect of long-term oxygen therapy on survival in patients with chronic obstructive pulmonary disease with moderate hypoxaemia. Thorax 52:674–9

Greenberg D, Lavi NG, Broides A, Blancovich I, Peled N, and Dagan R (2006) The contribution of smoking and exposure to tobacco smoke to *Streptococcus pneumoniae* and *Haemophilus influenzae* carriage in children and their mothers. Clinical Infectious Diseases 42:897–903

Greenwood D, Slack R and Peutherer J (2001) Medical Microbiology, A guide to Microbial Infections: Pathogenesis, Immunity, Laboratory Diagnosis and Control, 15th ed. Churchill Livingstone, USA

Griffiths TL, Burr ML, Campbell IA, *et al.* (2000) Results at 1 year of outpatient multidisciplinary pulmonary rehabilitation: a randomised controlled trial. Lancet 355:362–8

Growengen KH, Wouters EF (2003) Bacterial infection in patients requiring admission for an acute exacerbation of chronic obstructive pulmonary disease: 1-year prospective study. Respir Med 97:770-77

Grumelli S, Cony DB, Song LZ, Song L, Green L, Huh J, *et al*. (2004) An immune basis for lung parenchymal destruction in chronic obstructive pulmonary disease and emphysema. PLoS Med 1:e8

Gump DW, Phillips CA, Forsyth BR, McIntosh K, Lamborn KR, Stouch WH (1976) Role of infection in chronic bronchitis. Am Rev Respir Dis 113:465-7

Halbert RJ, Natoli JL, Gano A, Badamgarav E, Buist AS, Mannino D.M (2006) Global burden of COPD: systematic review and meta-analysis. Eur Respir J 28:523-532

Hanrahan J P, Twiger I, Seegal M R (1992) The effect of maternal smoking during pregnancy on early infant lung function. Am Rev Respir Dis 145:1129-1135

Harrison DJ, Cantlay AM, Rae F, *et al*. (1997) Frequency of glutathione S-transferase M1 deletion in smokers with emphysema and lung cancer. Hum Exp Toxicol 16:356-360

Hogg JC, Chu F, Utokaparch S, Woods R, Elliott WM, Buzatu L, *et al*. (2004) The nature of small-airway obstruction in chronic obstructive pulmonary disease. N Engl J Med 350:2645–53

Holt PG (1987) Immune and inflammatory function in lung functions. Thorax 42:241-49

Hnizdo E, Baskind E, Sluis-Cremer GK (1990) Combined effect of silica dust exposure and tobacco smoking on the prevalence of respiratory impairements among gold minners. Scand J Work Environ Health 16:411-422

Huang, SL, Su, CH, Chang, SC (1997) Tumor necrosis factor-alpha gene polymorphism in chronic bronchitis. Am J Respir Crit Care Med 156:1436-1439

Huo, JM, Shi, YZ, Liu, XH, *et al*. (1990) The phenotype and content of α_1-antitrypsin in patients with COPD. J Harbin Med Univ 24:246-248

http://www.who.int/topics/tobacco/facts/en/index.htlm (accessed 28 November 2008)

Isenberg HD (2004) Clinical Microbiology Procedures Handbook. 2nd edition. ASM press

Jha BK., Dey S, Tamang MD, Joshi ME, Shivananda PG, Brahmadatan KN (2006) Characterization of Candida species isolated from cases of lower respiratory tract infection. KUMJ 15:290-294

Jindal S K. Editorial, How appropriate is the gold standard appropriate for diagnosis of airway obstruction? *www.lungindia.com*

Jorenby DE, Leischow SJ, Nides MA, *et al*. (1999) A controlled trial of sustained-release bupropion, a nicotine patch, or both for smoking cessation. N Engl J Med 340:685–91

Kant S, Gupta B (2008) Role of lifestyle in the development of chronic obstructive pulmonary disease. Lung India 25:95-101

Kauffmann F, Drouet D, Lellouch J, Brille D (1979) 12 year spirometric changes among Paris area workers. Int J Epidemol 8:201-212

Kauffmann F, Drouet D, Lellouch J, Brille D (1982) Occupational exposure and 12 year spirometric changes among Paris area workers. Br J Ind Med 39:221-232

Keatings VM, Collins PD, Scott DM, Barnes PJ (1996) Differences in interleukin-8 and tumor necrosis factor alpha in induced sputum from patients with chronic obstructive pulmonary disease or asthma. Am J Respir Crit Care Med 153:530–4

Ko, F.W.S.; Hui, D.S.C.; Lai, C.K.W (2008) Worldwide burden of COPD in high- and low-income countries. Part III. Asia-Pacific studies. Int. J. Tuberc. Lung Dis 12:713-717

L Laviolette, Y Lacasse, M Doucet, *et al.* (2007) Chronic obstructive pulmonary disease in women. Can Respir J 14:93-98

Langhammer A, Johnsen R, Gulsvik A, Holmen TL, Bjermer L (2003) Sex differences in lung vulnerability to tobacco smoking. Eur RespirJ 21:1017-23

Lees AW, Mc Naught W (1959) Bacteriology of lower respiratory sections, sputum and upper respiratory sections in normals and chronic bronchitis. Lancet ii:1112-15

Levent E, Oguz U, Serhat F, Didem K, Ahmet S, Atilla GA (2008) Role of bacteria in acute exacerbations of COPD. Int Jour of COPD 3:463–467

Leynaert B, Bousquet J, Henry C, Liard R, Neukirch F (1997) Is bronchial hyperresponsiveness more frequent in women than in men? A population-based study. Am J Respir Crit Care Med 156:1413-20

Lim S, Roche N, Oliver BG, Mattos W, Barnes PJ, Chung KF (2000) Balance of matrix metalloprotease-9 and tissue inhibitor of metalloprotease-1 from alveolar macrophages in cigarette smokers. Regulation byinterleukin-10. Am J Respir Crit Care Med 162:1355–60

Llor C, Cots JM, Herreras A (2006) Bacterial etiology of chronic bronchitis exacerbations treated by primary care physicians. Archivos de Bronconeumologia :388-393

Love RG, Miller BG (1982) Longitudinal study of lung function in coalminers. Thorax 37:193-197

Lode H, Allewelt M, Balk S, Roux AD, Mauch H, Niederman M, Schmidt-Ioanas M (2007) A Prediction Model for Bacterial Etiology in Acute Exacerbations of COPD. Infection 35:143–149

World health organization (WHO) (2003) Lower Respiratory Tract Infections, Basic Laboratory Procedures in Clinical Bacteriology 2: 666-675

Liu, S, Li, B, Zhou, Y, *et al.* (2007) Genetic analysis of CC16, OGG1 and GCLC polymorphisms and susceptibility to COPD. Respirology 12:29-33

MacNee W, Calverley P M A (2003) Chronic obstructive pulmonary disease 7: Managementof COPD. Thorax 58:261–265

Macnee W, Rahman I (1999) Oxidants and antioxidants as therapeutic targets in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 160:S58–65

Mannino DM, Gagnon RC, Petty TL, Lydick E (2000) Obstructive lung disease and low lung function in adults in the United States: Data from the National Health and Nutrition Examination Survey, 1988-1994. Arch Intern Med 160:1683-9

Mannino DM, Buist AS (2007) Global burden of COPD: risk factors, prevalence, and future trends. Lancet 370:765-773

Maxfield RA (2004) New and emerging minimally invasive techniques for lung volume reduction. Chest 125:777–83

Meijer E, Kromhout H, Heederik D (2001) Respiratory effects of exposure to low levels of concrete dust containing crystalline silica. Am J Ind Med 40:133-140

Melissah H. Hunter and Ddana E. King (2001) COPD:Management of Acute Exacerbations and Chronic Stable Disease. Am Fam Physician 64:603-12

Menezes AM, Perez-Padilla R, Jardim JR, Muiño A, López MV, Valvidia G, Montes de Oca M, Talamo C, Hallal PC, Victoria CG, PLATINO team (2005) Chronic obstructive pulmonary disease in five Latin American cities (the PLATINO study): a prevalence study. Lancet 366:1875-1881

Menezes AMB, Perez-Padilla R, Hallal PC, Jardim JR, Muiño A, Lopez MV Valdivia G, Pertuze J, Montes de Oca M, Tálamo C for the PLATINO Team (2008) Worldwide burden of COPD in high- and low-income countries. Part II. Burden of chronic obstructive lung disease in Latin America: the PLATINO study. Int. J. Tuberc. Lung Dis 12:709-712

Milo A Puhan, Daniela Vollenweider, Tsogyal Latshang, Johann Steurer and Claudia Steurer-Stey (2007) Exacerbations of chronic obstructive pulmonary disease: when are Antibiotics indicated? A systematic review. Respir Res 8:30

Miravitlles M, Espinosa C, Fernández-Laso E, Martos JA, Maldonado JA and Gallego M (1999) Relationship between bacterial flora in sputum and functional impairment in patients with acute exacerbations of COPD. Chest 116:40-46

Miotto D, Ruggieri MP, Boschetto P, Cavallesco G, Papi A, Bononi I, *et al.* (2003) Interleukin-13 and -4 expression in the central airways of smokers with chronic bronchitis. Eur Respir J 22:602–8

Molet S, Belleguic C, Lena H, Germain N, Bertrand CP, Shapiro SD, *et al.* (2005) Increase in macrophage elastase (MMP-12) in lungs from patients with chronic obstructive pulmonary disease. Inflamm Res 54:31–6

Monso E, Ruiz J, Rosell A, *et al.* (1995) Bacterial infection in chronic obstructive pulmonary disease: a study of stable and exacerbated outpatients using the protected specimen brush. Am J Respir Crit Care Med 152:1316–1320

Morgan WJ (1998) Maternal smoking and infect lung function. Further evidence for an in uterus effect. Am J Respir Crit Care Med 158:688-690

Morgan MD (1999) The prediction of benefit from pulmonary rehabilitation: setting, training intensity and the effect of selection by disability. Thorax 54: S3–7

Morrison AJ, Wenzee RP (1984) Epidemiology of infection due to *Pseudomonas aeruginosa.* Rev Infect Dis 6: 627 S

Murphy RE, Katz S, Massaro DJ, Luchsinger PC (1962) Is emphysema a disease predominantly of the white male? Preliminary report. JAMA 181:726-7

Murphy TF, Sethi S and Niederman MS (2000) The role of bacteria in exacerbation of COPD. Chest 118:204-209

Murray CJ, Lopez AD (1997) Alternative projections of mortality and disability by cause 1990–2020: Global Burden of Disease Study. Lancet 349:1498–1504

National Emphysema Treatment Trial Research Group (2002) Patients at high risk of death after lung-volume-reduction surgery. N Engl J Med 345:1075–83

National institute for occupational safety and health. Work related lung disease surveillance report 1999. Cincinnati (OH): Division of Respiratory Disease Studies. National Institute for Occupational Safety and Health (NIOSH)

Pandey MR (1984) Domestic smoke pollution and chronic bronchitis in a rural community of the hill region of Nepal. Thorax 39:337-39

Papi A, Bellettato CM, Braccioni F, *et al.* (2006) Infections and airway inflammation in chronic obstructive pulmonary disease severe exacerbations. Am J Respir Crit Care Med 173:1114–1121

Parrott S, Godfrey C, Raw M, *et al.* (1998) Guidance for commissioners on the cost effectiveness of smoking cessation interventions. Health Educational Authority. Thorax 53:S1–38

Patel IS, Seemungal TAR, Wilks M, Lloyd-Owen JS, Donaldson GC, Wedzicha JA (2002) Relationship between bacterial colonisation and the frequency, character, and severity of COPD exacerbations. Thorax 57:759–764

Patrick Mallia and Sebastian L. Johnston (2006) How Viral Infections Cause Exacerbation of Airway Diseases. Chest 130:1203-1210

Pauwels RA, Buist AS, Ma P, Jenkins CR, Hurd SS (2001) Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. NHLBI/WHO Global Initiative for Chronic Obstructive Lung Disease (GOLD) Workshop summary. Am J Respir Crit Care Med 163:1256–76

Pauwels RA, Rabe KF (2004) Burden and clinical features of chronic obstructive pulmonary disease (COPD).Lancet 364:613–620

Pokharel BM (2004) A hand book of clinical bacteriology, 1st ed. Gorakhnath desktop and printers, Kathmandu

Prescott E, Bjerg AM, Andersen PK, Lange P, Vestbo J (1997) Gender difference in smoking effects on lung function and risk of hospitalization for COPD: Results from a Danish longitudinal population study. Eur Respir J 10:822-7

Pudasani D (2004) Microbial study of chronic obstructive pulmonary disease. MSc dissertation submitted to central department of Microbiology, TU, Kathmandu, Nepal.

Puljic R, Pahl A (2004) Smoke induced changes in epithelial cell gene expression: development of an in vitro model for COPD. ALTEX 21:3–7

Qiu Y, Zhu J, Bandi V, *et al.* (2003) Biopsy neutrophilia, neutrophil chemokine and receptor gene expression in severe exacerbations of chronic obstructive pulmonary disease. Am J Respir Crit Care Med 168:968–975

R. Andrew McIvor (2009). Antibiotics in acute exacerbations of chronic obstructive pulmonary disease Canadian Family Physician Le Médecin de famille canadien 55:15-16

Rafael Laniado-Laborín (2009) Smoking and Chronic Obstructive Pulmonary Disease (COPD) Parallel Epidemics of the 21st Century Int. J. Environ. Res. Public Health 6:209-224

Rahman I, MacNee W (1999). Lung glutathione and oxidative stress: implications in cigarette smoke-induced airway disease. Am J Physiol 277: L1067–88

Ravi Pathiyil Shankar, Praveen Partha, Nagesh Kumar Shenoy, Joshy Maducolil Easow, and Kottallur Narayanan Brahmadathan (2003) Prescribing patterns of antibiotics and sensitivity patterns of common microorganisms in the Internal Medicine ward of a teaching hospital in Western Nepal: a prospective study. Ann Clin Microbiol Antimicrob 2:7-12

Regional COPD working group (2003) COPD prevalence in 12 Asia-Pacific countries and regions: projections based on the COPD prevalence estimation model. Respirology 8:192-8

Rennard S, Decramer M, Calverley PM, *et al*. (2002) Impact of COPD in North America and Europe in 2000: Subjects' perspective of Confronting COPD International Survey. Eur Respir J 20:799-805

Rodriguez-Roisin R (2000) Toward a consensus definition for COPD exacerbations. Chest 117:398S–401S

Rohde G, Wiethege A, Borg I, Kauth M, Bauer TT, Gillissen A, *et al.* (2003) Respiratory viruses in exacerbations of chronic obstructive pulmonary disease requiring hospitalisation: a case-control study. Thorax 58:37–42

Saetta M (1999) Airway inflammation in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 160:S17–20

Saetta M, Baraldo S, Corbino L, Turato G, Braccioni F, Rea F, *et al.* (1999) CD8+ve cells in the lungs of smokers with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 160:711–719

Saetta M, Baraldo S, Turato G, Beghe B, Casoni GL, Bellettato CM, *et al.* (2003) Increased proportion of CD8 T-lymphocytes in the paratracheal lymph nodes of smokers with mild COPD. Sarcoidosis Vase Diffuse Lung Dis 20:28–32

Saetta M, Turato G, Maestrelli P, Mapp CE, Fabbri LM (2001) Cellular and structural bases of chronic obstructive pulmonary disease. Am J Respir Crit Care Med 163:1304–9

Saetta M, Di Stefano A, Maestrelli P, Turato G, Ruggieri MP, Roggeri A, *et al.* (1994) Airway eosinophilia in chronic bronchitis during exacerbations. Am J Respir Crit Care Med 150:1646–52

Schellenberg, D, Pare, PD, Weir, TD, *et al*. (1998) Vitamin D binding protein variants and the risk of COPD. Am J Respir Crit Care Med 157:957-9

Seemungal T, Harper-Owen R, Bhowmik A, *et al.* (2001) Respiratory viruses, symptoms, and inflammatory markers in acute exacerbations and stable

chronic obstructive pulmonary disease. Am J Respir Crit Care Med 164:1618–1623

Segura-Valdez L, Pardo A, Gaxiola M, Uhal BD, Becerril C, Selman M (2001) Upregulation of gelatinases A and B, collagenases 1 and 2, and increased parenchymal cell death in COPD. Chest 117:684–94

Sethi Sanjay Bacteria in Exacerbations of Chronic Obstructive Pulmonary Disease Phenomenon or Epiphenomenon? Proc Am Thorac 11:109–114

Sethi S (2004) New developments in the pathogenesis of acute exacerbations of chronic obstructive pulmonary disease. Curr Opin Infect Dis 17:113–91

Sethi S, Evans N, Grant BJ, Murphy TF (2002) New strains of bacteria and exacerbations of chronic obstructive pulmonary disease. N Engl J Med 347:465–71

Seyama, K (2001) State of α_1-antitrypsin deficiency in Japan. Respirology 6:35-S38

Siafakas, NM, Tzortzaki, EG, Sourvinos, G, *et al* (1999) Microsatellite DNA instability in COPD. Chest 116:47-52

Silverman, EK, Speizer, FE (1996) Risk factors for the development of chronic obstructive pulmonary disease. Med Clin North Am 80: 501-522

Sykes A, Mallia P and Sebastian L. Johnston (2007) Diagnosis of Pathogens in Exacerbations of Chronic Obstructive Pulmonary Disease Proc Am Thorac Soc 4:642–646

Smith, CA, Harrison, DJ (1997) Association between polymorphism in gene for microsomal epoxide hydrolase and susceptibility to emphysema. Lancet 350:630-633

Smith CB, Golden CA, Kanner RE, *et al* (1980) Association of viral and *Mycoplasma pneumoniae* infections with acute respiratory illness in patients with chronic obstructive pulmonary diseases. Am Rev Respir Dis 121:225–232

Spencer S, Calverley PM, Burge PS, Jones PW (2004) Impact of preventing exacerbations on deterioration of health status in COPD. Eur Respir J 23:698–702

Stockley RA, O'Brien C, Pye A, Hill SL (2000) Relationship of sputum color and outpatient management of acute exacerbations of COPD. Chest 117:1638–1645

Stringer KA, Freed BM, Dunn JS, Sayers S, Gustafson DL, Flores SC (2004) Particulate phase cigarette smoke increases MnSOD, NQO1, and CINC-1 in rat lungs. Free Radic Biol Med 37:1527–33

Tack G. ATS (2009) Conference Report. Respiratory Medicine : COPD update. Elseiver 5:62 – 66

Tamang MD, Dey S, Makaju RK, Jha BK, Shivananda PG, Bhramadatan KN (2003) Prevalence of *Moraxella catarrhalis* infections of the lower respiratory tract in elderly patients. KUMJ 3:39-44

Tan WC, Xiang X, Qiu D, *et al*. (2003) Epidemiology of respiratory viruses in patients hospitalized with near-fatal asthma, acute exacerbations of asthma, or chronic obstructive pulmonary disease. Am J Med 115:272–277

Tengs TO, Adams ME, Pliskin JS, *et al*. (1995) Five-hundred life-saving interventions and their cost-effectiveness. Risk Anal 15:369–90

Tsimogianni, Angeliki M (2009) Predictors of positive sputum cultures in exacerbations of chronic obstructive pulmonary disease. Respirology 14:1114-20

Turato G, Zuin R, Miniati M, Baraldo S, Rea F, Beghe B, *et al.* (2002) Airway inflammation in severe chronic obstructive pulmonary disease: relationship with lung function and radiologic emphysema. Am J Respir Crit Care Med 166:105–10

Turino GM (2002) The origins of a concept: the protease-antiprotease imbalance hypothesis. Chest 122:1058–60

Ulrik CS (2003) Smoking and mortality in women: “Smoke like a man (at least) die like a man”. Eur Respir J 8:103-109

Van Pelt W, Borsboom GJ, Rijcken B, Schouten JP, van Zomeren BC, Quanjer PH (1994) Discrepancies between longitudinal and crosssectional change in ventilatory function in 12 years of follow-up. Am J Respir Crit Care Med 149:1218-26

Van der Meer JW, Gyssens IC (2001) Quality of antimicrobial drug prescription in hospital. *Clin Microbiol Infect* Suppl **7**:12–15. [PubMed]

Vollmer WM, Enright PL, Pedula KL, *et al.* (2000) Race and gender differences in the effects of smoking on lung function. Chest 117:764-72

Wan C. Tan, Tze P. N (2008) COPD in Asia-Where East Meets West. Chest 133:517-527

White AJ, Gompertz S, Stockley RA (2003) Chronic obstructive pulmonary disease. The aetiology of exacerbations of chronic obstructive pulmonary disease. Thorax 58:73-80

Wongsurakiat P, Maranetra KN, Wasi C, Kositanont U, Dejsomritrutai W, Charoenratanakul S (2004) Acute respiratory illness in patients with COPD and the effectiveness of influenza vaccination: a randomized controlled study. Chest 125:2011–20

Woodhead MA, Arrowsmith J, Chamberlain R, *et al.* (1991) The value of routine microbial investigations in CAP. Respir Med 85:313-17

Woodhead M, Blasi F, Ewig S, *et al.* (2005) Guidelines for the management of adult lower respiratory tract infections. Eur Respir J 26:1138–1180

Xu X, Weiss ST, Rijcken B, Schouten JP (1994) Smoking, changes in smoking habits, and rate of decline in FEV1: new insight into gender differences. Eur Respir J 7:1056-61

Yim, JJ, Park, GY, Lee, CT, et al (2000) Genetic susceptibility to chronic obstructive pulmonary disease in Koreans: combined analysis of polymorphic genotypes for microsomal epoxide hydrolase and glutathione S-transferase M1 and T1. Thorax 55:121-125

Yin, QL, Liang, ZQ (1980) The frequency distribution of allelic gene of α_1-antitrypsin deficiency in Chinese. Chin Sci 10:897-903

Zejda JE, McDuffie HH, Dosman JA (1993) Respiratory effects of exposure to grain dust. Semin Respir Med 14:20-30

Zhang, H, Cai, B (2003) The impact of tobacco on lung health in China. Respirology 8:17-21

Zhao M, Gu G, Liu Z, Zhang X, Xu W, Fang Q (1999) A clinical study of bacterial infection in patients with chronic obstructive pulmonary disease. Zhonghua Jie He He Xi za Zhi 22:88-91

APPENDIX I

QUESTIONNAIRE

Name:

Age:

Sex:

Weight:

Height:

$SPO_{2:}$ ……. %

Address:

Occupation:

Number of People in your home:

Smoking Habit

1. Smoker
2. Ex-smoker
3. Non-smoker

Are there other smokers in the house?

1. Yes
2. No

Why have you come to the hospital?

1. Dyspnea - Breathlessness
2. Chest pain
3. Increase sputum production
4. Increase sputum purulence
5. Haemoptysis
6. Cough
7. Wheeze
8. Fever

No. of exacerbations last year:

What is the color of your sputum?

1. Clear
2. Green
3. Yellow

4. Other

What is the fuel used in your home for cooking purpose?

a. Firewood
b. Gas
c. Kerosene
d. Heater
e. Other

Who cooks the food most often?

1. You
2. Someone else

Previous history of Hospitalization:

1. Yes
2. No

RESULTS

Sputum

Gram's stain:

Culture:

Antibiotic sensitivity test:

Antibiotics used	Resistant	Sensitive

APPENDIX II

LIST OF EQUIPMENTS AND MATERIALS USED DURING STUDY

Glasswares

Beaker
Conical flask
Culture bottle
Glass rod
Glass slide
Measuring cylinder
Pipette
Petri plate
Test tube

Miscellaneous

Bacteriological loop
Bunsen burner
Cotton
Forceps
Gloves
Labeling sticker
Micropipette
Micropipette tip
Marker
Soap

Spirit lamp
Sterile cotton swab
Staining rack
Test tube holder

Equipments

Autoclave
Compound microscope
Hot air oven
Incubator
Refrigerator
Safety cabinet
Water bath

Chemicals and Reagents

Blood plasma
Distilled water
Gram's Iodine
3% Hydrogen peroxide
Lysol
Mac Farland's Nephelometer
α- Napthol solution
Crystal violet
Ethanol
Immersion oil
Paraffin oil
Safranin
Paraffin oil
40% Potassium hydroxide

Antibiotics (HiMedia Company)

Amikacin (30 mcg)
Ampicillin (10mcg)
Cefixime (5mcg)
Ceftazidime (30mcg)
Penicilin (10 unit)
Tetracyclin (30 mcg)
Piperacilliin/tazobactam(100/10mcg)
Amoxcillin/clavulanic acid (20/10mcg)
Ciprofloxacillin (5 mcg)
Chloramphenicol (30 mcg)
Cotrimoxazole (25 mcg)
Erythromycin (15 mcg)
Cephalexin (30mcg)

Media (HiMedia Company)

Blood agar base
MacConkey agar
Mueller Hinton agar
Sabouraud agar

Nutrient agar	Simmons Citrate agar
Mannitol salt agar	TSI Agar
Sabouraud dextrose agar	
MR/VP broth	
Nutrient broth	

APPENDIX-III

COMPOSITION AND PREPARATION OF DIFFERENT MEDIA AND TESTING REAGENTS

I Culture media

The culture media used were from Hi-Media Laboratories Pvt. Limited, Bombay, India.
(All compositions are given in grams per liter and at 25^0C temperature)

1. Blood agar (BA)

Blood agar base (infusion agar) + 5-10% sheep blood

Ingredients	gm/liter
Beef heart infusion	500.0
Tryptose	10.0
Sodium Chloride	5.0
Agar	15.0

Final pH (at 25^0C) 7.3±0.2

42.5 grams of the blood agar base medium was suspended in 1000 ml distilled water and sterilized by autoclaving at 121^0C (15lbs pressure) for 15 minutes. After cooling to 40-50^0C, 50 ml sterile defibrinated sheep blood was added aseptically and mixed well before pouring.

2. **Chocolate agar (CA)**

After adding the blood to blood agar base the, the medium was heated in a 70^0C water bath until the medium became brown in color. This took about 10-15 minutes during which time the medium was gently several times. The medium was allowed to cool to about 45^0C, remixed and dispensed in sterile Petri dishes.

3. **MacConkey agar (MA**)

Ingredients	**gm/liter**
Peptone	3.0
Pancreatic digest of gelatin	17.0
Lactose	10.0
Sodium chloride	5.0
Bile salt	1.5
Neutral red	0.03
Crystal violet	0.001
Agar	13.5

Final pH (at 25^0 C) 6.9-7.3

52 grams of the medium was dissolved in 1000ml distilled water and heated to dissolve the media. The media was autoclaved at 15 lbs at 121^0C for 15 minutes.

4. **Nutrient agar (NA)**

Ingredients	**gm/litre**
Peptone	10.0
Sodium Chloride	5
Beef Extract	10.0
Yeast Extract	1.5
Agar	12.0

Final pH (at 25^0C) 7.4±0.2

37 grams of the medium was suspended in 1000 ml of distilled water and then boiled to dissolve completely. Then the medium was sterilized by autoclaving at 121^0C (15 lbs pressure) for 15 minutes.

5. **Mannitol salt agar (MSA)**

Ingredients	**gm/litre**
Peptone	10.0
Beef Extract	1.0
D-Mannitol	10.0
Sodium chloride	75.0
Phenol red	0.025
Agar	15.0

Final pH at (25^0C) 7.4±0.2

111 grams of the medium was dissolved in 1000 ml distilled water and autoclaved at 121˚C for 15 minutes.

6. **Nutrient broth (NB)**

Ingredients	**gm/litre**
Peptone	5.0
Sodium Chloride	5.0
Beef Extract	1.5
Yeast Extract	1.5

Final pH (at 25^0C) 7.4±0.2

13 grams of the medium was dissolved in 1000 ml distilled water and autoclaved at 121˚C for 15 minutes.

7. **Sabouraud Dextrose agar (SDA)**

Ingredients	**gm/liter**
Glucose	20.0
Peptone	10.0
Agar	15.0

Final pH (at 25^0 C) 7.4±0.2

111 grams of the medium was suspended in 1000 ml distilled water and heated to dissolve the media. The media was autoclaved at 15 lbs at 121^0C for 15 minutes

II Biochemical test media

1. MR-VP medium

Ingredients	gm/litre
Buffered Peptone	7.0
Dextrose	5.0
Dipotassium Phosphate	5.0
Final pH (at 25^0C)	6.9±0.2

17 grams was dissolved in 1000 ml distilled water. 3 ml of medium was distributed in each test tube and autoclaved at 121^0C for 15 minutes.

2. Hugh and Leifson's medium

Ingredients	gm/litre
Tryptone	2.0
Sodium Chloride	5.0
Dipotassium Phosphate	0.3
Bromothymol Blue	0.08
Agar	2.0
Final pH (at 25^0C)	6.8±0.2

9.4 grams of the medium was rehydrated in 1000 ml cold distilled water and then heated to boiling to dissolve completely. The medium was distributed in 100 ml amounts and sterilized in the autoclave for 15 minutes at 15 lbs pressure (121^0C). To 100 ml sterile medium aseptically added 10ml of sterile Dextrose and mixed thoroughly and dispensed in 5 ml quantities into sterile culture tubes

3. Sulphide Indole Motility (SIM) medium

Ingredients	gm/litre
Beef Extract	3.0
Peptone	30.0
Peptonized Iron	0.2
Sodium Thiosulphate	0.025
Agar	3.0
Final pH (at 25^0C)	7.3±0.2

36 grams of the medium was suspended in 1000 ml distilled water and dissolved completely. Then it was distributed in tubes to a depth of about 3 inches and sterilized.

4. Simmon's Citrate agar

Ingredients	**gm/litre**
Magnesium Sulfate	0.2
Mono-ammonium Phosphate	1.0
Dipotassium Phosphate	1.0
Sodium Citrate	2.0
Sodium Chloride	5.0
Agar	15.0
Bromothymol Blue	0.08
Final pH (at 25^0C)	6.8±0.2

24.2 grams of the medium was dissolved in 1000ml distilled water. 3ml medium was distributed in test tubes and sterilized by autoclaving at 121^0C for 15 minutes. After autoclaving tubes containing medium were tilted to form slant.

5. Triple Sugar Iron (TSI) agar

Ingredients	**gm/litre**
Peptone	10.0
Tryptone	10.0
Yeast Extract	3.0
Beef Extract	3.0
Lactose	10.0
Sucrose	10.0
Dextrose	1.0
Ferrous Sulphate	0.2
Sodium Chloride	5.0
Sodium Thiosulphate	0.3

Phenol Red	0.024
Agar	12.0

Final pH (at 25^0C) 7.4±0.2

65 grams of the medium was dissolved in 1000ml of distilled water and sterilized by autoclaving at 15 lbs (121^0C) pressure for 15 minutes. The medium was allowed to set in sloped form with a butt about 1 inch of thickness.

6. Christensen Urea agar

Ingredients	**gm/litre**
Peptone	1.0
Dextrose	1.0
Sodium Chloride	5.0
Dipotassium Phosphate	1.2
Mono-potassium Phosphate	0.8
Phenol Red	0.012
Agar	15.0

Final pH (at 25^0C) 7.4±0.2

24 grams of the medium was suspended in 950 ml distilled water and sterilized by autoclaving at121^0C for 15 minutes. After cooling to about 45^0C, 50 ml of 40% urea was added and mixed well. Then 5 ml was dispensed in test tube and set at slant position.

III Staining and test reagents

1. For Gram's stain

(a) Crystal Violet solution

Crystal Violet	20.0 g
Ammonium Oxalate	9.0 g
Ethanol or Methanol	95 ml

Distilled Water (D/W) to make 1 litre

Preparation: In a clean piece of paper, 20 gm of crystal violet was weighed and transferred to a clean brown bottle. Then, 95 ml of ethanol was added and mixed

until the dye was completely dissolved. To the mixture, 9 gm of ammonium oxalate dissolved in 200 ml of D/W was added. Finally the volume was made 1 litre by adding D/W.

(b) Lugol's Iodine

Potassium Iodide	20.0 g
Iodine	10.0 g
Distilled Water	1000 ml

Preparation: To 250 ml of D/W, 20 gm of potassium iodide was dissolved. Then 10 gm of iodine was mixed to it until it was dissolved completely. Finally the volume was made 1 liter by adding D/W.

(c) Acetone-Alcohol Decoloriser

Acetone	500 ml
Ethanol (Absolute)	475 ml
Distilled Water	25 ml

Preparation: To 25 ml D/W, 475 ml of absolute alcohol was added, mixed and transferred into a clean bottle. Then immediately, 500 ml acetone was added to the bottle and mixed well.

(d) Safranin (Counter Stain)

Safranin	10.0 g
Distilled Water	1000 ml

Preparation: In a clean piece of paper, 10 gm of safranin was weighed and transferred to a clean bottle. Then 1 litre D/W was added to the bottle and mixed well until safranin dissolved completely.

2. Normal saline

Sodium Chloride	0.85 g
Distilled Water	100 ml

Preparation: The sodium chloride was weighed and transferred to a leak-proof bottle premarked to hold 100 ml. Distilled water was added to the 100 ml mark,

and mixed until the salt was fully dissolved. The bottle was labeled and stored at room temperature.

2. Test reagents

a. For Catalase test

Catalase Reagent (3% H_2O_2)

Hydrogen peroxide	3 ml
Distilled Water	97 ml

Preparation: To 97 ml of D/W, 3 ml of hydrogen peroxide was added and mixed well.

b. For Oxidase test

Oxidase Reagent (impregnated in Whatman's No. 1 filter paper)

Tetramethyl *p*-phenylene diamine dihydrochloride (TPD)	1 gm
Distilled Water	100 ml

Preparation: This reagent solution was made by dissolving 1 gm of TPD in 100 ml D/W. To that solution strips of Whatman's No. 1 filter paper were soaked and drained for about 30 seconds. Then these strips were freeze dried and stored in a dark bottle tightly sealed with a screw cap.

c. For Indole test

Kovac's Indole Reagent:

Isoamyl alcohol	30 ml
p-dimethyl aminobenzaldehyde	2.0 g
Hydrochloric acid	10 ml

Preparation: In 30 ml of isoamylalcohol, 2 g of *p*-dimethyl aminobenzaldehyde was dissolved and transferred to a clean brown bottle. Then to that, 10 ml of conc. HCl was added and mixed well.

d. For Methyl Red test

Methyl Red solution

Methyl red	0.05 g
Ethyl alcohol (absolute)	28 ml
Distilled Water	22 ml

Preparation: To 28 ml ethanol, 0.05 gm of methyl red was dissolved and transferred to a clean brown bottle. Then 22 ml D/W was added to that bottle and mixed well.

e. For Voges-Proskauer Test (Barritt's Reagent)

Solution A

α-Naphthol	5.0 g
Ethyl alcohol (absolute)	100 ml

Preparation: To 25 ml D/W, 5 g of *α*-Napthol was dissolved and transferred into a clean brown bottle. Then the final volume was made 100 ml by adding D/W.

Solution B

Potassium hydroxide	40.0 g
Distilled Water	1000 ml

Preparation: To 25 ml D/W, 40 gm of KOH was dissolved and transferred into a clean brown bottle. Then the final volume was made 100 ml by adding D/W.

f. McFarland tube (No. 0.5)

0.5 ml of 0.048 M $BaCl_2$ (1.17% w/v $BaCl_2.H_2O$) was added to 99.5 ml of 0.18 M H_2SO_4 (1% w/v) with constant stirring. The McFarland standard was thoroughly mixed to ensure that it is evenly suspended. Using matched cuvettes with a 1 cm light path and water as a blank standard, the absorbance was measured in a spectrophotometer at a wavelength of 625 nm. The acceptable range for the turbidity standard is 0.08-0.13. The standard was distributed into screw-cap tubes of the same

size and volume as those used to prepare the test inoculum. The tubes were sealed tightly to prevent loss by evaporation and stored protected from light at room temperature. The turbidity standard was then vigorously agitated on a vortex mixer before use. Standards may be stored for up to 6 months, after which time they should be discarded.

APPENDIX-IV

GRAM-STAINING PROCEDURE

First devised by Hans Christian Gram during the late 19th century, the Gram-stain can be used effectively to divide all bacterial species into two large groups: those that take up the basic dye, crystal violet (Gram-positive) and those that allow the crystal dye to was out easily with the decolorizer alcohol or acetone (Gram-negative). The following steps are involved in Gram-stain:

1. A thin film of the material to be examined was prepared and dried.
2. The material on the slide was heat fixed and allowed to cool before staining.
3. The slide was flooded with crystal violet stain and allowed to remain without drying for 10-30 seconds.
4. The slide was rinsed with tap water, shaking off excess.
5. The slide was flooded with iodine solution and allowed to remain on the surface without drying for twice as long as the crystal violet was in contact with the slide surface.
6. The slide was rinsed with tap water, shaking off excess.
7. The slide was flooded with alcohol acetone decolorizer for 10 seconds and rinsed immediately with tap water until no further color flows from the slide with the decolorizer. Thicker smear requires more aggressive decolorizing.
8. The slide was flooded with counter stain (safranin) for 30 seconds and washed off with tap water.
9. The slide was blotted between two clean sheets of bibulous paper and examined microscopically under oil immersion at 1000X.

APPENDIX-V

BIOCHEMICAL TESTS FOR IDENTIFICATION OF BACTERIA

A. Catalase test

During aerobic respiration, in the presence of oxygen, microorganisms produce hydrogen peroxide, which is lethal to the cell itself. Catalase enzyme breaks down hydrogen peroxide into water and oxygen. The enzyme catalase is present in most cytochrome containing aerobic and facultative anaerobic bacteria, the main exception being *Streptococcus* spp.

B. Oxidase test

This test is performed for the detection of cytochrome oxidase in bacteria which catalyzes the transport of electrons between electron donors. In the presence of redox dye Tetramethyl-*p*-phenylene diamine dihydrochloride, the cytochrome oxidase oxidizes it into a deep purple colored end product Indophenol which is detected in the test. Positive test is indicated by the appearance of blue-purple color within 10 seconds.

C. Oxidation-Fermentation test

This test is done to determine the oxidative or fermentative metabolism of carbohydrate resulting in production of various organic acids as end product. Some bacteria are capable of metabolizing carbohydrates (as exhibited by acid production) only under aerobic conditions, while others produce acid both aerobically and anaerobically. Most medical bacteria are facultative anaerobes.

D. Indole Production test

This test detects the ability of the organism to produce an enzyme: ‘tryptophanase’ which oxidizes tryptophan to form indolic metabolites: indole, skatole (methyl indole) and indoleacetic acid. A smooth bacterial colony was stabbed on SIM (Sulphide Indole Motility) medium by a sterile stab wire and the inoculated media was incubated at 37°C for 24 hours. After 24 hours incubation, 0.5 ml of Kovac's

reagent was added. Appearance of red color on the top of media indicates indole positive. The color reaction is based on the presence of the pyrrole structure present in indole.

E. Methyl Red test

This test is performed to test the ability of an organism to produce sufficient acid from the fermentation of glucose to give a red color with the indicator methyl red (denotes changes in degree of acidity by color reactions over a pH range of 4.4-6.0). A pure colony of the test organism was inoculated into 2 ml of MRVP medium and was incubated at 37°C for 24 hours. After incubation, about 5 drops of methyl red reagent was added and mixed well. The positive test was indicated by bright red color.

F. Voges-Proskauer (VP) test

This test is employed to detect the production of acetyl methyl carbinol (a neutral end product) or its reduction product 2, 3-butanidiol during fermentation of carbohydrates. A pure colony of the test organism was inoculated into 2 ml of MRVP medium and was incubated at 37°C for 24 hours. After incubation, about 5 drops of Barritt's reagent was added and shaken well for maximum aeration and kept for 15 minutes, positive test is indicated by the development of pink red color.

G. Citrate Utilization test

This test is performed to detect whether an organism utilizes citrate as a sole source of carbon for metabolism with resulting alkalinity. Organisms capable of utilizing citrate as its sole carbon source also utilizes the ammonium salts present in the medium as its sole nitrogen source, the ammonium salts are broken down to ammonia with resulting alkalinity. The pH indicator bromothymol blue has a pH range of 6.0-7.6, i.e. above pH 7.6; a blue color develops due to alkalinity of the medium.

H. Motility test

The motility media used for motility test are semisolid, making motility interpretations macroscopic. Motile organisms migrate from the stabline and diffuse into the medium causing turbidity. Whereas non-motile bacteria show the growth along the stabline.

I. Triple Sugar Iron (TSI) Agar

The TSI agar is used to determine the ability of an organism to utilize specific carbohydrate incorporated in the medium (glucose, sucrose and lactose in concentrations of 0.1%, 1.0% and 1.0% respectively), with or without the production of gas along with determination of possible hydrogen sulfide production. The test organism was streaked and stabbed on the surface of TSI and incubated at 37°C for 24 hours. Acid production limited only to the butt region of the tube is indicative of glucose utilization, while acid production in slant and butt indicates sucrose or lactose fermentation. Phenol red is the pH indicator which gives yellow reaction at acidic pH, and red reaction to indicate an alkaline surrounding.

J. Urea Hydrolysis test:

This test demonstrates the urease activity present in certain bacteria which decomposes urea, releasing ammonia and carbon dioxide. Ammonia thus produced changes the color of indicator incorporated in the medium. Positive organism shows pink red color due to the breakdown of urea to ammonia. With the release of ammonia the medium becomes alkaline as shown by a change in color of the indicator to pink.

K. Coagulase test

This test is used specifically to differentiate species within the genus *Staphylococcus*: *S. aureus* (usually positive) from *S epidermidis* (negative). A positive coagulase test is usually the final diagnostic criterion for the identification of *S. aureus.* Free coagulase and bound coagulase are the two types of coagulase possessed by this organism.

Slide Coagulase Test:

Bound coagulase (Clumping Factor) is detected by slide test. For slide coagulase test, a drop of physiological saline was placed on three places of a slide, and then a

colony of the test organism was emulsified in two of the drops to make thick suspensions. Later a drop of plasma was added to one of the suspensions and mixed gently. Then a clumping was observed within 10 seconds for the positive coagulase test. No plasma was added in second suspension. This was used for the differentiation of any granular appearance of the organism from true coagulase clumping. The third drop of saline was used for a known strain of coagulase positive staphylococci.

Tube Coagulase Test

This test is carried out to detect production of free coagulase. In the tube coagulase test, plasma was diluted 1 in 10 in physiological saline. Four small tubes were taken, one for test organism, one for positive control, one for negative control, and one to observe self clotting of plasma. Then 0.5 ml of the diluted plasma was pipetted into each tube and 0.5 ml of test organism, 0.5 ml of positive control (*S. aureus* culture) and 0.5 ml negative control (*S. epidermidis* culture) was added to three tubes, to the fourth tube, 0.5 ml sterile broth was added. After mixing gently, all tubes were incubated at 37^0C on a water bath for 6 hours and observed for gel formation in every 30 minutes.

APPENDIX VI

ZONE SIZE INTERPRETATION CHART FOR ANTIBIOTIC SENSITIVITY TEST

Antimicrobial Agents used	Symbol	Disc Content	Resistant (mm or less)	Intermediate (mm)	Susceptible (mm or more)
Amikacin	Ak	30mcg	14	15-16	17
Ampicillin	A	10mcg	18	19-25	26
Amoxycillin/ Clavulunic acid	Ac	20/10mcg	19	-	20
Ceftazidime	Ca	30mcg	14	15-17	18
Cefixime	Cfx	5mcg	15	16-18	19
Chloramphenicol	C	30mcg	12	13-17	18
Ciprofloxacin	Cf	5mcg	15	16-20	21
Cotrimoxazole	Co	1.25/23.75mcg	10	11-15	16
Erythromycin	E	15mcg	15	16-20	21
Gentamicin	G	10mcg	12	13-14	15
Methicillin	M	5mcg	9	10-13	4
Penicillin	P	10 units	19	20-27	28
Piperacillin/tazobactam	Pt	100/10mcg	17	-	18
Tetracycline	T	30mcg	18	19-22	23

(CLSI, 2007)