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A Microbiological and Clinical Study on Cases of Chronic Obstructive Pulmonary Disease (COPD) at Hilla City

A Thesis

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College of Medicine/ Babylon University
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of the Master Degree of Medical Microbiology*

by

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

((قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْنَا))

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We certify that this thesis was prepared under our supervision at the University of Babylon as partial Fulfillment of the requirements for the degree of M.Sc. in Medical Microbiology....

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ABSTRACT

In this study, seventy five sputum and 75 blood samples were obtained from seventy-five (51 males and 24 females) Chronic Obstructive Pulmonary Disease patients above the age of 30 years who were admitted to Mirjan Teaching Hospital for Internal Medicine at Hilla City at the period from November, 2006 up to May 2007. Acid fast test was done for all patients, patients with positive results were excluded from the study.

The collected sputum samples were cultured for bacterial analysis, the results revealed that 65:75 (86.66%) gave positive sputum culture, whereas 10:75 (13.34%) gave negative growth, among the positive sputum cultures 58:65 (89.23%) got single bacterial isolate, and the predominant type were *Moraxella catarrhalis* (23 isolates), then *Haemophilus influenzae* (9 isolates), *Streptococcus pneumoniae* (8 isolates), *Enterobacter spp.* (4 isolates), *Klebsiella pneumoniae* (3 isolates), *Pseudomonas aeruginosa* (2 isolates), *Acinetobacter spp.* (2 isolates), and *Staphylococcus aureus* (1 isolate), in addition to yeasts, *Candida albicans* (5 isolates), and *Geotrichum* (1 isolate).

On the other hand, the other group of positive sputum culture were 7:65 (10.77%) which are mixed bacterial isolates with predominance of the *Moraxella catarrhalis* and viridans streptococci. Antimicrobial sensitivity test has been performed, where Azithromycin was the most effective antibiotic on most of the

bacterial isolates which were tested, however, other antibiotics showed a variable degree of sensitivity and resistance.

H.influenzae isolates were higher among ex-smokers patients compared with those who were current and non smokers.

Sputum cytology showed that sputum neutrophilia occurred in 51: 75 (68%), whereas, blood film examination showed that 47:75 (62.66%) of the blood samples had blood neutrophilia. Sputum neutrophilia were predominantly associated with *Haemophilus influenzae* (88.8%), followed by *Moraxella catarrhalis* (78.62%), and *Streptococcus pneumoniae* (62.5%).

Likewise, C-reactive protein was done on the sera of 52 Chronic Obstructive Pulmonary Disease patients and showed that all 52 samples had a positive reaction.

N-acetylcysteine was performed to detect its effect on the growth of 20 bacterial isolates, and 90% of these isolates had no growth in the presence of of this material, whereas, 10% gave positive growth even in the presence of this substance.

In addition, some of the clinical parameters were studied, it was found that the percentage of the disease increased with increasing age, the disease affected males higher than females, the percentage of the disease was higher in both current and ex-smokers in comparison to non smoker patients, all of the patients had productive cough , shortness of the breath, and expectoration of sputum.

الخلاصة

تم في هذه الدراسة جمع 75 عينة قشع و75 نموذج دم من 75 مريضاً (51 ذكور و 24 إناث) من المصابين بمرض الانسداد الرئوي المزمن وبأعمار فوق الثلاثين سنة للمرضى الذين أدخلوا إلى مستشفى مرجان التعليمي للأمراض الباطنية والقلبية في مدينة الحلة للفترة ما بين تشرين الثاني 2006 ولغاية أيار 2007، تم إجراء فحص AFB لجميع المرضى الذين تم شمولهم بهذه الدراسة وقد تم استبعاد المرضى الذين كان لديهم فحص موجب من الدراسة.

تم زرع نماذج القشع التي تم جمعها للتحري عن البكتيريا، حيث بينت النتائج أن 65 : 75 (86,66%) أعطت نتيجة زرع موجبة للقشع، بينما 10 : 75 (13,34%) أعطت نتيجة زرع سالبة، بين نتائج الزرع الموجبة 58 : 65 (89,23%) أعطت عزلات بكتيرية مفردة، والنوع السائد كان *Moraxella catarrhalis* (23 عزلة)، *Haemophilus influenzae* (9 عزلات)، *Streptococcus pneumoniae* (8 عزلات)، *Enterobacter spp.* (4 عزلات)، *Klebsiella pneumoniae* (3 عزلات)، *Pseudomonas aeruginosa* (عزلتين)، *Acinetobacter spp.* (عزلتين)، *Staphylococcus aureus* (عزلة واحدة)، بالإضافة إلى الخمائر، *Candida albicans* (5 عزلات)، *Geotrichum* (عزلة واحدة).

من الناحية الأخرى، فإن المجموعة الأخرى للزرع الموجب كانت 7 : 65 (10,77%) والتي كانت عزلات بكتيرية مختلطة (متعددة)، حيث كان النوع السائد *Moraxella catarrhalis* و *Viridans streptococci*. تم دراسة فحص الحساسية للمضادات الحيوية لبعض العزلات البكتيرية حيث تبين أن الأثرورومايسين (*Azithromycin*) كان من أكثر المضادات الحيوية التي كان لها تأثير على معظم العزلات البكتيرية، أما المضادات الحياتية الأخرى فكانت بدرجات متباينة من حيث الحساسية والمقاومة.

كانت نسبة عزل بكتيريا *Haemophilus influenzae* لدى المرضى الذين تركوا التدخين كبيرة مقارنة مع المرضى المستمرين على التدخين والمرضى الغير مدخنين أصلاً. بين الفحص الخلوي للقشع بأن زيادة الخلايا العدلة في القشع كانت 51 : 75 (68%)، بينما فحص فيلم الدم بين أن 47 : 75 (62,66%) من نماذج الدم كانت مصاحبة بازدياد نسبة الخلايا العدلة في الدم. كانت نسبة زيادة الخلايا العدلة في القشع مرتبطة بصورة سائدة مع بكتيريا

الخلاصة

Haemophilus influenzae (88,8%)، متبوعة بـ *Moraxella catarrhalis* (78,62%)، و *Streptococcus pneumoniae* (62,5%).

على نفس النمط، تم إجراء فحص البروتين التفاعلي-C (C-reactive protein) على مصل دم 52 مريض مصاب بالانسداد الرئوي المزمن حيث أن كل العينات كانت بنتيجة موجبة. تم إجراء تجربة آل N-acetylcysteine لتبيان تأثيرها على نمو 20 عزلة بكتيرية، حيث أن (90%) من هذه العزلات كانت نتيجة نموها سالبة بوجود هذه المادة، بينما (10%) أعطت نتيجة نمو موجبة على الرغم من وجود هذه المادة.

بالإضافة, تم دراسة بعض المعايير السريرية, لقد وجد نسبة المرض تزداد مع تقدم العمر, المرض يصيب الإناث أكثر من الذكور, نسبة المرض بين المدخنين والذين تركوا التدخين لمدة سنة كانت عالية بالمقارنة مع الغير المدخنين, كل المرضى كان لديهم سعال, ضيق التنفس, وبصاق القشع.

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LIST OF ABBREVIATIONS

Abbreviation	Meaning
AFB	Acid Fast Bacilli
BAL	Broncho alveolar lavage
BHIA	Brain Heart Infusion Agar
BHIB	Brain Heart Infusion Broth
CD3+	Cluster of differentiation 3
CD4+	Cluster of differentiation 4
CD8+	Cluster of differentiation 8
COPD	Chronic Obstructive Pulmonary Disease
CO₂	Carbon dioxide
CRP	C-reactive protein
DAMB	Dimethyl-amino benzaldehyde
DM	Diabetes Mellitus
DNA	Deoxy ribo nucleic acid
EDTA	Ethylene diamine tetra acetic acid
EX	Previous
FEV1	Forced Expiratory Volume in one second
FVC	Forced vital capacity
GSH	Reduced glutathione
gm	Gram
H₂O₂	Hydrogen peroxide
hr	Hour
ICAM-1	Intracellular adhesion molecule-1
IgE	Immunoglobulin E
IL-1Beta	Interlukin-1B
IL-6	Interlukin-6
IL-8	Interlukin-8
LOS	Lipooligosaccharide
LPS	Lipopolysaccharides

NAC	N-Acetylcysteine
NADH	Reduced nicotine amide dinucleotide
NADPH	Reduced phosphorelated nicotine amide dinucleotide
NCCLS	National Committee for Clinical Laboratory Standards
O2	Molecular Oxygen
PMN	Polymorphnuclear leukocytes
PPMO	Potentially pathogenic microorganism
RO	Reactive oxygen
RPM	Round per minute
RV	Residual volume
Spp.	Species
TGF	Transforming growth factor
Th-2 type	T-helper subtype-2
TLC	Total lung capacity
TMX	Trimethoprim-Sulphamethaxozole
TNF	Tumor necrosis factor

CONCLUSIONS

From this study the following findings can be concluded :

1. Chronic obstructive pulmonary disease (COPD) can affect the age group above 30 yrs, and the percentage of the disease increased with increasing the age.
2. COPD can affect both sexes with male predominance.
3. Smoking cigarettes and history of smoking are the major risk factors for the disease.
4. C-reactive protein (CRP) was elevated in all tested patients with COPD.
5. *Moraxella catarrhalis* was the major pathogen recovered from the sputum of patients with COPD, followed by *H.influenzae*, and *S.pneumoniae*.
6. Azithromycin was the most effective antibiotic that had an effect on nearly all isolates recovered from the sputa of COPD patients in this study.
7. Sputum neutrophilia and Blood neutrophilia showed a high degree of the elevation among COPD patients, where sputum neutrophilia was associated mainly with *H.influenzae*.
8. N-acetylcysteine showed a great effect in vitro on most of the bacterial isolates being tested in this study by inhibiting their growths.

RECOMMENDATIONS

1. Oral Azithromycin should be given as a major line of antibiotic treatment of COPD exacerbation.
2. Sputum cytology for sputum neutrophilia may aid the diagnosis of bacterial cause of exacerbating and severity of COPD.
3. N-Acetylcysteine is recommended due to its effect in inhibition the growth of predominant potentially pathogenic microorganism.
4. Further investigations like molecular and immunological techniques which may give the accurate and fast diagnosis of COPD.

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1.1. Introduction

Chronic obstructive pulmonary disease (COPD) is defined as a disease state that is characterized by airflow limitation that is not fully reversible, the airflow limitation usually is progressive and associated with abnormal inflammatory response, primarily caused by cigarette smoking (Celli and MacNee, 2004). COPD encompasses chronic bronchitis and emphysema, chronic bronchitis defined as a productive cough that is present for a period of three months in each of two consecutive yrs in absence of other identifiable causes of excessive sputum production, whereas emphysema is defined as alveolar wall destruction with irreversible enlargement of the air spaces distal to the terminal bronchioles without evidence of fibrosis.

Indeed, COPD is already the fourth most common cause of death in the United States that has increased over the last 30 years (Murray *et al.*, 2001). COPD in the individuals under 45 years is low, while it is high over 65 years old (Hurd, 2000) and, a difference has always existed in the prevalence of COPD in males and females with males having higher rate, however the increased rates of female smoking cigarettes in the last 20-30 years have associated with steadily increasing rate of COPD in the females (Mannino *et al.*, 1997).

The single most important risk factor of COPD is a cigarette smoking and accounts for about 90% of the COPD risk. Other environmental factors associated with high rate COPD; include, air pollution, occupational dust or chemical exposure, or some severe childhood respiratory viral infections. Likewise, COPD may be due to

alpha-1anti- trypsin enzyme deficiency (Coakley *et al.*, 2001), other factors include ; low socioeconomic status, poor nutrition and male sex .

COPD is characterized by acute exacerbations which are defined as an increased symptoms and worsening of lung function and it is a common cause of hospital admission, and featured by increase amount of sputum purulence which is due to bacterial infections of lower respiratory tract, upper respiratory tract viral infections and due to environmental factors (Seemungal *et al.*, 1999). COPD is associated with increased level of the proinflammatory cytokines like ;C-reactive protein, oxidative stress, and activation of the inflammatory cells like; neutrophils(Agusti *et al.*, 2003).

Bacteriological examination of sputum obtained from COPD patients must be taken in account that bacteria are isolated from the sputum of patients with COPD in 40-60% of acute exacerbations of COPD which include ;non-typeable *Haemophilus influenzae*, *Moraxella catarrhalis*,and *Streptococcus pneumoniae*, other infrequently isolated bacteria like ;*Pseudomonas aeruginosa*, *Staphylococcus aureus* ,*Klebsiella pneumonia*, in addition to the Enterobacteriaceae (Sethi , 2004).

Treatment of COPD includes ;smoking cessation which is the only measure that will slow the progression of the disease , use of bronchodilators like, B2-agonists and anticholinergics , antibiotics are commonly used for exacerbation of COPD with bacterial infection , and use of inhaled and oral corticosteroids.

1.2 The aims of the study:

The studies that carried out about COPD in Iraq were very little if they were compared to other respiratory diseases, so this study was the first study in Hilla city and aimed to the following :

- A. Studying the nature and some of clinical features of the disease .
- B. Studying some of the risk factors of the disease like; smoking.
- C. Determining the bacterial causes that are responsible for some cases of acute exacerbations of COPD..
- D. Cytological studying of the sputum to determine the sputum neutrophilia, and blood sampling for blood neutrophilia.
- E. Antibiotic sensitivity of bacterial isolates.
- F. Effect of N-Acetylcysteine(NAC) on the growth of some bacterial isolates.

2.1. The Lungs (Anatomic and Physiologic Overview) :

The lungs are two spongy organs in the chest which are surrounded by a thin and moist membrane called the pleura. The major function of the lungs is to exchange oxygen O₂ and carbon dioxide CO₂ between the atmosphere and the blood (Franklin, 2004).

The lungs are the largest organs in the body. Each one is composed of smooth and shiny lobes ; the right lung has three lobes and the left has two. Approximately 90% of the lung is filled with air and only 10% is solid tissue, and during the process of inhalation, the air travels through the following structure, the air is carried from the trachea into the lung through flexible airways called the bronchi which are like the branches of tree, then the bronchi further divided successively into over a million of smaller airways called bronchioles (Young and Health, 2002). The bronchioles lead to grape-like clusters of microscopic sacs called alveoli. Each alveolus has a thin membrane through which oxygen and carbon dioxide pass to and from capillaries, and during deep breathing , the alveoli unfold and expand to allow fresh oxygen to pass into capillaries and remove carbon dioxide waste to pass out of the body through the lungs. The oxygenated blood is carried back through blood vessels to the heart, where it is pumped from the heart to the body (Franklin, 2004).

Likewise, breathing or (ventilation) which includes two phases inspiration

and expiration and during each phase the body changes the lung dimensions to produce a flow of air either in or out of the lungs, where, Inspiration initiated by contractions of the muscles of the diaphragm and intercostal muscles where the diaphragm moves downward and this movement will enlarge the thoracic cage and as the thoracic wall moves away from the lung which increases the space between the thoracic wall and lungs and decreases the pressure in the intrapleural cavity, this decrease in the pressure causes the pressure in the alveoli to become greater than the elastic recoil that is inherent in lung tissue, and expiration during quiet breathing is normally a passive process and does not require muscles to work, when the lungs are stretched and expanded, the stretchy receptors within the alveoli send an inhibitory nerve impulses to the medulla oblongata, causing the rib cage and diaphragm to relax and rise and this elastic recoil causes the lungs and chest cavity to shrink and increase the air pressure within the lungs pushing the air out of the lungs(Graaf and Kent, 2002).

2.2.Definitions of Chronic Obstructive Pulmonary Disease (COPD):

The term COPD refers to a disease state characterized by progressive airflow limitation that is not fully reversible, and is associated with abnormal response of the lungs to the noxious particles or gases, primarily cigarette smoke (Celli and MacNee, 2004)), likewise, Devereux, (2006) defines COPD as a group of diseases characterized by limitation of airflow in the airways that is not fully reversible and it is an umbrella term for chronic bronchitis, emphysema,

it is most often due to tobacco smoking but can be due to other airborne irritants such as coal dust, asbestoses or solutions. COPD is a lifelong disease with significant cost and morbidity and in COPD patients acute bacterial infections of the respiratory tree are common and have a negative impact on quality of life and on the progression of the disease (Devereux, 2006).

Besides, Hurd, (2000) describes COPD that is the fourth leading cause of death in the United States and mortality rate expected to continue to increase, both in the United States and worldwide. On the same manner, COPD is an all-inclusive and non-specific term that refers to a defined set of breathing-related symptoms; chronic cough, expectoration, varying degrees of exertional dyspnea, and a significant and progressive reduction in expiratory airflow where most of COPD patients are smokers and ex-smokers (Lapperre *et al.*, 2006).

2.3. Exacerbation of COPD :

2.3.1. Definition :

An exacerbation of COPD is an event in the natural course of the disease characterized by a change in the patient's baseline dyspnea, cough, and or / sputum beyond day-to-day variability sufficient to warrant a change in management (Celli and MacNee, 2004).

2.3.2. Epidemiology and Risk Factors :

Exacerbations are a common cause of morbidity and mortality in COPD patients (Mannino *et al.*, 2002). Despite, aggressive medical treatment, approximately, one third of patients discharged from the emergency department with acute exacerbation have recurrent symptoms within 14 days and 17% have relapse and require hospitalization (Miravittles, 2002).

Risk factors of exacerbation include, infectious process such as ; viral infections (*Rhinovirus spp.*, *Influenza V.*) , Bacteria (*Haemophilus influenzae*, *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Enterobacteriaceae spp.*, and *Pseudomonas spp.*) (Adams and Anzueto, 2000). In addition to environmental conditions, air pollution exposure, lack of compliance with long-term oxygen therapy, and failure to participate in pulmonary rehabilitation . Furthermore, the incidence of relapse rate of exacerbation ranges from 21-40%, where the risk factors for relapse are, low pretreated forced expiratory volume in one second, increase corticosteroids use, previous exacerbation (more than 3 in the last 2 years), prior antibiotic treatment (mainly ampicillins), and presence of comorbid conditions (congestive heart failure) (Miravittles, 2002).

2.3.3. Assessment and Treatment of Exacerbation :

It includes several clinical elements like, severity of underlying COPD, the presence of comorbidity and history of previous exacerbation, and the

physical examination should evaluate the effects of the episode on the haemodynamic and respiratory systems (Adams and Anzueto, 2000).

Treatment of COPD exacerbation is based on the clinical presentation of the patient and includes, oxygen therapy, rehydration, bronchodilators, corticosteroids, Antibiotics which may be initiated in patients with altered sputum characteristics and the choice should be based on local bacteria resistance patterns, and the main antibiotics used, Amoxicillin, Cephalosporins, Macrolides, Doxycycline, and in case of failure consider the use of Amoxicillin/Clavulenate, and Fluoroquinolones (Swanson *et al.*, 2002).

2.4. Types of COPD :

The term COPD encompasses chronic obstructive bronchitis, with obstruction of small airways, and emphysema, with enlargement of airspaces and destruction of lung parenchyma, loss of lung elasticity, and closure of small airways.

2.4.1. Chronic obstructive bronchitis :

Chronic bronchitis can be defined as a clinical diagnosis characterized by a cough productive of sputum for over three months duration during two consecutive years and airflow obstruction, these requisites exclude more transient causes of cough associated with sputum production such as acute bronchitis, airflow obstruction in chronic bronchitis is caused by excessive

tracheobronchial mucous production (Ingram, 1994).

Likewise, chronic bronchitis is the inflammation and eventual scarring of the lining of the bronchial tubes and once the bronchial tubes have been irritated over a long period of time excessive mucous is produced constantly and the lining of the bronchial tubes becomes thickened with impairment of the airflow and eventual scarring of the lungs (Sat Sharma, 2006).

2.4.2. Emphysema :

Emphysema is the abnormal permanent enlargement of air spaces distal to the terminal bronchioles, accompanied by the destruction of the alveolar walls, without obvious fibrosis .

There are three morphological types of emphysema ;

- Centiaccinar emphysema which begins in the respiratory bronchioles and spreads peripherally and called centilobular emphysema and is associated with long lasting cigarette smoking and it involves the upper half of the lung.
- Panaccinar emphysema that destroys all of the alveoli uniformly and predominates in the lower half of the lung.
- Distal accinar emphysema or paraseptal type, which involves preferentially the distal airways structures, alveolar ducts , and alveolar sacs (Sat Sharma, 2006).

2.5. Pathogenesis of COPD :

Pathogenesis of COPD includes three important mechanisms which are: inflammatory mechanisms, oxidative stress, and immune mechanisms.

These different mechanisms can produce the pathological changes which in turn, give rise to many physiological abnormalities like ; mucous hypersecretion, ciliary dysfunction, airflow limitation and hyperinflation, and systemic effects (O'Donnell *et al.*, 2001).

2.5.1. Inflammatory Mechanisms :

COPD is a chronic inflammatory disease characterized by an increase in the neutrophils, macrophages, and T-lymphocytes (CD8+) in various parts of the lung, which are driven by inflammatory mediators, particularly cytokines, chemokines, and oxidants (Hogg *et al.*, 2004), and this abnormal inflammatory reaction to the stimuli is believed to be responsible for the most important pathologic abnormalities of COPD, bronchitis, and emphysema (Hogg, 2004).

On the same manner, Woodruff *et al.*, (2005) showed that smoking can induce a remarkably consistent and distinctive pattern of alveolar macrophage activation that might be relevant in COPD pathogenesis, likewise, the decreased expression of transforming growth factor (TGF)-beta type II is associated with bronchial gland enlargement in smokers with COPD, and induce structural changes in the bronchial glands, which, in turn may promote mucous hypersecretion , and also, the imbalance of proteinases and antiproteinases are

also important in the pathogenesis of COPD (Baraldo *et al.*, 2005).

2.5.2. Oxidative Stress :

The most important environmental factors related to COPD is active and passive cigarette smoking which act through the generation of oxidative stress or reduction of antioxidant capacity (Lindberg *et al.*, 2005).

Indeed, the role of oxidative stress in COPD is the increased ability of neutrophils to synthesize the chemotactic factor Leukotriene B4 which may contribute to the infiltration and activation of neutrophils into the airways, particularly in conjunction with exacerbations, and the effect of oxidative stress may extend to dysfunction of respiratory skeletal muscle particularly in hypoxic conditions (Koechlin *et al.*, 2005).

2.5.3. Immune Mechanism :

CD8 T- lymphocytes have been related to the progressive reduction of the pulmonary function in both COPD and Asthma (van Rensen *et al.*, 2005). CD4 T- lymphocytes are also increased in COPD especially, in severe cases (Hogg, 2004).

Sever emphysema is associated with apoptosis of alveolar epithelial cells and inflammation involving T-lymphocytes and these T-cells accumulate in the lung secondary to conventional antigenic stimulation which might be involved in the persistent inflammation of severe emphysema, and CD4+ cells may be involved in the development of COPD that points to a possible immune

Inflammation which persists even after cessation of the risk factor like ; the cessation of cigarette smoking (Willemse *et al.*, 2002).

2.6. Epidemiology of COPD :

The estimates of the prevalence of COPD depend on the definitions and used criteria that is based on the presence of airflow limitation are the most accurate, since symptoms and self-report or clinical diagnosis lack sensitivity and specificity (Pauwels *et al.*, 2001). The estimated prevalence in United states for those aged 25-75 years of mild COPD (defined as $FEV_1/FVC < 70\%$ and $FEV_1 \geq 80\%$ predicted) was 6.9%, and for moderate COPD ($FEV_1/FVC < 70\%$ and $FEV_1 \leq 80\%$ predicted) was 6.6% where , the prevalence of both mild and moderate COPD was higher in males than females (Mannino *et al.*, 2002).

The morbidity of COPD increases with increasing age and still greater in males than females, and the risk of hospital admission due to COPD increases with the decrease in the lung function, COPD death rates are very low under the age of 45 years, and increase with age and COPD is the fourth leading cause of death worldwide where it is estimated to be the third leading cause of death by the year of 2020 (Feenstra *et al.*, 2001; Murray and Lopez, 1997).

2.7. Risk Factors of COPD :

A- Smoking :

Smoking is considered by far the most important risk factor associated with the functional decline in COPD, as well as having more respiratory

symptoms, where smokers also have a greater annual rate of lung function decline assessed by changes in FEV1 and greater risk of premature mortality due to COPD than non-smokers, middle-aged smokers who continue smoking have an accelerated loss in FEV1 compared with non-smokers, in addition, individuals with COPD are at increased risk of death from other causes such as lung cancer and cardiovascular disease which may complicate the problem and irrespective of such conditions, stopping smoking has a positive benefits on functional decline in individuals with COPD (Ramsey and Hobbs, 2004).

80- 90 % of COPD may be caused by cigarette smoking both current and ex-smokers, but 15-20% of all smokers have COPD (Shahab *et al.*, 2006 ; Mannino *et al.*, 2002). Smoking induces inflammation which thought to play an important role in the pathogenesis of COPD and characterized by the influx of the neutrophils into the airways lumen and increased macrophages and T-lymphocytes number in the airways and, the cigarette smoking can act through the generation of oxidants and / or reduction of antioxidant capacity (Hogg *et al.*, 2004).

B- Occupational Dusts and Chemicals :

Exposure to occupational dusts, to chemicals and air pollution are important risk factors that can cause COPD independently of tobacco smoke, and such exposure results in inflammation which is a key factor in the pathogenesis of COPD (Wongsurakiat *et al.*, 2003).

C- Outdoor and Indoor Air Pollution :

The role of outdoor air pollution in causing COPD is unclear, but appears to be small when compared with cigarette smoking, whereas indoor air pollution biomass fuel burned from cooking and heating has been implicated as a risk factor for COPD (Chen and Mamino, 1999).

D- Infections :

A history of severe childhood respiratory infections has been associated with reduced lung function and increased respiratory symptoms in adulthood (Tager *et al.*, 1988).

E- Genetic Factor :

It is believed that many genetic factors increase or decrease the person's risk of COPD, and the genetic risk factor that is best documented is a rare hereditary deficiency of alpha-1 antitrypsin (McElvaney and Crystal, 1997). The exploratory studies have revealed a number of candidate genes that influence a person's risk of COPD and several of these genes are thought to be involved in inflammation and related to potential pathogenic mechanisms of COPD (Sandford *et al.*, 1997).

F- Airway Hyperresponsiveness :

Asthma and airway hyperresponsiveness identified as risk factors that contribute to the COPD development, and they are complex disorders related

to a number of genetic and environmental factors, in addition, airway hyperresponsiveness may also develop after exposure to tobacco smoke or other environmental insults (Tager *et al.*, 1988).

G- Gender :

Worldwide, COPD is more prevalent in males than females, however, it may be a consequence of the marked difference in smoking exposure between males and females, and recent data suggest that females may in fact be more susceptible to the effects of tobacco smoke than males, but now the male sex is not a risk factor for COPD development (Prescott, 2000).

H- Age :

COPD is low under 45 years, while it is high above 65 years old, and COPD is the most rapidly rising cause of death in the individuals above 60 years of age (Hurd, 2000). Indeed, aging associated with decrease elastic recoil of the lung, decrease compliance in chest wall, and decrease in the strength of the respiratory muscles (Janssens *et al.*, 1999).

2.8. Clinical Features of COPD :

2.8.1.Chronic Cough :

Chronic cough may be the first symptom to develop, and initially it is intermittent but may progress daily, and cough in COPD may have a protective role in some patients (Connolly, 2003).

The airway inflammation and excessive bronchial secretions are likely to activate the afferent limb of the cough reflex (Higgenbottom, 2002), on the same manner, Foster, (2002) shows that the presence of airflow obstruction may lead to ineffective cough as a result of decreased expiratory flow and this coupled with impaired mucociliary clearance which results in further retention of the secretions and a vicious cycle of chronic coughing will happen.

2.8.2. Sputum Production :

Sputum production occurs initially in the morning but later presents at all the day, usually it is tenacious and mucoid and in small quantities, the production of sputum for 3 months in 2 consecutive years suggests chronic bronchitis and changes of the sputum into purulent indicates an infectious exacerbation (Celli *et al.*, 1995). The presence of purulent (green) sputum was sensitive and specific for the yield of a high bacterial load which indicates a subset of which likely to benefit from antibiotic therapy (Robert, 2000), on the same manner, Anthonisen *et al.*, (1987) points that the change of the sputum from mucoid into purulent is associated with the presence of bacteria which is accompanied by the activation of secondary host defenses that include neutrophils recruitment to the airways.

2.8.3. Dyspnea :

Chronic dyspnea (breathlessness) is the most clinically important feature of COPD, and patients experience chronic ventilation- perfusion mismatch as a

result of mechanisms associated with emphysema, chronic bronchitis, or a combination of underlying pathologies where the resulting dyspnea reflects decreased pulmonary function with poor prognosis and it is the main symptom of COPD or it is the primary symptom in which COPD seek medical attention and it may be described by the patients as air hunger or gasping for breath where, it becomes worse with activity and in the presence of respiratory infections (O'Donnell *et al.*, 2001).

2.8.4. Weight Loss :

The rate of weight loss is one to four people with COPD where COPD patients with emphysema weighs too little for good health and emphysematous patients with weight loss are at high risk of death (Barnes, 2000). Likewise, the causes of weight loss in COPD maybe due to using more energy, eating less due to dyspnea, need for steroids as a part of treatment which increase the muscle breakdown, in addition to hepatic venous congestion (Barnes, 2000).

2.8.5. Peripheral Oedema :

It occurs in a substantial number of COPD patients particularly in those with hypercapnia and hypoxemia affects the venous return to the right side of the heart with worsening of right ventricular function with hepatic venous congestion (MacNee, 1994).

2.9. Diagnosis and Assessment of COPD :

2.9.1. Clinical Assessment :

It is based on medical history and physical examination, where complete examination is indicated for all patients, these two components are important for patients suspected with COPD (Pauwels *et al.*, 2001).

2.9.1.1. Medical History : includes the following (Pauwels *et al.*, 2001);

A- Symptoms like; cough, sputum, and dyspnea.

B- Past medical history and review of systems: which includes :

- Any family history of COPD or other respiratory disease.
- Any history of exacerbation of COPD or hospitalizations.

Any comorbidities like; heart disease, peripheral disease.

- History of un-explained weight loss because if it is caused by COPD, it has a poor prognosis.
- Any other, non-specific symptoms, like; wheezing and chest tightness.

C- Exposure history :

The history of exposure to risk factors, like; smoking, or occupational or environmental noxious agents, should be noted. A detailed smoking history is essential (pack-years). Pack-years are calculated by multiplying the number of packs equivalent smoked every day by the total number of years.

2.9.1.2. Physical Examination :

A normal physical examination is frequent in early COPD, and as the disease progress some signs become apparent and in the advanced stages become almost pathognomonic (Calverley and Georgopoulos, 1998).

A- Respiratory Signs : which includes the following :

- **Inspection :** check for barrel chest deformity, pursed lips breathing, chest/abdominal wall paradoxical movement and use of accessory respiratory muscles and these signs are of severe airflow limitation, hyperinflation and impaired the mechanisms of breathing.
- **Percussion :** decreased motion of the diaphragm and tympanetic sounds due to hyperinflation .
- **Auscultation :** adventitious rhonchi and wheezing may help to differentiate COPD from congestive heart failure which is often associated with crackles.

B- Systemic Signs :

- Neck vein distension, liver enlargement and peripheral oedema could be due Cor pulmonale or due to severe hyperinflation.
- Loss of muscle mass and peripheral muscle weakness with malnutrition.
- Cyanosis or bluish color of the mucosal membranes may indicate hypoxemia.

2.9.2. Diagnosis and Investigations of COPD :**2.9.2.1. Diagnosis :**

Diagnosis of COPD should be considered in any patient who has ;

symptoms of cough, sputum production, dyspnea, and history of exposure to risk factors of the disease. The diagnosis requires spirometry, post-bronchodilator $FEV_1 / FVC < 0.7$ confirms the presence of airflow limitation that is not fully reversible (Pauwels *et al.*, 2001).

Spirometric classification has been proved useful in predicting health status, utilization of health care resources, development of exacerbation and mortality (Dewan *et al.*, 2000), and it is intended to be applicable to populations and not to substitute clinical judgment (Celli *et al.*, 2003).

2.9.2.2. Investigations :

A- Tests for all patients :

Spirometry should be performed in all patients suspected of COPD and this is necessary for diagnosis, assessment of severity, chest radiography that should be obtained in all patients and it is helpful in excluding other diseases (Pauwels *et al.*, 2001; Dewan *et al.*, 2000).

B- For Selected patients :

Alpha-1 anti-trypsin level measurement, blood gases, and computed tomography (Celli *et al.*, 2003 ; Pauwels *et al.*, 2001).

2.10. Treatment of COPD :

Treatment of stable COPD includes the following issues ; smoking cessation, pharmacological therapy, long-term oxygen therapy, pulmonary rehabilitation, nutrition, and surgery for COPD (Swanson *et al.*, 2002).

2.10.1. Smoking Cessation :

Quitting smoking can slow the progressive loss of lung function and can reduce symptoms at any point of time, and the beneficial impact of smoking cessation on the natural history of COPD is greatest the earlier cessation is achieved (Barker *et al.*, 1991). The most comprehensive guidelines for smoking cessation is "Treating Tobacco Use and Dependence" (Fiore, *et al.*, 2000).

2.10.2. Pharmacological therapy of COPD :

COPD medications can reduce or abolish symptoms, increase exercise capacity, reduce the number and severity of exacerbations, and improve health status (Burge *et al.*, 2000). It includes ; Bronchodilators which are used for the treatment of COPD, inhaled glucocorticoids also used for COPD therapy and they act on multiple points within the inflammatory cascade and can benefit the COPD, also the use of combined inhaled steroids and bronchodilator in one inhaler that serves a convenient way of treatment (Calverley *et al.*, 2003 ; Belman *et al.*, 1996). Mucolytic and antioxidant therapy has been assessed in the reduction the number of COPD exacerbation (Poole and Black, 2001).

2.10.3. Others :

Other treatment includes long-term oxygen therapy, and surgery like; bullectomy and Lung-volume reduction surgery (Vermeran *et al.*, 2001 ; Balter *et al.*, 1994).

2.11. The Role of C-reactive Protein (CRP) in COPD :

CRP is a member of an ancient family of molecules called pentraxin, and it was discovered in humans in 1930 as a serum component that binds the C-polysaccharides of *Streptococcus pneumoniae*, where it is composed of five identical subunits that linked non-covalently to form a disc-like pentagonal ring (Carlson *et al.*, 2005).

CRP is a member of a diverse class of defense molecule called acute-Phase protein, where, the levels of acute-phase protein rises rapidly during infection, and after injury (Broekhuizen *et al.*, 2005).

Furthermore, CRP level increases in stable COPD and in ex-smokers with COPD, and the diseased lung tissue itself provides the sustained and danger signal that driven CRP synthesis where the damaged lung tissue might generate endogenous fibronectin, or heat shock protein triggering direct inflammation whereas, in COPD current smokers the high level of CRP may be due to the inducing of IL-6 by cigarette smoke (Torres *et al.*, 2006).

2.12. The Role of Bacteria in COPD :

The presence of bacteria in the lower bronchial airways of patients with stable COPD has been labeled as colonization (Cabello *et al.*, 1997).

2.12.1. Potential Roles of Bacterial Infections in COPD :

Five potential roles contribute to the course and pathogenesis of COPD.

2.12.1.1. Childhood Lower Respiratory Tract Infection and Adult Lung Function :

The association between childhood lower respiratory tract infection and impaired lung function in adulthood could be explained by damage caused to a vulnerable lung undergoing rapid postnatal growth and maturation by the infectious process (Jonhston *et al.*, 1998).

2.12.1.2. Bacterial Pathogens as a Cause of Acute Exacerbations of COPD :

Bacteria can be isolated from the sputum in 40-60% of acute COPD exacerbation (Sethi, 1999). The incidence of bacterial isolation from sputum during exacerbations of COPD was not different from the incidence during the stable state (McHardy *et al.*, 1980). Moreover, several investigators have re-examined whether the bacterium causes acute exacerbations of COPD by using new diagnostic modalities or new research techniques like, bronchoscopic sampling of the lower respiratory tract, immune response to bacterial pathogens, molecular epidemiology of bacterial pathogens, and airway inflammation measurement and correlation with bacteriology (Fagon *et al.*, 1990).

2.12.1.3. Vicious Circle Hypothesis :

Central to vicious circle hypothesis is the notion that bacterial pathogen has gained a foothold in the lower respiratory tract from impaired mucociliary clearance due to tobacco smoking (Aebi *et al.*, 1998).

2.12.1.4. Chronic Bacterial Infection of Respiratory Tissues :

Nontypeable *H.influenzae* has always been regarded as an extracellular pathogen that infects the airway lumen in COPD and binds with specificity to mucin, and adherence to the surface of respiratory epithelial cells and bacteria passes through confluent layers of epithelial cells with no effect on the permeability or viability and it is protected from the bactericidal activity of several antibiotics (van Schilfgaarde *et al.*, 1999).

2.12.1.5. Hypersensitivity to Bacterial Antigens :

Inefficient removal of the bacteria from the lower respiratory tract is the characteristic of chronic bronchitis which results in prolonged contact between the airway lymphoid tissue and bacterial antigens where this could lead to the emergence of IgE antibodies to bacterial antigens which induces eosinophilic infiltration and mast cell degranulation on repeated exposure to the bacterial antigens. Histamine release and IgE to bacterial antigens suggest that bacterial pathogens, directly or indirectly via Th2-type immune response could contribute to the eosinophilic airway hyper-reactivity and bronchoconstriction seen in COPD (Hargreave and Leich, 1999).

2.12.2. Types of Bacterial and other Pathogens :

Bacterial pathogens in COPD include, both potentially pathogenic microorganisms (PPMO) like, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*,

Acinetobacter spp., *Enterobacter* spp., and *Staphylococcus aureus*. whereas, the other group is non-potentially pathogenic microorganisms(non-PPMO) like, Viridans streptococci, *Nisseria* spp., *Corynebacterium* spp., and *Candida* spp. (Banerjee *et al.*, 2004; Soler *et al.*, 1999 ; Cabello *et al.*, 1997).

2.12.2.1. *Haemophilus influenzae* :

Nontypeable *H.influenzae* strains are common inhabitants of the human upper respiratory tract which being present in up to three to fourths of healthy adults (Cabello *et al.*, 1997). The adherence of this bacteria to human respiratory tract mucosal surface is the result of a complex interaction of bacterial adhesions and host molecules, and the bacteria has some mechanisms of adherence like, mucin binding, adherence to respiratory mucosa, and intracellular and intercellular invasion (St.Geme, 1997). Hill *et al.*, (2000) points that the bacteria has antigenic variation of Lipoolygosaccharide in structure, phase variation, molecular mimicry of host tissues, and the, adults with COPD make strain-specific antibody responses to surface exposed epitope following infection with *H.influenzae*. Furthermore, the bacteria can induce sputum neutrophilia in COPD patients which is higher than other pathogens in both mucoid and purulent sputum (Stockley *et al.*, 2001), indeed, LOS from this bacteria has intracellular adhesion molecule (ICM-1) expression and release of IL-6, IL-8, and TNF-alpha where their concentrations were significantly increase by higher concentration of LOS (Khair *et al.*, 1996), and persistence

of this bacteria in the lower airways appear to stimulate airway epithelium to produce proinflammatory cytokines ,especially those that promote neutrophil chemotaxis (Sethi *et al.*, 2006).

2.12.2.2. *Moraxella catarrhalis* :

It is the second most common cause of exacerbations after *H.influenzae* ,and 30% of exacerbations were caused by this bacteria (Verghese *et al.*, 1990).

Regarding the epidemiology of colonization, the bacterium colonizes mainly the respiratory tract and rarely recovered from genitourinary tract, the upper respiratory tract colonization with the bacteria is common throughout the infancy where the prevalence of colonization decreases with age and nearly 1-5 percent of healthy adults are colonized with this bacterium (Smith, 1987).

Indeed, *M.catarrhalis* are more likely to be recovered from the sputa of patients with chronic lung disease than from healthy adults (Boyle *et al.*,1991).Moreover, the outer membrane of the bacteria contains lipooligosaccharides (LOS) which lacks the long O-polysaccharide side chain that is present in enteric lipopolysaccharides(LPS) where LOS of this bacterium has one epitope in common with LOS of other non enteric gram negative bacteria and the lipid A portion of LOS molecule of this bacteria is similar in structure to the lipid A of other gram negative bacteria and is responsible for profound biological effects of LOS (Holme *et al.*, 1999).

Furthermore, *M.catarrhalis* is the most common pathogen encountered

in Saudi Arabia (Al-Amoudi, 2007), whereas some studies put this bacteria in the second or in the third place, and the rate of isolation is much higher in the winter than in spring and summer which possessed high seasonal variation in isolation which is less pronounced in other bacteria (Murphy *et al.*, 2005).

2.12.2.3. *Streptococcus pneumoniae* :

These bacteria can be recovered from the sputum of patients with chronic bronchitis even in the absence of clinical infection, and the rate of respiratory tract colonization by *S.pneumoniae* varies with age, where 20-40% of healthy children and 10-20% of healthy adults are colonized at any time, and the bacteria can be recovered from the sputum of patients with COPD exacerbation at a rate of 7-26% (Aebi *et al.*, 1998), besides, the major virulence factor for the organism is polysaccharide capsule and it accounts for the resistance to phagocytosis and survival in blood stream, and antibodies to capsular polysaccharides are protective from infection and cell wall polysaccharides which are major surface antigens among all serotypes where both cell wall and cell wall polysaccharides can induce inflammation (Musher *et al.*, 1990).

S.pneumoniae that colonizes lower airways in COPD is continuously dividing and shedding cell wall fragments into the airways and their antigens may lead to airway inflammation observed in COPD with less neutrophils in the sputum than *H.influenzae* and *M.catarrhalis* (Carlsen *et al.*, 1992).

2.12.2.4. Other Potentially Pathogenic Microorganisms :

Include, *P.aeruginosa*, *K.pneumoniae*, *Acinetobacter* spp., and *S.aureus* where they account for a significant number of isolates with severe airflow obstruction.

A- *P.aeruginosa*:

It accounts for a significant number of isolates in patients with severe airflow obstruction, but rare in mild cases (Miravittles *et al.*, 1999), and its LPS is more potent than E-coli LPS in stimulating release of monocyte chemotactic activity from various cell lines (Koyama *et al.*, 1999).

Soler *et al.*, (1999) showed that this bacteria is isolated at a high rate from the respiratory samples in patients with severe exacerbation COPD especially, those on mechanical ventilation with severely impaired pulmonary function.

B- *K.pneumoniae* :

It is one of the potential pathogens which are isolated from the sputum of patients with COPD exacerbations, and it occurs with gradual degree of the deteriorating pulmonary function and also affects severely ill patients who need mechanical ventilation (Soler *et al.*, 1999).

C- *S.aureus* :

It is one of the less frequently potential pathogens, and it is rarely implicated in other respiratory mucosal infections and unlikely to be pathogens of major importance in acute exacerbation of COPD (Sethi and Murphy, 2001).

D- Others :

Like ; *Acinetobacter* spp. and other gram negative bacteria are in mild cases and it may ascribe to frequent giving of antibiotics in patients with severe airflow obstruction has driven the bacteria to colonize the lower airways (Fagon *et al.*, 1990).

2.12.2.5. Non-Potentially Pathogenic Microorganisms :

These microorganisms are implicated in COPD mostly in association with other potentially pathogenic microorganisms, and the presence of yeasts either as a part of non-PPMO isolated from the patients sputa or may be due to contamination during the processing and culturing of the sputum, and the non-PPMO sputum neutrophilia and inflammatory response with cytokines level lower than that of PPMO (Banerjee *et al.*, 2004 ; Soler *et al.*, 1999).

2.13. Antibiotics Treatment of COPD :

Antibiotics are commonly prescribed empirically to patients with an acute COPD exacerbation to treat presumed bacterial infection (Wilson, 2001).

Bacterial infections are judged to cause half of exacerbations, and it is advised that the choice of antibiotic is important because the rise in bacterial resistance has made older agents less effective (Ball *et al.*, 1995). In recent years, there has been a dramatic rise in antibiotic resistance among common respiratory pathogens, which led to a relative increase in the clinical features in the treatment of lower respiratory tract infection (Ewig *et al.*, 1999).

Anthonisen *et al.*, (1987) shows that the antibiotics should be used if the patient has two or more of ; increased breathlessness, increased sputum volume, and development of sputum purulence, likewise, Adams *et al.*, (2000) indicates that the antibiotic choice for COPD exacerbations should depend on the severity of underlying disease, exacerbation, sputum character, and the local susceptibility pattern.

Amoxicillin and penicillins are the most common antibiotics used in the exacerbation, but the development of B-lactamase by *M.catarrhalis* and *H.influenzae* limits their activity in addition to the dosing because these drugs Should be given three to four times daily (Sonnesyn and Gerdin, 1994).

Amoxicillin-Glavulenate (Augumentin) has been proved to be effective against B-lactamase producing organisms and there are limited data concerning the activity of these antibiotics on penicillin-resistant *S.pneumoniae*, in addition to the frequent gastrointestinal side effects (Doern *et al.*, 1996).

Cephalosporins like cephalexin and cefaclor have been used greatly in the treatment of acute exacerbation, but in sever infections these antibiotics have been shown to be ineffective and newer Cephalosporins like, cefixime and cefprozil have shown improved efficacy and safety in the treatment of acute exacerbations of chronic bronchitis (Ball *et al.*, 1995).

Trimethoprim-Sulphamethaxozole (TMX) has no significant difference in comparison to Cephalosporins and amoxicillin where penicillin resistant

S.pneumoniae are highly resistant to TMX in addition to the toxicity of TMX in causing severe skin reaction especially in elderly patients (Balter *et al.*, 1994).

Tetracyclines which include, naturally occurring molecules like, tetracyclin and semi-synthetic molecule like, doxycyclin and minocyclin, sputum concentrations of doxycyclin are low compared with minocyclin and it is more active against *H.influenzae* and *S.pneumoniae*, whereas doxycyclin is effective against *M.catarrhalis* with no difference in the response rate between doxycyclin and minocyclin in the treatment of acute exacerbation (Chopra *et al.* 1992).

Macrolides are used to inhibit RNA-dependant protein synthesis by reversibly binding to 50s ribosomal subunits of susceptible microorganisms, erythromycin has poor activity against *H.influenzae* and *M.catarrhalis* and can not be considered one of the drug choice in the treatment, while Azithromycin, Clarithromycin, and dirithromycin are semi-synthetic derivatives of Erythromycin with structural modifications to improve pharmacokinetic tissue penetration. Azithromycin has enhanced potency against *H.influenzae*, whereas Clarithromycin has an intermediate in vitro activity against the same bacteria (Boswell and Wise, 1998).

Resistance to Macrolides by the microorganisms is either, by modification of antimicrobial agent (by antimicrobial inactivation), or by active efflux of the antimicrobial agent (Leclercq and Courvalin, 1993).

Flouroquinolones should be a first-line treatment of acute exacerbation of chronic bronchitis especially, in patients who have chronic bronchitis with comorbid illness like, severe obstruction ($FEV_1 < 50\%$), or old age > 65 years old or have recurrent exacerbations (Sethi, 1999).

Flouroquinolones are broad-spectrum antibiotics with high lung tissue penetration and oral bioavailability where the bactericidal activity of quinolones is obtained by inhibition of topoisomerase IV and DNA gyrase which prevent microbial DNA replication and transcription (Nix, 1998).

2.14. Sputum Neutrophilia and Blood Neutrophilia in COPD :

Sputum neutrophilia is the presence of more than 25 neutrophils per high power field in the sputum, and neutrophils normal value in the sputum should be less than 35% of the total cells where the neutrophils in the specimen is quite variable among healthy people (Robert, 2000).

Sputum neutrophilia can be seen in clinically stable COPD and during exacerbation where neutrophils increase in exacerbation and the neutrophils number is related to disease severity where the appearance of purulent and mucopurulent sputum in patients with COPD reveal more intense sputum neutrophilia (Papi *et al.*, 2006 ; Stockley *et al.*, 2001).

Sputum neutrophilia can be induced by cigarette smoking and loosely related to the degree of chronic airflow limitation, also it can be induced by air pollution, endotoxin, some occupational exposure, viral and bacterial infections

(Jayaram *et al.*, 2000). Airway obstruction with chronic expectoration and decline in lung function are associated with increase number of neutrophils in the sputum of smokers and ex-smokers (Stanescu *et al.*, 1996).

Furthermore , sputum neutrophilia is dominant among *H.influenzae* which is higher than other pathogens in both mucoid and purulent sputum, also *M.catarrhalis* is associated with sputum neutrophilia but less than that of *H.influenzae*. Exacerbation of COPD due to bacterial causes is also associated with high percentage of sputum neutrophilia dominated mainly among *H.influenzae* followed by *M.catarrhalis* and *S.pneumoniae* (Papi *et al.*, 2006 ; Stockley *et al.*, 2001).

Blood neutrophilia can also present in the peripheral blood among COPD patients during exacerbation and it is correlated with the severity of the exacerbation in relation to lung function reduction (Papi *et al.*, 2006), also peripheral blood neutrophils in acute exacerbations of COPD have a specific apoptotic pattern supported by the fact that COPD is an inflammatory disease with systemic impact (Pletz *et al.*, 2004). Besides, exacerbation is associated with infection has a greater blood neutrophilia response compared with non infectious exacerbation which depends also on the disease severity (Papi *et al.*, 2006).

2.15. The Role of N-Acetylcysteine (NAC) in COPD :

N-Acetyl-L-Cysteine (NAC) is an altered form of the amino acid cysteine which is commonly found in food and synthesized by the body, and it degrades the mucous (Grandjean *et al.*, 2000).

NAC is used in the medical treatment of chronic bronchitis, cancer, and paracetamol intoxication where it is one of the smallest drug molecule in use which has antibacterial properties because this molecule is a thiol-containing antioxidant that disrupts disulphide bonds in mucous and competitively inhibits the utilization of cysteine (Riise *et al.*, 2000 ; Naszal *et al.*, 2000).

Likewise, NAC is effective in the treatment of liver failure due to causes other than acetaminophen poisoning like ; hepatitis, and other drug toxicity , also supplementation with NAC has been shown to reduce the proliferation of certain cells lining the colon and may reduce the risk of colonic cancer in people with recurrent polyps in the colon (Ben-Ari *et al.*, 2000).

NAC exhibits direct and indirect antioxidant properties where it's free thiol group is capable of interacting with the electrophilic group of reactive oxygen (RO) which leads to the intermediate formation of NAC thiol with NAC disulphide as a major end product, NAC also exerts indirect antioxidant effect related to its role as a precursor of reduced glutathione (GSH) (Auroma, *et al.*, 1989).

Furthermore, NAC has an effect in lowering the intrabronchial bacterial growth particularly in patients with chronic bronchitis (Riise *et al.*, 1994), on the same manner, Riise *et al.*, (2000) states that the use of NAC as a drug in vitro has a negative effect on bacterial adhesive capacity, especially in *S.pneumoniae* and *H.influenzae*, and the rate of positive sputum culture will be low compared with those who do not use NAC as a medicine among COPD patients (Zheng *et al.*, 1999).

3.1. Materials :**3.1.1. Equipments and Apparatuses :**

The equipments and apparatuses used in the study are shown in table (3-1) :

Equipment	Company (origin)
Autoclave	Stermite, Germany
Incubator	Memmert, Germany
Centrifuge	Hermle, England
Electronic balance	Memmert, Germany
Hot air oven	Memmert, Germany
Light microscope	Olympus, Japan
Refrigerator	Concord, Lebanon
Water bath	Memmert, Germany
Millipore filter	Satorius membrane filter Gm, bH Germany
pH meter	Hoelezeand Cheluis, KG, Germany
Micropipette	Oxford, USA
Water distiller	Ogawa Seiki, Japan

Automated differential counter (Mardel blood cell calculator)	Mardel blood cell calculator company, USA
Neubauer improved chamber WBC slide	Marienfal, Germany

3.1.2. Chemicals materials :

Table (3-2) shows the chemicals materials used throughout the study :

Chemical materials	Company (origin)
I-Chemicals : - Acetic acid, acid fuchsin, amyl alcohol, Ethanol alcohol (95%), glucose, lactose. - NaCl , KH ₂ PO ₄ , K ₂ HPO ₄ , KOH, HCl. - Tetramethyl-P- paraphenylene diamine dihydrochloride, alpha- naphthanol, Dimethyl-amino benzaldehyde (DAMB) - Ethylene diamine tetra-acetic acid (EDTA), glacial acetic acid. - H ₂ O ₂ , 99% ethanol alcohol, 70% alcohol. - Sodium citrate, methyl red. - C-reactive protein (CRP) latex slide agglutination. - N-Acetyl-L-cysteine (NAC	BDH, England Merk-Darmstadt BDH, England AFMA-DISPO, Jordan Fluka chemika-Switzerland BDH, England SPIREACT, Spain Sandoz Co.

II- Culture media : - Blood agar base medium, Brain heart infusion agar, Brain heart infusion broth, , Cimon citrate medium, MacConky agar medium, Kligler`s iron agar (KI agar), Muller -Hinton agar, Glucose phosphate peptone water. - Nutrient agar media, Nutrient broth. - MR – VP broth.	 OXOID, England Fluka, France Diffco-Michigan, USA
III- Stains : - Gram stain : - Zheil-Nelson stain : - Leishman stain :	 BDH, England Crescent, Morocco BDH, England

IV-Antibiotic discs :			
Antibiotic	Symbol	Disc potency ((μ g)	Company (origin)
Amoxicillin	Amx	10	Oxoid, England
Doxycyclin	Dox	30	Bioanalyse, India
Erythromycin	Ery	15	Oxoid, England
Clarithromycin	CLR	15	Bioanalyse, India
Azithromycin	AZM	15	Bioanalyse, India
Cefotaxime	CTX	30	Oxoid, England
Cephalothin	KF	30	Oxoid, England
Trimethoprim-Sulphamethaxazole	TMX	1.25+23.75	Oxoid, England
Sparfloxacin	Spar	5	Span diagnostics, India
Lomefloxacin	Lom	10	Bioanalyse, India

3.1.3. Solutions, Reagents, and Culture media:

3.1.3.1. Solutions :

3.1.3.1.1. Phosphate buffer solutions :

This solution was prepared by dissolving of : 80 gm of NaCl, 0.34 gm of KH_2PO_4 , and 1.12 gm of K_2HPO_4 in 1000 ml of distilled water. The pH was 7.3. then sterilized in autoclave (Baron *et al.*, 1994).

3.1.3.1.2. Normal saline :

It was prepared by dissolving 8.5gm of sodium chloride in one liter of distilled water. The pH was 7.0 and sterilized in autoclave(Merk-Darmstadt).

3.1.3.1.3. Stains :

The stains that have been used in this study were : Gram stain, Zheil-Nelson attain, and Leishman stain. The procedures of these stains were done according to Cruickshank *et al.*, (1975).

3.1.3.2. Reagents :**3.1.3.2.1. Catalase reagent :**

It has been prepared by adding 3ml of hydrogen peroxide (H_2O_2) to 97ml of distilled water, when it's concentration became 3%. It had been used to detect catalase enzyme (Baron *et al.*, 1994).

3.1.3.2.2. Oxidase reagent :

It has been prepared by dissolving of 0.1gm of Tetramethyl-paraphenylene diamine dihydrochloride in 10ml distilled water and stored in a dark container, and used to detect oxidase enzyme (Baron *et al.*, 1994).

3.1.3.2.3. Methyl Red reagent :

This reagent is prepared by dissolving of 0.1gm of methyl red in 300ml of 99% alcohol and then the volume was completed to 500ml by distilled water, it was used to detect the complete fermentation of carbohydrates and the production of acid in the media (MacFaddin, 2000).

3.1.3.2.4. Vogas-Proskauer (VR) reagent :

A- 5gm of alpha-naphthol has been dissolved in 100ml of 99% alcohol.

B- 40gm of KOH has been dissolved in 100 ml of distilled water. This reagent had been used to detect the production of acetoin that is produced by incomplete fermentation of carbohydrates (Collee *et al.*, 1996).

3.1.3.2.5. Kovac`s Reagent :

This reagent has been prepared by dissolving 10gm of Dimethyl amino benzaldehyde (DAMB) in 150 ml of amylalcohol, 50ml of concentrated HCl were gradually added to this mixture. The solution had been stored in a dark bottle , and gently shaken before use. It had been used the production of indole production (MacFaddin, 2000).

3.1.3.3. Cultures Media :**3.1.3.3.1. Blood Agar medium :**

It has been prepared by suspending 40gm of blood agar base in one liter of distilled water, heating to boiling and sterilizing in autoclave at 121 C°, 15 pound/square inch for 15 minutes and supplemented with 5% of fresh human blood after cooling 45 C°. Final pH was adjusted to 7.1. It was used to show the colonial morphology and haemolysin production (Collee *et al.*, 1996).

3.1.3.3.2. Chocolate Agar medium :

It has been prepared by dissolving 40gm of blood agar base in one liter of distilled water and sterilized by autoclave, then 5% of fresh human blood was added to the medium after cooling to 80 C°. It was used for culturing and isolation of fastidious bacteria (MacFaddin, 2000).

3.1.3.3.3. Brain Heart Infusion Agar (BHIA) :

It has been prepared by dissolving 52gm of (BHIA) in one liter of distilled water, heating to boiling and sterilizing by autoclave, it was used for the cultivation of fastidious bacteria and used as a storage media after addition of 5% glycerol (Cruickshank *et al.*, 1975).

3.1.3.3.4. Brain Heart Infusion Broth (BHIB) :

It has been prepared by suspending 34.5gm of (BHIB) in one liter of distilled water, heating to boiling and sterilizing by autoclave and used as a transfer media for clinical specimens (Cruickshank *et al.*, 1975).

3.1.3.3.5. MacConky Agar medium :

It has been prepared by dissolving 50gm of the medium in 1000ml of distilled water, heating to boiling and sterilizing in autoclave, and used to detect the growth of gram-negative bacteria (MacFaddin, 2000).

3.1.3.3.6. Simmon`s Citrate medium :

It has been prepared by dissolving 12.5gm of this medium in one liter of distilled water, distributed in test tubes and autoclaved. It was used for testing

the ability of bacteria to utilize citrate as a sole source of carbon for energy and growth (MacFaddin, 2000).

3.1.3.3.7. Kligler Iron agar (KI agar) :

It has been used to determine the glucose and lactose fermentation and possible hydrogen sulphide H₂S production as a first step in the identification of gram-negative bacilli, and it was prepared according to MacFaddin, (2000).

3.1.3.3.8. MR-VP medium :

It has been prepared by dissolving of 5gm of peptone, and 5gm of K₂HPO₄ in 500ml of distilled water, the pH was adjusted to 7.6, then the final volume was completed into one liter by distilled water, and autoclaved. Fifty ml of glucose 10% (sterilized by filtration) was added to this medium after cooling to 50C°, and the medium had been distributed into sterilized test tubes. It was used to detect the partial or complete hydrolysis of sugars, production of acids, and production of Acetyl-methyl carbon (MacFaddin, 2000).

3.1.3.3.9. Peptone Water medium :

It has been prepared by dissolving 8gm peptone in 1liter of distilled water, and distributed into test tubes, and autoclaved. It was used to show the bacterial ability to decompose the amino acid tryptophane to indole (MacFaddin, 2000).

3.1.3.3.10. Nutrient Agar medium :

It has been prepared by suspending 28 gm of this medium in 1liter of distilled water, heating to boiling and sterilizing by autoclave. It was used for the cultivation of bacteria (MacFaddin, 2000).

3.1.3.3.11. Muller Hinton Agar medium :

It was prepared by dissolving of 35 gm of this medium in 1liter of distilled water, heating to boiling and sterilizing by autoclave. This medium had been used for testing the antimicrobial susceptibility of the bacterial isolates.

3.1.3.3.12. Motility Medium :

It was prepared by adding 4 gm of agar- agar to 100 ml of nutrient broth in 1000ml distilled water, then sterilized by autoclave and distributed into test tubes. It was used to detect the bacterial motility (MacFaddin, 2000).

3.2. Patients and Methods :**3.2.1. Patients :**

A total of seventy five patients from adult males and females whose age ranged from thirty years to ninety who have Chronic Obstructive Pulmonary Disease (COPD) with productive cough in a period of 3 successive months in each of two consecutive yrs in absence of identifiable causes of sputum production and also the disease was clinically diagnosed by chest physicians in Mirjan teaching hospital at Babylon province from November 2006 to May 2007.

All the necessary informations have been obtained from the COPD patients according to the following forma :

A. Name of the patient.

B. Age of the patient.

- C. Sex , residence , and occupation of the patient.
- D. clinical features like, cough, shortness of breath, sputum expectoration, the presence of leg edema, and history of weight loss.
- E. History of smoking, duration, and number of packs per day, in addition to the Ex-smokers patients who stopped smoking at least for one yr according to (Wise *et al.*, 2003).
- G. Physical examination: to assess the decrease chest movement with respiration, and acting of intercostals muscles, with the presence or absence of barrel chest.
- H. History of antibiotic intake, where patients with antibiotics intake less than one week prior to the sample obtaining would be excluded.
- I. AF stain was done for all COPD patients with exclusion of positive samples.

3.2.2. Controls:

Ten COPD patients giving sputum culture with negative results and neutrophilia (sputum and blood) were considered as a control group.

3.2.3. Samples :

3.2.3.1. Sputum sample :

It had been obtained by instruction of the COPD patient to give the early morning sputum after rinsing of the mouth thoroughly and before eating any meal, then the sputum put in a clean container and transferred to the laboratory immediately, and the obtained sputum was examined grossly for, amount, color and consistency (purulent, mucoid), then the sputum being divided into three

parts, the first part is being obtained by wooden applicator and cultured on the ordinary media (blood, chocolate, and macConky media) at 37C° for 24 hours, the other part was also obtained by wooden applicator and smeared on glass slide and left to dry and then fixed by passing over flame for many times and the smear has been stained by Zheil-Nelson stain to exclude Acid Fast bacilli (AFB), whereas the last part of the sputum is used for the sputum cytology to detect the sputum neutrophilia where the sputum was smeared over a glass slide by the use of wooden applicator, then the smear left to dry and fixed by passing it over the flame for many times, after that the slide is ready to be stained by Leishman stain by immersion the slide in the stain, and the stain is mixed well by the blowing with pasture pipette, then it was left to dry for nine minutes then rinsed with tap water, and the slide cleaned from below to remove the debris, then dry by air and examined under oil immersion of the microscope (Cruickshank *et al.*, 1975).

3.2.3.2. Blood sample :

The venous blood sample had been obtained from antecubital vein by means of disposable plastic syringe with wide bore needle, the skin had been cleaned with 70% alcohol and 3ml of the blood being withdrawn, 1ml was used for blood film by transferring this 1ml immediately into the potassium EDTA tube (1.2mg of the EDTA per 1ml of the blood), then one drop of the blood had been taken and smeared over a glass slide by a glass spreader to make film and

then left to dry for 10 minutes and stained with Leishman stain for 5 minutes then immersed with phosphate buffer solution for 10 minutes and washed with tap water and left to dry and then examined under the microscope to detect blood neutrophilia and differential white blood cells with the aid of automated differential counter. Also, 0.02ml of the blood had been taken from EDTA tube and then mixed with 0.4ml of glacial acetic acid for the determination of white blood cells count (Dacie and Lewis, 1995). The other 2ml of blood was delivered into disposable sterile plain tube and allowed to clot at 37C° for 30-45 minutes, then the serum was separated by centrifugation for 15 minutes at 3000 rounds per minute (RPM), and the serum obtained is put in a disposable plain tube to be ready for the qualitative determination of CRP according to the kit, where haemolysed serum was avoided (Dacie and Lewis, 1995).

3.3. Laboratory Diagnosis :

3.3.1. Bacterial Diagnosis :

According to the diagnostic procedures recommended by (MacFaddin, 2000 ; Collee *et al.*, 1996 ; Baron *et al.*, 1994, and Baron and Finegold, 1990) the isolation and identification of bacteria was performed as follows :

3.3.1.1. Colonial Morphology and Microscopic Examination :

From each primary positive culture after 24 hours incubation a single colony had been taken where its identification depended on the morphology properties (colony size, shape, color, and texture), then the colonies which were

requested or suspected to be pathogen had been selected and investigated by gram stain to observe a specific shape, color, and aggregation by examining under the microscope. After staining the bacteria by gram stain, specific biochemical tests had been performed to reach the final diagnosis.

3.3.1.2. Biochemical Tests :

3.3.1.2.1. Catalase Test :

This test has been used to detect the ability of bacteria to produce the enzyme catalase. It had been carried out by mixing a single isolated colony transferred by wood stick with a drop of hydrogen peroxide (3% H₂O₂) on the surface of dry and clean slide, the production of gas bubbles indicate the positive result (Collee *et al.*, 1996).

3.3.1.2.2. Oxidase Test :

This test was performed by transferring a small portion of bacterial colony by sterilized wooden stick into filter paper saturated with a recently prepared indicator (3.1.3.2.2), the appearance of purple color indicated a positive result (Collee *et al.*, 1996).

3.3.1.2.3. Methyl Red Test (M.R) :

This test was done on 1ml of MR-VP broth cultured by the organism and then incubated for 24 hours at 37 C°. After that (6-8) drops of methyl red reagent being added to the culture, the change of the color to orange-red indicated a positive result, whereas the change to yellow (pH over 6.0) revealed

a negative result (MacFaddin, 2000).

Chapter three

Material and Methods

3.3.1.2.4. Vogas-Proskauer Test (V.P) :

The tubes of (MR-VP broth) has been seeded with a specific bacterial culture and incubated at 37 C° for 48 hours. The result was read by adding (0.6 ml of α -naphthanol reagent) and (0.2ml of 40% KOH solution), the appearance of red color after 15 minutes indicate a positive due to partial analysis of glucose which produces acetone or (Acetyl methyl-carbinol) (MacFaddin, 2000).

3.3.1.2.5. Indole Test :

This test was performed by inoculating peptone water medium as in (3.1.3.3.9) with bacterial growth, and incubated at 37 C° for 48 hours. Then, 0.5ml of Kovac`s reagent had been added, the tubes were gently shaken, the formation of red-ring between the medium and reagent indicated the positive result (MacFaddin, 2000).

3.3.1.2.6. Simon Citrate Utilization Test :

This test was performed by inoculating citrate agar slant with bacterial growth and incubated at 37 C° for 24-48 hr. The color change from green to blue indicated the positive result, whereas unchanged color indicated a negative result (MacFaddin, 2000).

3.3.1.2.7. Kligler`s Iron Agar Test :

This test was performed on the colonies which were grown on the MacConky agar where it was done by touching them with a straight wire and

inoculated on Kligler iron medium by stabbing the bottom and streaking the slant. Fermentation can be detected by a change in the color of the indicator (phenol red) from red to yellow. The pH changes in the bottom and the slant of the medium and detected after 18-24hrs of incubation of gas formation which was visualized as bubbles in the medium caused by gas formed in the agar. Organisms that can produce H_2S form a black precipitate in the bottom (Baron *et al.*, 1994).

3.3.1.2.8. Motility Test :

It was performed by stabbing the tubes containing motility test medium once at the center with an inoculating needle and incubated at 35 C° for 24-48 hrs, the motile bacteria speed out from the line of inoculation, while, non-motile bacteria grow only along the stab line, and the surrounding medium remained clear (Baron *et al.*, 1994)).

3.3.2. Antimicrobial Susceptibility Test (Kirby-Bauer method) :

It has been performed by using a pure colony of previously identified bacterial organism. The inoculum to be used in this test was prepared by adding growth from 5 isolated colonies grown on blood agar plate to 5ml of nutrient broth, then this culture incubated for 2hrs to produce bacterial suspension with moderate turbidity, then a sterile swab was used to obtain an inoculum from the standardized culture and streaked on a Muller-Hinton agar plate, then the antibiotic discs placed on the surface of the medium at

evenly spaced intervals with sterile flamed forceps or disc applicator, then incubated for 18-24hrs at 37C° .

Antibiotic inhibition zones were measured, and the zones sizes were compared to standard zones to determine the sensitivity of the organism to each antibiotic (NCCLS, 1990 ; MacFaddin, 2000).

3.3.3. Qualitative Determination of C-reactive Protein (CRP) :

The presence of CRP in the sera of the tested individuals was evaluated by CRP-latex slide agglutination kit, which was a rapid test for the qualitative and semi-qualitative measurement of CRP in serum by agglutination of latex particles on the slide, where it was carried out according to the mentioned procedure in the kit (SPINREACT).

A-principle :

The CRP- latex is a slide agglutination test for the qualitative and semi-qualitative detection of C- reactive protein (CRP) in human serum. Latex particles coated with goat IgG anti-human CRP which are agglutinated when mixed with samples containing CRP.

B-Procedure of Qualitative Method : (SPINREACT, Spain) :

It was carried out according to procedure recommended by the company.

3.3.4. Determination of Total White Blood Cells Count and Differential Count with Detection of Blood Neutrophilia :**3.3.4.1. Detection of Total White Blood Cells Count :**

The routine measurement of the total white blood cells count among COPD patients was performed according to (Dacie and Lewis, 1995). It was used for the detection of absolute blood neutrophilia.

3.3.4.2. Blood Film for Differential White Blood Cells and Blood Neutrophilia:

Blood film was prepared by taking a drop from the amount of the blood (1ml) which was mixed with EDTA (3.2.2.2), and by the use of the automated differential counter, where, the absolute neutrophils number was calculated by multiplying the total white blood cells (WBC) by the percentage of the neutrophils detected and compared to the reference range in adult (normal level of relative neutrophilia 40%-75%, and absolute neutrophilia 2000-7000 cells/cubic centimeter) (Dacie and Lewis, 1995).

3.3.5. Detection of the Sputum Neutrophilia :

The sputum sample which was obtained from the COPD patients was processed according to (Cruickshank *et al.*, 1975) as in (3.2.2.1). This was used to detect the sputum neutrophilia where the neutrophils in the sputum is variable among healthy subjects, but the neutrophilia can be indicated by the presence of either more than 35% of the neutrophils to the total cell count in the sputum or more than 25 neutrophils per high power field per field (Robert, 2000 ; Baron *et al.*, 1994).

3.3.6. Effect of N-Acetylcysteine(NAC) on some bacterial isolates :

This chemical material was prepared by dissolving 0.1gm of this NAC in 10ml distilled water (1%) and put in a sterile plain tube. It has been used to detect it's effect invitro on the growth of some bacterial isolates that had been recovered from some sputa of COPD patients after reculturing these sputa in the presence of this material on both blood and chocolate media as suggested by our supervisors.

Procedure:

- A. After obtaining the sputum from the COPD patients, a mixture of the sputum and normal saline had been prepared.
- B. 0.5ml of this mixture was obtained and mixed with 0.5 ml of NAC (1%) and incubated for 2 hrs.
- C. The final mixture had been transferred in Brain heart infusion broth (BHIB) and incubated at 37 C° for 24 hrs.
- D. The results were scored according to the presence and absence of the bacterial growth.
- E. The positive result (growth) had been recultured on additional media (Blood and MacConky agars) to detect the type of growth and effect of NAC.

4.Results and Discussion**4.1. Distribution of COPD According to the Age:**

The distribution of COPD patients according to age groups was showed in table (1). The incidence of COPD increased with increasing age . Age group ≥ 70 years old was the dominant which included highest COPD patients with 33 out of 75 (44%) followed by the other age group of (60-69) years old which included 20 out of 75 COPD patients (26.6%), whereas the first and second age groups showed the lowest percentages with 7 out of 75 (9.33%) and 15 out of 75 (20%) respectively as shown in table (4-1).

Table (4-1) : Distribution of COPD Patients According to Age

Age group (year)	patients Number of
< 50	7 (9.33%)
50 – 59	15(20%)
60 – 69	20 (26.6%)
≥ 70	33 (44%)
Total	75 (100%)

The high percentage of COPD patients in age group ≥ 70 years as shown in this study was in accordance to the Kim *et al.*, (2005), who they stated that the prevalence of COPD was (7.2%) among subjects older than 45 years old.

Likewise, the prevalence increased with increasing age as reported by Lindberg *et al.*, (2005). Moreover, in 1995, 553000 patients were treated for COPD in the United States and two thirds of them were more than 65 years of age, where the prevalence of those over 65 years was fourfold that in 45 to 65 years old group (Hurd, 2000), and this increase with advancing age and may reach as high as 30-40% in individuals older than 70 years of age as reported by Vanden Boom *et al.* (1998).

The most physiological changes associated with age ; a decrease in the static elastic recoil of the lung, a decrease in the compliance of the chest wall, and a decrease in the strength of the respiratory muscles, moreover these physiological changes that occur in the process of aging and those due to effect of exposure to noxious environmental factors lead to high incidence and prevalence of acute and chronic respiratory diseases in the elderly as compared to young age group (Janssens *et al.*, 1999).

4.2. Distribution of COPD Patients in Relation to Sex :

This study showed the relation between COPD and it's distribution among sex where, the highest frequency of COPD expressed in male in comparison to

the female , with 51 out of 75 COPD patients were male (68%), and 24 of 75 were female (32%) with male to female ratio **2.57:1** as shown in the figure (4-1).

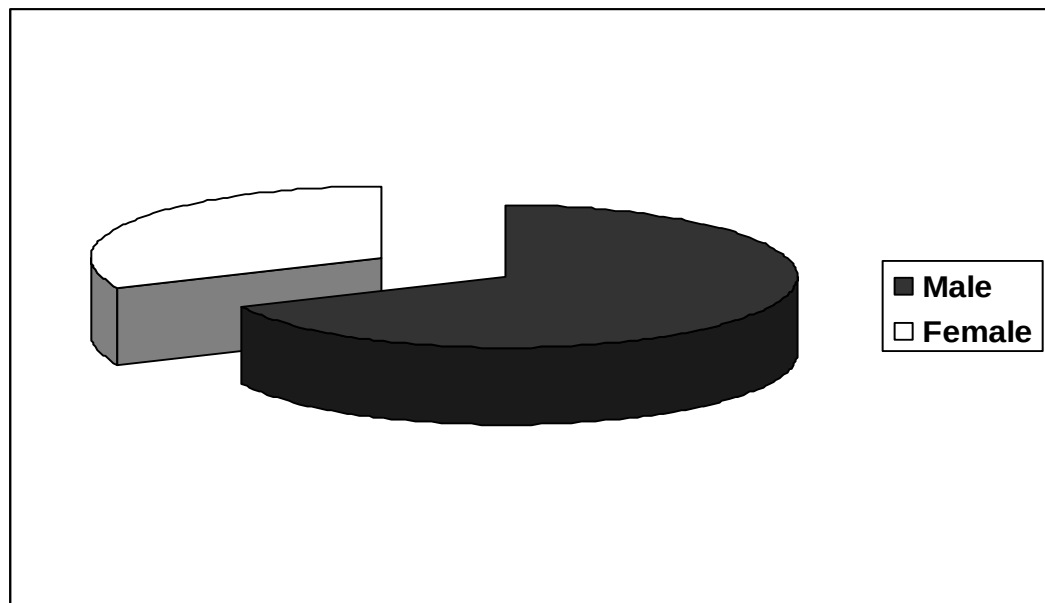


Figure (4-1) : Distribution of COPD Patients in Relation to the Sex

The result was in accordance to study by Murray and Lopez, (1997) who reported that the worldwide prevalence of COPD at 1990 was estimated to 59% in men and 41% in women and at 1987 the incidence of COPD was 61% in male and 39% in female, it may be due to higher incidence of smoking in male than female.

Actually, several recent studies have been suggested sex differences in the diagnosis and clinical manifestations of COPD which is more prevalent in males than in females as reported by Soriano *et al.*, (2000).

Moreover, in the developed countries the prevalence of COPD is equal in men and women, which probably reflects the changing pattern of tobacco smoking, really, some studies reported that women are more susceptible to the effects of tobacco smoke than men, and there is an increasing rate of smoking among women in both the developed and developing countries (Xu *et al.*, 1994).

4.3.Distribution of Smoking Status among COPD Patients:

This study showed the distribution of the types of smoking status which were included ; current smokers, ex-smokers with at least one year duration of smoking cessation and non-smokers.

Results in table (4-2) showed that the number of COPD patients who were current smokers were 32:75 (42.66%), whereas the number of ex-smokers among COPD patients were 31:75 (41.33%), and the rest of COPD patients in relation to smoking status who were non smokers were 12:75 (16%) which was the lowest group in regard to other groups of COPD patients as shown in table (4-2).

The present study showed the distribution of COPD patients among the three groups of smoking status (current smokers, ex-smokers, and non smokers), the group of smokers (current and ex-smokers) were 84% which was the highest in comparison to never smokers group and this result was in accordance to almost all studies which showed also high incidence of COPD, and 80-90% of COPD caused by cigarette smoking both current and ex-smoker groups (Shahab *et al.*, 2006).

Table (4-2) : Distribution of COPD Patients in Relation to Smoking Status

Smoking status	Number of patients	%
Current smokers	32	(42.7%)
Ex-smokers	31	(41.3%)
Non smokers	12	(16%)
Total	75	100%

Woodruff *et al.*, (2005) showed that the current cigarette smoking is significantly higher among people with COPD than among those without COPD and they reported that the smokers with COPD have a higher level of cigarette dependence. Indeed, smoking induced inflammation is thought to play important role in the pathogenesis of COPD and is characterized by influx of neutrophils in the airway lumen and increased macrophages and T-lymphocytes number in the airway wall (Hogg *et al.*, 2004), likewise, for middle age who are smokers (who continued smoking for 5years) had further loss of FEV1 of several milliliters, and cigarette smoking was considered as one of the most environmental factor related to COPD and acts through the generation of the oxidative stress and /or reduction

of antioxidant capacity (MacNee, 2005), furthermore, the macrophages may be activated by the cigarette smoking and other irritants to release of neutrophil-chemotactic factors that can release proteases that breakdown connective tissue in the lung and stimulate mucous secretion (Liu *et al.*, 1999).

The high percentage of ex-smokers among COPD patients can be explained by the fact that some patients with COPD were smokers claimed to be ex-smokers rather than non smokers (Shahab *et al.*, 2006), besides, the large airways inflammation in COPD persists even after smoking cessation as indicated by Lapperre *et al.*, (2006), moreover, it has been shown by Hogg *et al.*, (2004) that the apoptosis of the epithelial cells of the airway persists after smoking cessation in patients with COPD which may induce persistent inflammation and the process of COPD continues even after smoking cessation.

However, patients who quit smoking experience less respiratory symptoms and hyperresponsiveness than those who continue to smoke and the largest improvement in lung function and symptoms occur within the first year after the smoking cessation (Wise *et al.*, 2003).

In addition, this study showed the third group of the smoking status included the non smokers who constituted 16% of the total COPD patients and COPD may be due to another factors other than smoking like ; genetic risk factor as severe alpha-1 anti-trypsin deficiency, air pollution, occupational dust or chemical exposure and some severe childhood respiratory viral infections(Celli *et al.*, 2003).

4.4. Distribution of Some Clinical Features on COPD Patients :

This current study showed the distribution of some clinical features of COPD among patients especially, cough, sputum production, dyspnea and others like; weight loss and bilateral peripheral edema, as shown in table (4-3).

Result in table (4-3) showed that all COPD patients had a productive cough in a (75:75 COPD patients 100%), for sputum production which were purulent sputum occurred in 45 out of 75 COPD sputum samples (60%) which was the predominant ,and mucoid sputum at 30:75 sputum samples (40%).

All the patients had dyspnea at a rate of 100% (75:75) which was of two types exertional dyspnea and dyspnea at rest, exertional dyspnea occurred in 45:75(60%) which was the dominant, while dyspnea at rest it occurred in 30:75 (40%).

The other features studied in this study were weight loss which occurred in 13:75 of COPD patients (17.33%) and bilateral leg edema occurred 13:75 (17.33%) as shown in table (4-3).

The clinical part of this current study showed the frequency of some clinical features of COPD, and their percentages of distribution on the 75 COPD patients, chronic productive cough was the predominant feature of all COPD patients and it may be the first symptom to develop where, initially it may be an intermittent but may progress to daily occurrence , where the airway inflammation and excessive bronchial secretions are likely to activate the afferent limb of the cough reflex (Higgenbottan, 2002).

Table (4-3): Distribution of COPD Patients in Relation to Some Clinical Features

Clinical feature	Number	%
Productive cough:	75	100%
Sputum :	75	100%
a. Purulent	45	60%
b. Muroid	30	40%
Dyspnea:	75	100%
a. Exertional	45	60%
b. At rest	30	40%
Weight loss :	13	17.33%
Bilateral leg edema :	13	17.33%

Furthermore, when airflow obstruction is present, it often leads to an ineffective cough as a result of decreased expiratory flow and this coupled with impaired mucociliary clearance which results in further retention of the secretions and a vicious cycle of chronic recurrent coughing (Foster, 2002).

Besides, the other most important feature associated with COPD patients is the sputum production which was of two types ; mucoid and purulent where the latter was the predominant, and the presence of bacteria that causes an infection would be expected to be accompanied by the activation of secondary host defenses which include neutrophils recruitment to the airways and this neutrophil influx should be associated with a change the secretions from mucoid to the to purulent sputum because the myeloperoxidase from the neutrophils is green (Anthonisen *et al.*, 1987), on the same manner, the presence of purulent sputum would be consistent with the reported increase in neutrophils seen microscopically by presence of other markers in assumed bacterial exacerbations (Fagon *et al.*, 1990).

The important symptom being observed in this study was dyspnea which was of two types ; exertional dyspnea and dyspnea at rest where the former was the dominant and it was in accordance with many studies showed that the presence of both types of the dyspnea among COPD patients , indeed, shortness of breath is a common symptom with the perception of the respiratory discomfort and expiratory flow limitation arises because of the combined effect of the reduced

elastic lung recoil and an increased airway resistance and it is the primary symptom limiting the exercise in patients of advanced COPD and leads oftenly to avoidance of activity with consequent skeletal muscle deconditioning (Denis, 2006).

On the same manner, dyspnea is the cardinal symptom of COPD and it is the primary symptom in which COPD patients seek medical attention, dyspnea may be described by the patients as air hunger or gasping for breath and it worsens with activity and respiratory infections (O'Donnell *et al.*, 2004),

Besides, the other symptom being shown in this study was weight loss which was shown in few COPD patients at a rate of 17.33% where this study was agreed with Barnes, (2000) who reported that one in four people with COPD weighs too little for good health, especially those with emphysema who are at higher risk of death than are people with COPD who have a normal weight.

Indeed, Barnes, (2000) reported that the weight loss in COPD occurred as a result of combinations of factors like ; using more energy (due to increased energy required to breath), eating less because of the shortness of breath, the need for corticosteroids that increased the breakdown of muscle tissue in the body, COPD patients are less interest in food because people who have COPD may be depressed(causing lack of interest in eating), weight loss is a concern in COPD as they lose weight they lose muscle mass where the loss of the muscle may result in feeling weaker and leads to more difficulty in breathing and weakness that can lead to a greater chance of infection.

In addition, peripheral leg edema has been shown in this study among COPD patients but at a rate of 17.33% which was agreed with some studies related to the presence of bilateral leg edema among COPD patients, indeed, the peripheral leg edema occurs in a substantial number of COPD patients, especially in those who had hypercapnia and hypoxemia, (MacNee, 1994), and also it may be due to right sided heart failure secondary to respiratory diseases, where the pulmonary pressure raises with the contraction of the right ventricle of the heart is less efficient and becomes unable to pump the blood to meet the requirements of the liver and the kidney and lead to the edema of the ankles, and the legs (Sat Sharma, 2006).

4.5. Correlation of COPD with C-reactive Protein (CRP) :

This current study showed the reaction of C-reactive protein (CRP) on (52) patients with COPD , and the reaction depended on the qualitative reaction of the CRP with the serum of these patients. It was shown by this study that all 52 patients with COPD showed positive reaction at a percentage of (100%) which was in accordance to other previous studies that showed an elevation in CRP level among COPD patients.

CRP is a serum constituent originally defined by its ability to precipitate the pneumococcus C polysaccharides , and acts as an opsonin, it is an acute phase protein present in normal serum and increased significantly after most forms of tissue injuries, bacterial and viral infections, where the high serum CRP level is associated with lung inflammation in stable COPD (Dahi *et al.*, 2007) .

Moreover, Torres *et al.*, (2006) found that CRP levels are elevated in clinically stable COPD patients and that CRP levels correlate best with arterial oxygen tension,

Besides, Mannino *et al.*, (2003) pointed that CRP increases and becomes positive in all cases of COPD and the serum of CRP increases in COPD cases and decreased after treatment with steroids, and is related to the presence of the comorbidities, and thus might contribute to a more accurate assessment of the systemic effect and the comorbidities in this disorder.

4.6. Distribution of Bacterial Growths and Others among COPD Patients :

Bacterial growths which showed in this study in term of positive growths on culture and negative growths, positive growth group was the predominant that found in of 65:75 at a percentage of 86.66%, whereas the rest of the sputum cultures were negative in 10:75 (13.34%) as shown in figure (4-3). The positive bacterial growth was further divided into single growth (single isolate) and mixed growth (multiple isolates).

The results of positive culture in other studies differ from one to another for example, the study reported by Wilson, (2001) reported that about half of the exacerbations yield positive sputum culture and the isolation rate may be increased by the selection of the purulent samples.

The remainder of the sputa which was obtained in this study from the patients with COPD were negative and comprised 13.34%, and these negative cultures may be ascribed to the prior intake of antibiotics soon before obtaining the sample and the patient did not told us about them, also exacerbation of COPD patients may be due to non-bacterial causes like ; viral infections, atypical bacteria such as *Chlamydia pneumoniae* , *Mycoplasma pneumoniae* environmental conditions, excessive smoking, and air pollution exposure (Murphy *et al.*, 2004).

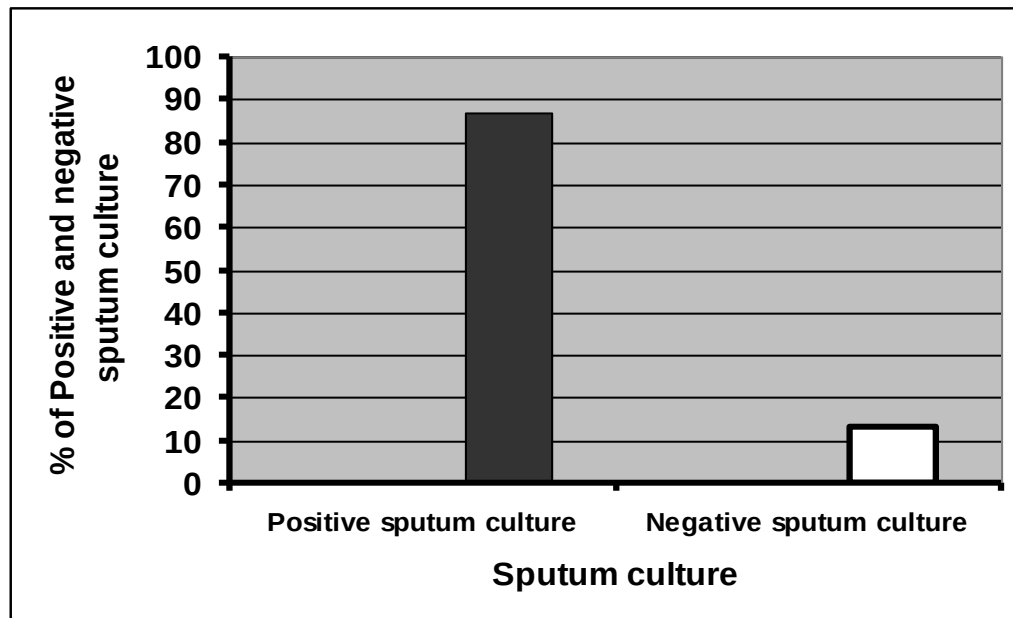


Figure (4-2) : Distribution of Positive and Negative Sputum Cultures among COPD Patients.

4.6.1. Distribution of Single Isolates types among COPD Patients :

By this study it was shown that single growth or single isolates occupied the predominant percentage where the number of these isolates was 58:65 (89.23%) and the predominant single isolates were *Moraxella catarrhalis* which were occupied 23 :58 of total single isolates (39.65%), followed by *Haemophilus influenzae* that were occupied the second rank among single isolates 9:58(15.51%)

and the third single bacterial isolate which was revealed by this study was the *Streptococcus pneumoniae* 8:58 (13.79%) and those above bacterial isolates were the major pathogens recovered in COPD patients that were comprised 40:58 (68.96%) as shown in table (4-4), the other group of single isolates expressed as follows ; *Klebsiella pneumoniae* 3:58 (5.17%), *Enterobacter Sp.* 4 : 58 (6.89%), *Pseudomonas aeruginosa* 2:58 (3.44%), *Acinetobacter Sp.* 2:58 (3.44%), *Staphylococcus aureus* 1 : 58 (1.72%), and the other group of single isolates were included fungal isolates like ; *Candida albicans* 5:58 (8.62%), and *Geotrichum* 1:58 (1.72%) as shown in table (4-4).

This study also showed the isolation of single isolates in the sputum of patients at a percentage of 89.23%, and the predominant type of single isolate which was revealed by this study was *Moraxella catarrhalis* that constituted 39.65%, this study was differed from other previous studies in which the *Moraxella catarrhalis* was either in the second or in the third place, the result of this current study was in accordance to the study done in Saudi Arabia reported by Alamoudi, (2007) who showed that the most common pathogen isolated from sputum culture from patients with COPD was *Moraxella catarrhalis* and this may be attributed to the geographical variations, indeed, the rate of *Moraxella catarrhalis* isolation are much higher in winter than in spring and summer where, this study was done also in winter time and this seasonal variation in the isolation is less pronounced with *Streptococcus pneumoniae* and *Haemophilus Influenzae*.

infection and the characteristics of those patients did not differ from other COPD as a whole (Murphy *et al.*, (2005). Moreover, Sporter *et al.*, (1995) observed that the *Moraxella catarrhalis* is the single most isolated pathogen in COPD, also, this study revealed the isolation of the other single isolates included *Haemophilus influenza* and *Streptococcus pneumoniae* at a variable rates 15.51% for *Haemophilus* and 13.79% for *S.pneumoniae* which was in accordance to many previous studies, and these three above isolates called potentially pathogenic microorganisms (PPMO) which were recovered from the sputum at a rate of 68.9% which was higher than the percentages of the previous studies, really, the above three species are also the predominant bacterial pathogens implicated in other infections like ; respiratory mucosal infections, otitis media, and sinusitis (Sethi and Murphy, 2001).

The actual study classified the isolated organisms from the sputa of COPD patients according to (Banerjee *et al.*, 2004 and Soler *et al.*, 1999) into potentially pathogenic microorganisms (PPMO) which included *M.catarrhalis*, *H.influenzae*, *S.pneumoniae* (predominant PPMO), *K.pneumoniae*, *Enterobacter spp.*, *S.aureus*, *P.auroginosa*, and *Acinetobacter spp.*, (other PPMO) and non-potentially pathogenic microorganisms (non-PPMO) like ; Viridians streptococci, Diphtheroid, *Corynebacterium spp.*, *Candida albicans*, and *Geotricum*.

The recovery of other single isolates from the sputum of the COPD patients such as ; *Enterobacter spp.*, isolates which were recovered at a rate higher than that

of other potentially pathogenic bacteria and the result of study was in accordance with the study reported by Soler *et al.*, (1999) who said that these pathogens are considered as one of the potentially pathogenic microorganisms (PPMO).

On the other hand, the study also revealed other group of single isolated which were also considered as a PPMO like ; *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter spp.*, and *Staphylococcus aureus*, where they recovered from the sputum of COPD patients together with the above the predominant PPMO at a rate higher than that of Banerjee *et al.*, (2004) who observed that the rate of recovery of these isolates 40% which was lower than this study.

Furthermore, this study revealed the isolation of other isolates in the sputa of COPD patients included ; Viridans streptococci, *Corynebacterium spp.* , Diphtheroid, and yeasts like ; *Candida spp.* , and *Geotrichum.*, and this result was in accordance with the study of Soler *et al.*, (1999) who pointed that these isolates are non - potentially pathogenic microorganisms (non-PPMO) and they were recovered from the sputum at a rate higher than the rate of recovery of these isolates in this study and the isolation of the yeasts were either as a part of non-PPMO or may be due to the contamination during the processing and culturing of the sputum.

Moreover, the precise role of bacterial infections in COPD has been a source of controversy for several decades and several putative roles of bacterial infections in the etiology, pathogenesis, and the clinical course of COPD which can

be identified like; childhood lower respiratory tract infections impairs lung growth reflected in lower FEV1 in the adulthood, chronic colonization of the lower respiratory tract by these pathogens that induce a chronic inflammatory response with lung damage (vicious circle hypothesis), chronic infection of the respiratory tissue by bacterial pathogen contribute to the pathogenesis of COPD by altering the host response to cigarette smoke a chronic or by inducing a chronic inflammatory response , bacteria can cause acute exacerbations of chronic bronchitis, which contribute significantly to the morbidity and mortality, and the bacterial antigens in the lower airways induce hypersensitivity that enhance airway hyperreactivity (Murphy and Sethi, 1992).

However, bacterial infection is one of several important causes of exacerbation of COPD where the incidence of bacterial infection is not uniform ,and the mucus hypersecretion may be an important risk factor, lower airway bacterial colonization may be a stimulus for chronic inflammation in COPD (Wilson, 2001).

Table (4-4) :Distribution of Single Isolates Types among COPD Patients

Type of single isolate	Number	%
Bacteria :		
<i>Moraxella Catarrhalis</i>	23	39.65%
<i>Haemophilus Influenza</i>	9	15.51%
<i>Streptococcus Pneumoniae</i>	8	13.79%
<i>Enterobacter Sp.</i>	4	6.89%
<i>Klebsiella Pneumoniae</i>	3	5.17%
<i>Pseudomonas aurogenosa</i>	2	3.44%
<i>Acinetobacter Sp.</i>	2	3.44%
<i>Staphylococcus aureus</i>	1	1.72%
Yeasts :		
<i>Candida albicans</i>	5	8.62%
<i>Geotrichum</i>	1	1.72%
Total	58	100%

4.6.2. Distribution of Mixed Growth (multiple isolates)Types among COPD Patients:

Mixed growths with their multiple isolates occurred in 7:65 (10.77%), the predominant type was *M.catarrhalis* and Viridans streptococci 2 : 7 (28.57%), the others included ; *M.catarrhalis* and Diphtheroid , *M.catarrhalis* and *Corynebacterium Sp.*, *H.influenzae* and *C.albicans*, *S.pneumoniae* and

Corynebacterium Sp., and Viridians streptococci and *C.albicans*, where all these isolated recovered at a rate of 1:7 (14.28%) for each type as shown in table (4-5).

This study revealed the presence of mixed isolates in a single sputum culture at a rate greatly less than that of single isolate 7:65 (10.77%), and the majority of these isolates were for *M.catarrhalis* with other non -PPMO, on the same manner, the other PPMO like ; *H.influenzae* and *S.pneumoniae* also were recovered from the sputum with other non-PPMO, this study was in accordance to that reported by Banerjee *et al.*, (2004) who mentioned that 26.38% of sputa of COPD patients revealed more than one isolates where this result higher than the rate of recovering of mixed isolates in this current study.

Furthermore, *M.catarrhalis* colonizes the respiratory tract of 5-32% of adult who had COPD at any time which may lead to the increase the frequency of recovering of this pathogen, and the acquisition of new strains of *H.influenzae* and *M.catarrhalis* with the occurrence of exacerbations (Sethi *et al.*, 2002) , also, it has been shown by Murphy *et al.*, (2005) that adult with COPD has acquisition of new strain of *M.catarrhalis* with absence of acquisition of new strain of other pathogen and presence of a symptomatic colonization has more often associated with the development of a mucosal immune response compared with exacerbation.

Table (4-5) : Distribution of Mixed Isolates Types among COPD Patients

Mixed isolates types	Frequency	%
<i>Moraxella catarrhalis</i> and Viridans Streptococci	2	28.57 %
<i>Moraxella catarrhalis</i> and Diphtheroid	1	14.28 %
<i>Moraxella catarrhalis</i> and <i>Corynebacterium</i> spp.	1	14.28 %
<i>Haemophilus influenza</i> and <i>Candida albicans</i>	1	14.28 5
<i>Streptococcus pneumoniae</i> and Diphtheroid	1	14.28 %
Viridans streptococci and <i>Candida albicans</i>	1	14.28 %
Total	7	100 %

4.7. Distribution of Potentially Pathogenic Microorganisms among COPD Patients in Relation to Smoking Status :

This current study showed the distribution of predominant PPMO and other PPMO among COPD patients in relation to the smoking status, the most types of predominant PPMO were among ex-smokers at a rate of 26:31 (83.87%), followed the current smokers of 20:32 (62.5%), whereas, the never smokers group showed

the least in having these pathogens at a rate of 6:12 (50%) as shown in table (4-6).

M.catarrhalis isolates were shown at a comparable rates between three groups of COPD patients in relation to smoking status and this study was in agreement with Nicolas *et al.*, (1991) who said that this bacteria can be isolated in the same frequency in both current and ex-smokers COPD especially, at old age group, on the same manner, Barnes, (2004) said that *H.influenzae* isolates were the most frequently isolated species in ex-smokers compared with both current and never smokers COPD patients which was in accordance with this study in which this pathogen being isolated with a highest frequency in ex-smokers, whereas, *S.pneumoniae* was isolated at a rate slightly higher in current smokers that the other two groups .

The rest of PPMO which occurred in both current and ex-smokers without never smokers, were, that *K.pneumoniae* was isolated in current smokers higher rate than ex-smokers, while, *P.aeruginosa*, *Enterobacter spp.*, and *Acinetobacter spp.*, were isolated at the same rate in both current and ex-smokers, whereas, the single isolate of *S.aureus* was isolated only in ex-smokers as shown in table (4-6), this result was in comparable to Miravittles *et al.*, (1999) who observed that smoking is a risk factor in patient with COPD for colonization with *P.aerugenosa* and other predominant PPMO and other gram negative bacilli, in addition, the zero rate of isolation of above microorganisms in never smokers may be due to the

absence of the smoking effect on the respiratory epithelium in predisposing to more colonization and increasing the rate of infection, especially in these other PPMO which are less frequently isolated in COPD.

Table (4-6) : Distribution of Potentially Pathogenic Microorganisms among COPD Patients in Relation to the Smoking Status

PPMO types	Total	Current smokers (32)	Ex-smokers (31)	Never smokers (12)
<i>M.catarrhalis</i>	23	9	10	4
<i>H.influenzae</i>	9	1	7	1
<i>S.pneumoniae</i>	8	4	3	1
<i>P.aeruginosa</i>	2	1	1	0
<i>Enterobacter spp.</i>	4	2	2	0
<i>K.pneumoniae</i>	3	2	1	0
<i>Acinetobacter spp.</i>	2	1	1	0
<i>S.aureus</i>	1	0	1	0
Total	52	20:32 (62.5%)	26:31 (83.87%)	6:12 (50%)

Smoking is the major stimulus for the inflammatory process in COPD and the persistent inflammatory process seen in the central and small airways of ex-smokers which is indistinguishable from inflammation seen in current smokers, and inflammatory mucous exudates have been demonstrated in ex-smokers with COPD where these findings related to the microbial colonization with alteration of the host inflammatory response to tobacco smoking and/or serve as an additional inflammatory stimulus enabling microbial pathogen to persist in the lower airways in COPD (Barnes, 2004 ; Murphy *et al.*, 2004).

Moreover, the isolation of PPMO which shown by this study were high among ex-smokers may be attributed to the fact that ex-smokers with COPD had higher CD3T, CD4T and plasma cell number than current smokers where showed no difference in other inflammatory cells, which may be due to the reversal of the immunosuppression which returned to a similar level in long term quitters of as in current smokers where this reversal seems transient for lymphocytes but persist for CD3T, CD4T, and plasma cells, and smoking cessation may result in reversal of the smoking induced harmful effects on airway epithelial cells such as a reduction in metaplastic secretory cell numbers in small airways leading to improved lung defense mechanisms where the effector mechanisms of immunity to environmental antigens was stimulated more efficiently in the absence of smoking leading to high number of immunocompetent cells (Wise *et al.*, 2003 ; Wright and Churg, 2002).

Airway inflammation in COPD patients and its effects on the mucociliary clearance and other host defense mechanism can potentially increase the risk of the recurrent infectious exacerbations (Murphy and Sethi, 1992).

Moreover, Sethi *et al.* (2006) showed that the isolation of *M.catarrhalis* was the most pathogen encountered in ex-smokers with stable COPD, and bacterial colonization which was associated with the presence of neutrophilic airway lumen inflammation in ex-smokers is similar to the inflammatory process seen in current smokers where ongoing inflammation is present in the bronchoalveolar lavage (BAL) fluid in bronchial biopsies among ex-smokers with COPD.

Likewise, Anthonisen *et al.*, (2002) showed that the persistent symptoms and recurrent exacerbations that are seen in ex-smokers with moderate to severe COPD suggesting that persistent inflammation has clinical consequences of the bacterial pathogens that colonize the lower airways in COPD.

4.8. Distribution of Sputum Neutrophilia and Blood Neutrophilia among COPD Patients :

Results in figure (4-3) showed the distribution of sputum neutrophilia and blood neutrophilia, where the study showed that sputum neutrophilia occurred 51:75 (68%), whereas the blood neutrophilia occurred in 47: 75 (62.66%) as shown in figure (4-3).

The current study showed one of the important issues in the course of the COPD by showing sputum neutrophilia in a high percent (68 %) as well as blood neutrophilia that occurred in a significant percentage (62.66%) and it was in accordance to study reported by Robert, (2000) who pointed that sputum neutrophilia (with neutrophil count more than 25per high power field per field and neutrophils normal value should be less than 35% of the total cells in the sputum sample where normal cell count is quite variable among subjects), were seen at a percentage of 76.4% which were higher than the incidence of sputum neutrophilia in this study.

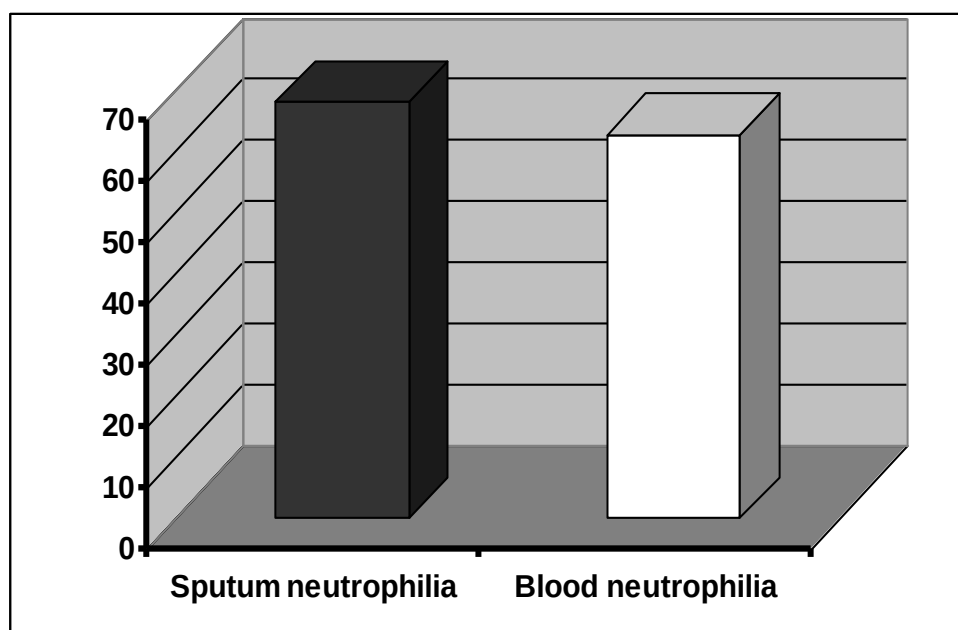


**Figure (4-3) : Distribution of Sputum Neutrophilia and Blood Neutrophilia
among COPD Patients**

Indeed, it has been shown that neutrophils are usually present in the secretions of patients with clinically stable COPD as well as during exacerbations which were associated with impaired lung function, and sputum neutrophilia increased in all exacerbations that were related mainly to disease severity and a significant relationship between neutrophils infiltration and exacerbation severity was demonstrated by significant correlation between severity of reduction in the lung function during exacerbation and increase in sputum neutrophils(Papi *et al.*, 2006).

Furthermore, Stockley *et al.*, (2001) observed that the appearance of purulent sputum in COPD patients usually indicates a more intense sputum neutrophilia.

Besides, the neutrophils are highly labile cells indicated by the variability seen among healthy persons and by stimuli that cause it to increase, where their number increased at hours after sputum induction by cigarette smoke, air pollution, endotoxin, some occupational exposure, and viral or bacterial infections, and the neutrophilia is non specific parameter for COPD, the degree of neutrophilia due to cigarette smoke with COPD related loosely to the degree of chronic airflow limitation and these activated neutrophils in the bronchial tree is a frequent finding in patients in both stable state and exacerbation and this may be attributed to an increased neutrophil migration and or a prolonged survival due to inhibition of the spontaneous apoptosis (Pauwels *et al.*, 2001).

On the other hand, this study showed a significant proportion of the peripheral blood neutrophilia among COPD patients and the result in this study was in

accordance to Papi *et al.*, (2006) who pointed that there is a significant increase in the peripheral blood neutrophils during exacerbation and it is correlated to the severity of exacerbation in relation to lung function reduction. Likewise, it has been indicated by Pletz *et al.*, (2004) that the peripheral blood neutrophils in acute exacerbations of COPD should also present a specific apoptotic pattern and this presumption supported by the fact that COPD is an inflammatory pulmonary disease with systemic impact and cytokines and other signaling factors that suppress neutrophils apoptosis such as soluble IL-6 where they have a detectable levels in the serum and plasma of COPD patients (Yasuda *et al.*, 1998).

Furthermore, an exacerbation that was associated with infection had a greater blood neutrophils responses compared with non infectious exacerbation, where the blood neutrophilia which showed during exacerbation were significantly related to the exacerbation severity (Papi *et al.*, 2006).

4.8.1. Distribution of Sputum Neutrophilia among Predominantly Potentially Pathogenic Microorganisms :

This study showed the distribution of sputum neutrophilia among COPD patients in relation to the predominant PPMO, where results in table (4-7) showed high percentage of sputum neutrophilia was appeared among COPD patients with *H.influenzae* at a level 8:9 (88.8%) followed by *M.catarrhalis* with sputum neutrophilia of 18:23 (78.26%) whereas, the lowest percentage of sputum neutrophilia was seen in *S.pneumoniae* at 5:8 (62.5%) as shown in table (4-7).

This study showed the distribution of sputum neutrophilia among the three predominant PPMO in patients with COPD where the dominant percentage of sputum neutrophilia was in association with *H.influenzae* and this study was in

Table (4-7) : Distribution of Sputum Neutrophilia among Predominantly Potentially Pathogenic Microorganisms

Type of single PPMO isolate	No.	Sputum neutrophilia (51)	%
<i>H.influenzae</i>	9	8	88.8%
<i>M.catarrhalis</i>	23	18	78.26%
<i>S.pneumoniae</i>	8	5	62.5%
Total	40	31	77.5%

accordance with the study of Stockley *et al.*, (2001) who stated that the sputum neutrophilia was dominant among *H.influenzae* which was higher than other other pathogens, also this study was in accordance with the study mentioned by Papi *et al.*, (2006) who showed that COPD exacerbations due to bacterial causes associated with increase sputum neutrophilia and high rate of sputum neutrophilia demonstrated among *H.influenzae* followed by *S.pneumoniae* and *M.catarrhalis* during both exacerbation and stable state .

Indeed, bacterial products in the airways may be a potent stimulus for the neutrophil migration into the airways, and elastase released from the neutrophils which can act synergistically with bacterial products and cause further inhibition of tracheobronchial ciliary function (Amitani *et al.*, 1991), on the same manner, Khair *et al.*, (1996) showed that LOS from nontypeable *H.influenzae* has intracellular adhesion molecule-1 (ICAM-1 which is CD54) expression and release of IL-6 and IL-8, and TNF-alpha and their concentrations were significantly increased by higher concentration of LOS and the levels of inflammatory mediators attained in the culture media is adequate to increase neutrophil chemotaxis and adherence in vitro.

Besides, sputum neutrophilia was observed in a higher number of patients with COPD having infection with *M.catarrhalis* at a percent of 78.26% and this may be due to the presence of LOS in its outer membrane and it lacks the long O-poly saccharides side chains present in other enteric lipopolysaccharides and thus it is

similar to LOS of *H.influenzae* in inducing airway inflammation, and the lipid A portion of the LOS molecule of *M.catarrhalis* is similar in structure to the lipid A of other gram negative bacteria and is responsible for the profound biological effects of LOS which may induce a stimulatory effect for proinflammatory mediators like in *H.influenzae* (Holme *et al.*, 1999).

In addition, *S.pneumoniae* was also showed sputum neutrophilia but less than that of *H.influenzae* and *M.catarrhalis*, where cell wall and cell wall polysaccharides of this bacteria induce inflammation and their cell wall consists of peptidoglycan which consists of glycopeptides and it has a potent biological effects and play a major role in the inflammation observed in pneumococcal infection, the lower airway of adults with COPD are colonized by pneumococci which are continuously dividing and shedding cell wall fragments into the airways where these bacterial antigens may contribute to the airways inflammation observed in COPD (Carlsen *et al.*, 1992).

4.9. Antimicrobial Susceptibility Test for Potentially Pathogenic Microorganisms :

Antibiogram profile was performed to show the effect of some antibiotics on some bacterial isolates from sputa of COPD patients by using Kirby-Bauer method (Disc diffusion method), the antibiotics which were used in this study included ; Amoxicillin, Doxycycline, Erythromycin, Clarithromycin, Azithromycin, Cephalothin, Cefotaxime, Trimethoprim-Sulphamethoxazole, Sparfloxacin, and Lomefloxacin.

4.9.1. Susceptibility to Amoxicillin :

Result in figure (4-4-A) showed the susceptibility of some bacterial isolates to amoxicillin in vitro where the study showed that (33.3%) of *M.catarrhalis* were sensitive to amoxicillin, *H.influenzae* was sensitive to amoxicillin at a percent of (60%), *S.pneumoniae* isolates were sensitive at a percent of (55.5%), (50%) of the *Enterobacter spp.* were susceptible to this antibiotic, *K.pneumoniae* sensitive at a (33.3%), whereas, *P.aeruginosa*, *Acinetobacter spp*, *S.aureus* isolates had no response (fully non susceptible) to this antibiotic as shown in figure (4-4-A).

This result was in agreement with some previous studies which showed the variability in response of such isolates towards amoxicillin, where this result was in accordance with the study of Jawad, (2007) who stated that *M.catarrhalis* isolates were susceptible to amoxicillin at a rate of (22%) which was lower than the result in this study with full susceptibility of *S.pneumoniae* to this antibiotic, with (30%) of *K.pneumoniae* sensitivity to amoxicillin, with full resistance of *S.aureus*.

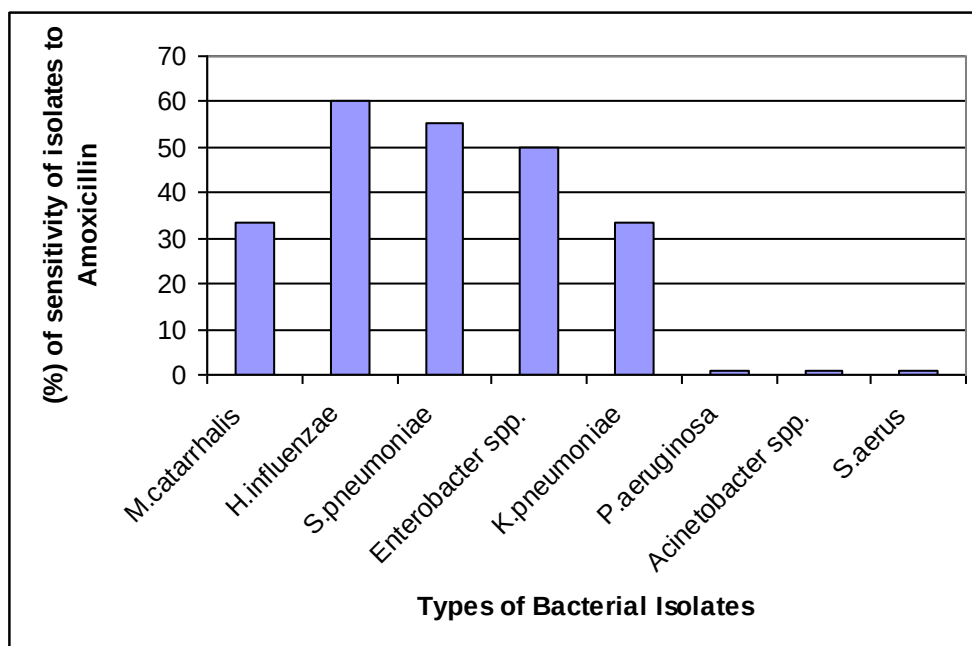


Figure (4-4-A) : The sensitivity of bacterial isolates to Amoxicillin

On the other hand, Al-Hamawandi, (2005) showed that *K.pneumoniae* isolates were fully sensitive to amoxicillin with full non susceptibility of both *P.aeruginosa* and *Acinetobacter spp.*, whereas, *S.aureus* isolates were entirely resistant to amoxicillin which were agreed with this study.

Likewise, Al-Rubaey, (2005) stated that *H.influenzae* isolates were sensitive to penicillins at a rate of (61%), where as, Thornsberry *et al.*, (1999) pointed that the susceptibility rate of *H.influenzae* to amoxicillin were less than (70%) which was also agreed with this study, in addition, Frazer, (2007) showed that amoxicillin can be used as one of the treatment options of *Enterobacter spp.*

It has been observed by Anthonisen *et al.*, (1987) that the low susceptibility of *M.catarrhalis* to amoxicillin may be ascribed that 92% of this pathogen was

B-lactamase producers which were active against B-lactam antibiotics such as ; penicillins and amino-penicillins with a considerable geographical variation in the resistance pattern where resistance increases towards commonly prescribed drugs.

Besides, Karlowsky *et al.*, (2003) showed that the resistance of *H.influenzae* to this antibiotic may be due to the production of B-lactamase in (25.6%) of the isolates and this non susceptibility decreased above 10 yrs of age by 10%, also, the non susceptibility of B-lactamase negative *H.influenzae* to amoxicillin may be due to the alteration of penicillin binding protein(PBP) (Devarajan, 2007), whereas, the non susceptibility of *S.pneumoniae* to amoxicillin may be due to the alteration of PBP that decreased it's binding affinity to B-lactam antibiotics(Doern, 1995).

Brooks *et al.*, (2004) had shown that the non susceptibility of *K.pneumoniae* and *S.aureus* to amoxicillin may be due to the production of B-lactamase and penicillinase which hydrolyze and inactivate the drug, also, James, (1999) who stated that the full non susceptibility of *P.aeruginosa* to this antibiotic may be due to the B-lactamase production. The non susceptibility of *Acinetobacter spp.* to B-lactam antibiotics amoxicillin may be due to the B-lactamase production where extended spectrum B-lactamase found to be disseminated within this bacteria (Shah *et al.*, 2001), in addition, Mallea *et al.*, (1998) said that the resistance of *Enterobacter spp.* to this drug was also due to B-lactamase production, where amoxicillins can act by having a bactericidal activity with inhibition of cell wall synthesis.

4.9.2. Effect of Doxycyclin on Some Bacterial Isolates :

Results in figure(4-4-B) showed that (59.2%) of *M.catarrhalis* were sensitive to doxycyclin, (70%) of *H.influenzae* were sensitive, *S.pneumoniae* were sensitive at (77.7%) with full susceptibility (100%) of *K.pneumoniae*, *Acinetobacter spp.* showed sensitivity at a percent of (50%), whereas, *Enterobacter spp.*, *P.aeruginosa* and *S.aureus* showed no susceptibility (fully resistant) to this antibiotic.

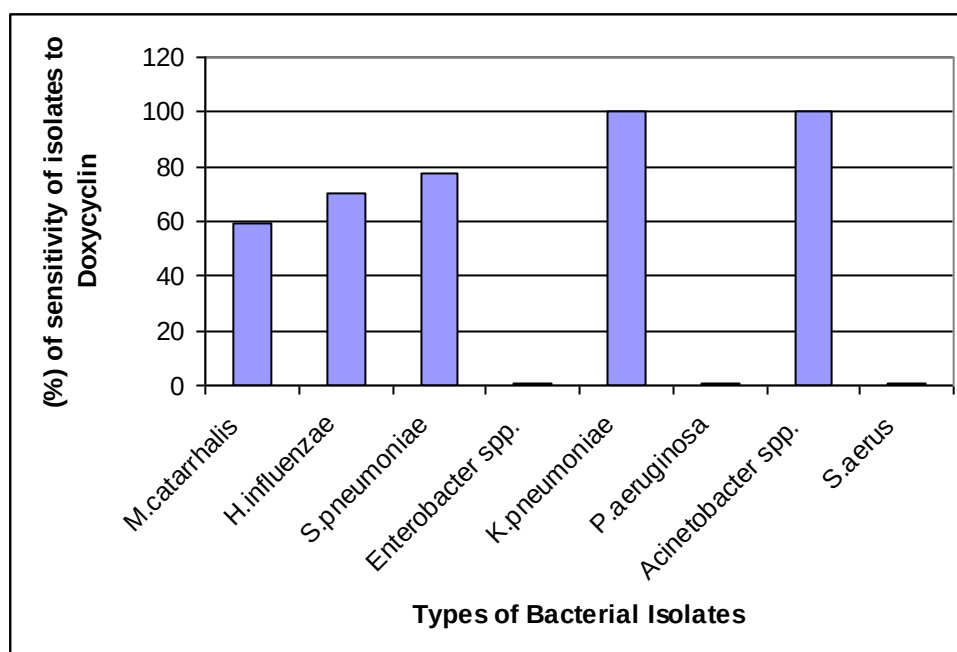


Figure (4-4-B) : The rate of Susceptibility of bacterial isolates to Doxycyclin

The result in this study was comparable with Skerret, (2006) who pointed that *M.catarrhalis* were highly susceptible to doxycyclin, likewise, this result was agreed with Gold and Moellering, (1996) who observed that *H.influenzae* showed high degree of susceptibility to doxycyclin where the drug acts by inhibiting the

protein synthesis by blocking amino-acyl-tRNA and preventing the introduction of new amino acids to adjacent peptides. On the same manner, Jawad,(2007)indicated that (71%)of *S.pneumoniae* were susceptible to this antibiotic with very low rate of susceptibility of *S.aureus* to doxycyclin, whereas, most of the *S.pneumoniae* isolates were susceptible to tetracyclines (Al-Rubaey, 2005).

It has been mentioned by Appelbaum, (2002) that about half of *S.pneumoniae* isolates in COPD acute exacerbation are not completely susceptible to doxycyclin especially, penicillin resistant strains, in addition, Alles *et al.*, (2000)who stated that all isolates of *K.pneumoniae* were susceptible to doxycyclin which was agreed with this study, also, Govan, (2004) who showed that *P.aeruginosa* was a multi-drug resistant to commonly prescribed antimicrobial agents either by alteration of outer membrane porins or reduction of cellular permeability to prevent the entry of antibiotics into the bacterial cell. Besides, the full resistance of *Acinetobacter spp.* to this antibiotic was agreed with Govan, (2004)who mentioned that the resistance of this bacteria to antibiotics was widely common among clinical isolates with an inherited resistance to many antibiotics, the non susceptibility of *Enterobacter spp.* towards doxycyclin may be due to porin deficiency or due to active efflux as shown by Mallea *et al.*, (1998) which was agreed with this study.

4.9.3. Susceptibility to Macrolides :

It has been shown by this study, that (40.7%) of *M.catarrhalis* were sensitive to erythromycin, *H.influenzae* , *Acinetobacter spp.*, and *Enterobacter spp.* were sensitive at a percent of (50%), highest rate of susceptibility were seen among the isolates of *S.pneumoniae* (66.6%), (33.3%) of *K.pneumoniae* were sensitive to this antibiotic whereas, *P.aeruginosa* and *S.aureus* showed full non susceptibility to this antibiotic as shown in figure (4-4-C).

On the other hand, these isolates showed a variable percentages of sensitivity to clarithromycin, (48.1%) of *M.catarrhalis* were sensitive to this drug, *H.influenzae* were sensitive at (60%), (55.5%) of *S.pneumoniae*, and the highest rate of the susceptibility were observed among *K.pneumoniae* isolates (66.6%), (50%) of the *Acinetobacter spp.* whereas, the rest of isolates tested; *P.aeruginosa*, *Enterobacter*, and *S.aureus* showed full non susceptibility (100%) to clarithromycin as shown in figure (4-4-D).

In addition, this current study showed the susceptibility to azithromycin, where *M.catarrhalis* showed high rate of sensitivity to this antibiotic (81.4%), (30%) of *H.influenzae* were susceptible, *S.pneumoniae* and *K.pneumoniae* were susceptible at a percent of (66.6%), (50%) of both *P.aeruginosa* and *Acinetobacter spp.* were susceptible to this antibiotic, *Enterobacter spp.* were sensitive (75%), whereas the single isolate of *S.aureus* was sensitive to this drug as shown in figure (4-4-E).

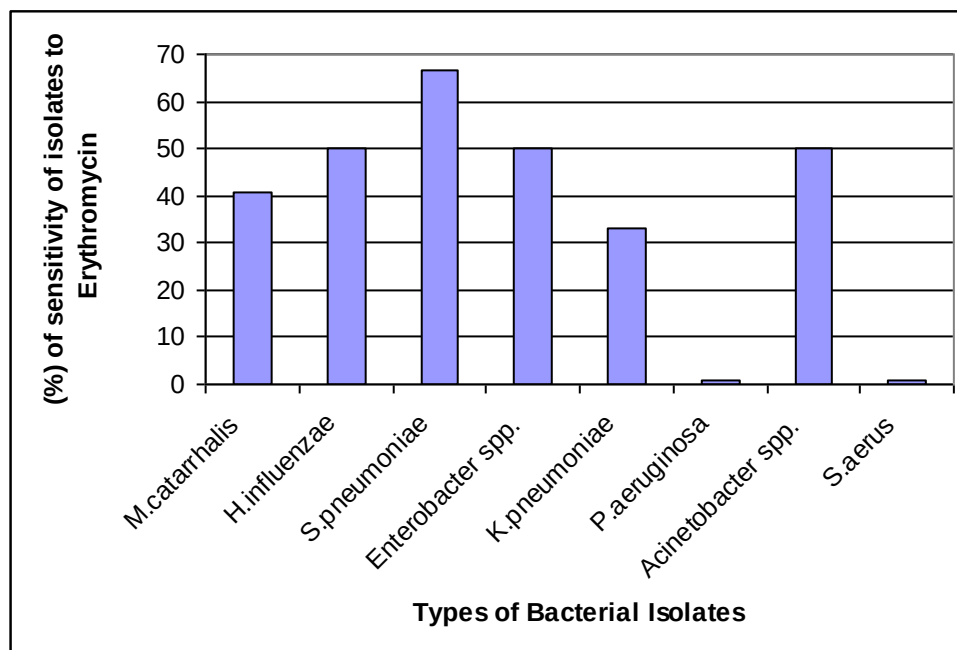


Figure (4-4-C) : The sensitivity of bacterial isolates to Erythromycin

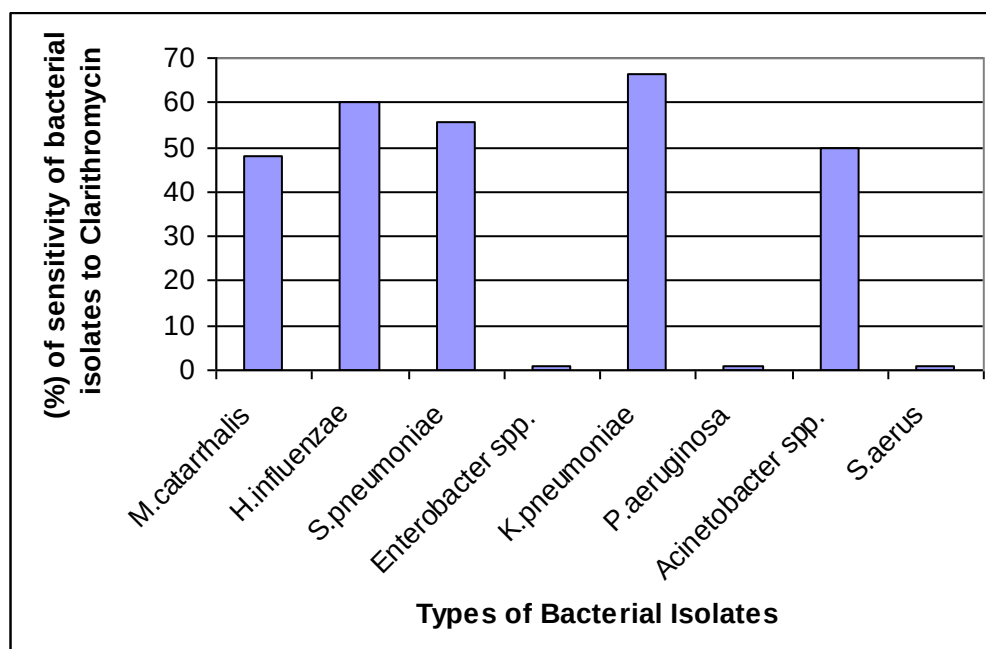


Figure (4-4-D) : The sensitivity of bacterial isolates to Clarithromycin

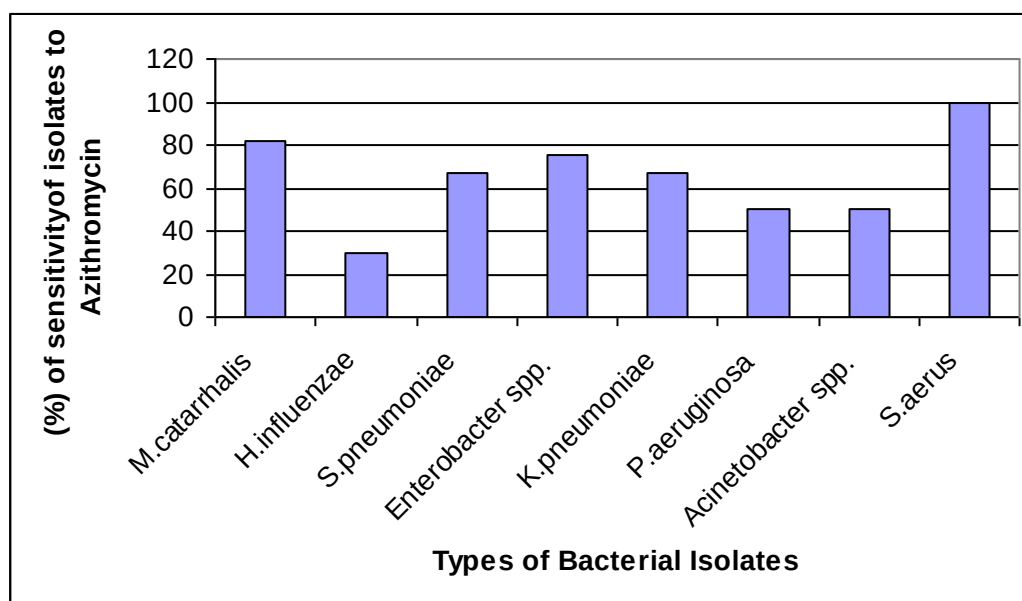


Figure (4-4-E) : The sensitivity of bacterial isolates to Azithromycin

The results in this study were agreed with Skerret, (2006) who stated that the *M.catarrhalis* isolates were fully sensitive to clarithromycin and azithromycin, where as, Anzueto, (2000) showed that *M.catarrhalis* were less susceptible to erythromycin, on the same manner, Peters and Clissold, (1992) showed that the isolates of *H.influenzae* were highly sensitive to clarithromycin, and this antibiotic may act by having both additive and synergistic effects in vitro against this isolate probably because adults have acquired antibodies to this bacteria from previous infections which was agreed with this study, also Thornsberry *et al.*, (1999) observed that *H.influenzae* were highly susceptible to azithromycin (99%) which was higher than the result of this study, whereas Al-Rubaey, (2005) stated that this bacteria had low susceptibility to erythromycin (18.4%) which lower than the

result in this study, and (33.3%) of *K.pneumoniae* isolates were sensitive to erythromycin which was agreed with this study.

Likewise, this study was in accordance with Imamura, (2005) who showed that azithromycin had an effect on *P.aeruginosa* causing bronchitis where it acts as a bactericidal agents in the stationary phase but not in the growth phase and this may be attributed to the differences between genes expression of the bacteria in the stationary phase, on the other hand, Frazer, (2007) mentioned that the choice of the appropriate antimicrobial agents against *Enterobacter spp.* is complicated by the fact that majority of the bacteria may develop resistance during the antimicrobial therapy.

In addition, it has been stated by Al-Shukri,(2003)that *Acinetobacter spp.* were susceptible to erythromycin at a rate of (27.3%) which was comparable with this study, whereas, Koelman *et al.*, (2001) observed that *Acinetobacter spp.* showed susceptibility to most of the antibiotics like ; macrolides which was related to the presence or absence of integron within the bacteria where integron positive bacteria showed higher resistance than itegron negative, also, Al-Hamawandi, (2005) who showed that *S.aureus* were entirely non susceptible to erythromycin which was with this study, and this single isolate recovered in this study does not reflect the real state of the sensitivity or resistance to the used antibiotics in this study.

Furthermore, Anzueto, (2000) observed that the action of erythromycin on the bacterial isolates is by inhibiting RNA-dependant protein synthesis by reversible

binding to 50s ribosomal subunits of susceptible microorganisms, on the other hand , Doern *et al.*, (1996) mentioned that the non susceptibility of the bacterial isolated to macrolides may be due to that some isolates may acquire degradation enzymes that hydrolyze these drugs or the bacteria can resist erythromycin by the active efflux (active removal of the antibiotic). Likewise, the non susceptibility of *K.pneumoniae* isolates to macrolides may be attributed to the production of the esterases enzymes which hydrolyze these antibiotics (Chambers, 2001).

4.9.4. Susceptibility of to Cephalosporins :

In this study, two types of cephalosporins had been studied one of them is first generation, cephalothin, and the second is third generation, cefotaxime.

Results in figure (4-4-F) showed the sensitivity of isolates to cephalothin where, the isolates in this study exhibited the lowest rates of the sensitivity in comparison to other drugs, both *M.catarrhalis* and *S.pneumoniae* had sensitivity at a rate of (11.1%), (20%) of *H.influenzae* were susceptible to this antimicrobial agent, *Enterobacter spp.* and *Acinetobacter spp.* were sensitive at a rates of (25%) and (50%) respectively, whereas, *K.pneumoniae*, *P.aeruginosa*, and *S.aureus* showed full resistance (100%) to this antibiotic, on the other hand, cefotaxime had a higher rates of effect than cephalothin, *M.catarrhalis* were sensitive at (51.8%), (80%) of *H.influenzae*, and (55.5%) of *S.pneumoniae* were susceptible, *Enterobacter spp.* and *K.pneumoniae* were sensitive at (50%) and (33.3%), full sensitivity for both *Acinetobacter spp.* and *S.aureus* to this

antibiotic ,whereas, *P.aeruginosa* isolates were fully non susceptible (100%) to this agent as shown in figure (4-4-G).

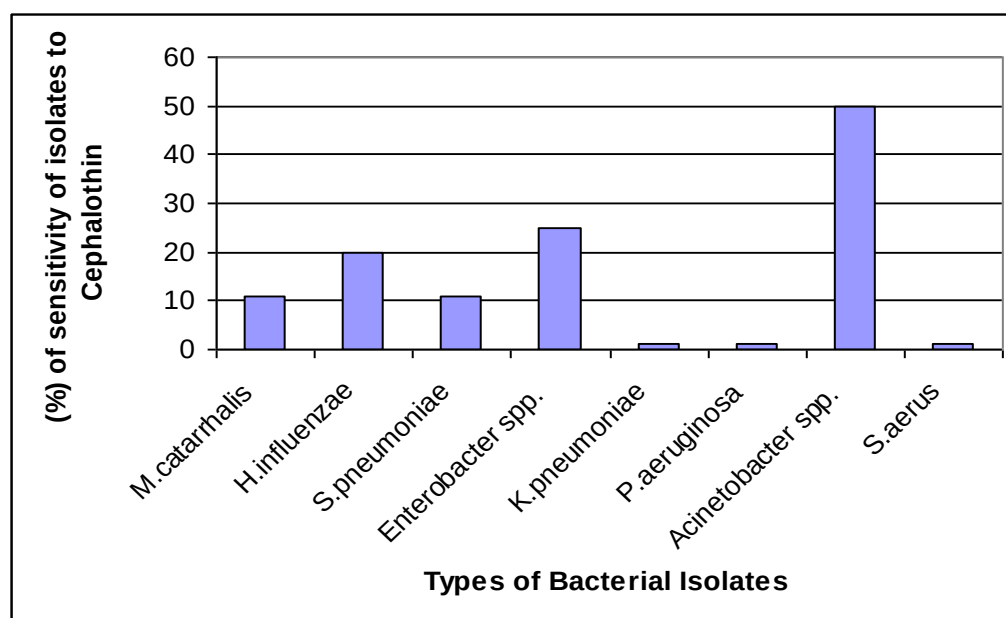


Figure (4-4-F) : The sensitivity of Bacterial isolates to Cephalothin

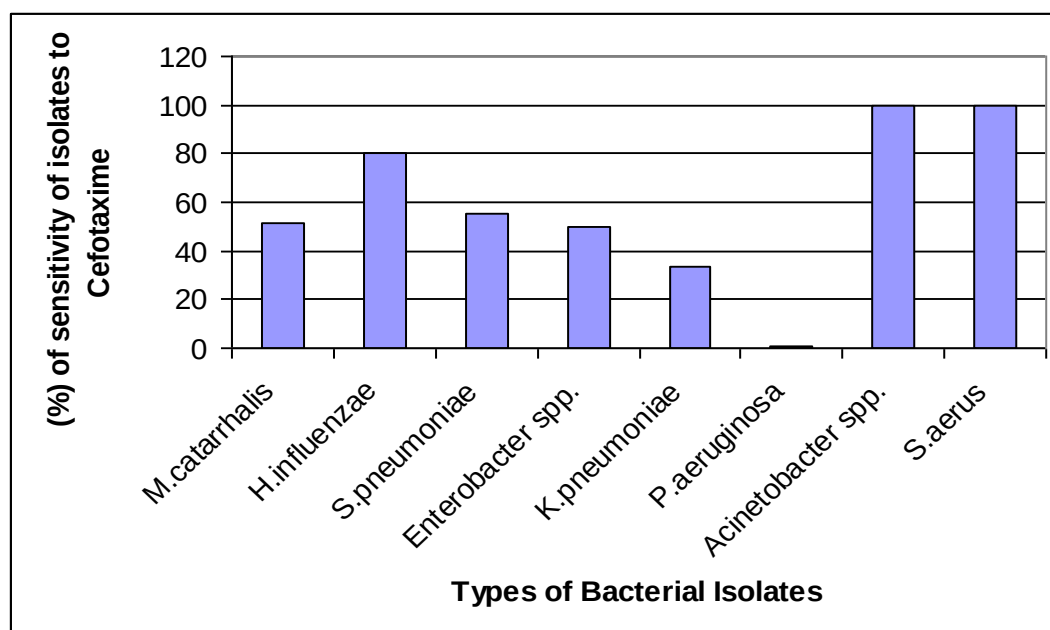


Figure (4-4-G) : The sensitivity of bacterial isolates to Cefotaxime

This result was agreed with Al-Rubaey, (2005) who indicated that *H.influenzae* isolates were fully sensitive to cefotaxime, whereas, *S.pneumoniae* were sensitive at a rate (85.7%), and, *K.pneumoniae* were susceptible to cefotaxime at (33.3%) also, Jawad, (2007) stated that the isolates of *M.catarrhalis* were fully susceptible to cefotaxime which was higher than the result in this study with very low rate of susceptibility *K.pneumoniae* to cephalothin which was agreed with this study.

On the same manner, Block *et al.*, (1996) observed that the incidence of B-lactamase produced by *H.influenzae* has increased to 55% where these enzymes confer resistance to amoxicillins and first and second generations cephalosporins like, cephalothin having high non susceptibility which was agreed with this study.

Moreover, the full non susceptibility of *P.aeruginosa* to both cephalothin and cefotaxime may be attributed that the bacteria can resist these antibiotics by having cephalosporinase enzyme that is able to resist and inactivate the cephalosporins (James, 1999), in addition, Al-Hamawandi, (2005) stated that *Acinetobacter spp.* and *S.aureus* were fully susceptible to cefotaxime which were in accordance with this study, whereas the full resistance of this bacteria to cephalothin was ascribed to the prior exposure of the first and second generations cephalosporins which were commonly prescribed and may be due to special B-lactamase which hydrolyze and inactivate the drug (Brooks *et al.*, 2004). The sensitivity of *Enterobacter spp.* to cephalosporins were in comparable to Mallea *et al.*, (1998) who showed that (50%) of this bacteria were sensitive to cefotaxime, whereas, Frazer, (2007) stated

that first generation cephalosporins like, cephalothin can be used as one of the treatment options for these bacteria, and the resistance of the bacteria to cefotaxime may be due to the expression of the gene AmpC is repressed and induced by B-lactams which lead to the hyperproduction of B-lactamases.

Furthermore, the low susceptibility of most isolates to cephalothin may be due to the alteration in the receptor sites for antibiotics on the cellular membrane, and prevention the uptake of antibiotics by blocking the pores of outer membrane as shown by Shah *et al.*, (2001). On the other hand, Schleiss, (2002) pointed that the high susceptibility of *H.influenzae* to cefotaxime may be due to its potent bactericidal activity.

4.9.5. Effect of Trimethoprim-Sulphamethaxozole (TMX) on Some Bacterial Isolates :

Results in figure (4-4-H) showed that, highest rate of sensitivity to this antimicrobial agent was among *Enterobacter spp.* (75%), followed by isolates of *K.pneumoniae* which were sensitive at a rate of (66.6%), *M.catarrhalis*, *H.influenzae*, and *S.pneumoniae* were sensitive at a rate (29.6%), (30%), and (11.1%) respectively, whereas, the rest of bacterial isolates tested in this study showed full non susceptibility (100%) to this antibiotic.

The study of Jawad, (2007) showed that *M.catarrhalis* was sensitive to TMX at a rate of (68%) which were higher than the results in this study, and the sensitivity of *K.pneumoniae* was (86.7%) which was

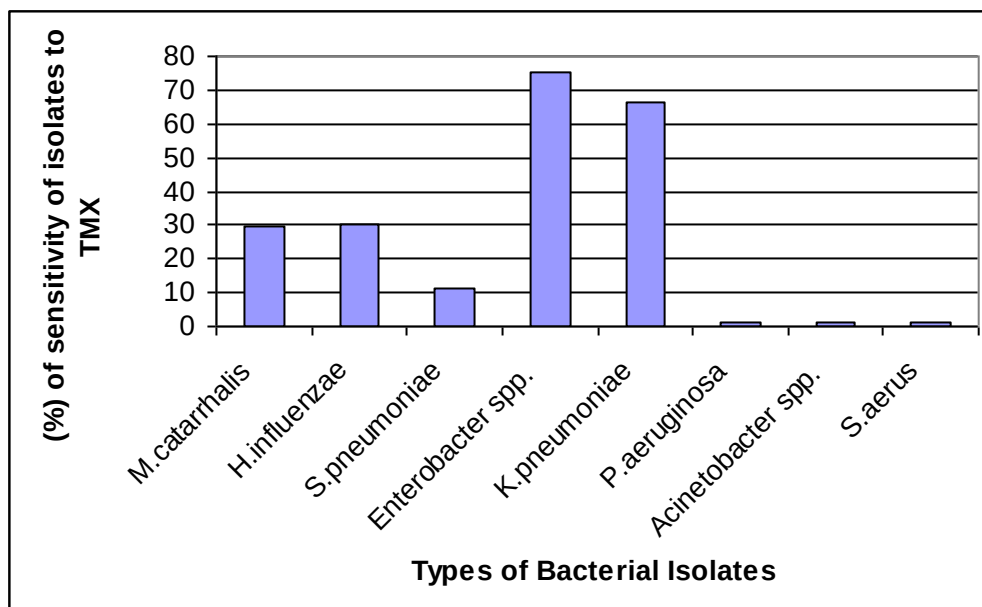


Figure (4-4-H) : The sensitivity of Bacterial isolates to TMX

higher than the result in this study, whereas, Thornsberry *et al.*, (1999) pointed that *H.influenzae* isolates were susceptible to this antibiotic at a rate higher than this result (90%), on the same manner, Doern *et al.*, (1996) showed that *S.pneumoniae* isolates were sensitive to TMX at a rate of (69%) which was higher than this result, and resistance of penicillins resistant strains to non-B-lactam antibiotics were higher than penicillins sensitive strains like in case of resistance of this bacteria to TMX, and high resistance being encountered among isolates of *S.pneumoniae* which have been recovered from the respiratory tract than the isolates recovered from the cerebrospinal fluid and blood. In addition, Al-Hamawandi, (2005) observed that (33.3%) of *P.aeruginosa* were susceptible to TMX, with complete

sensitivity of *Acinetobacter spp.* to the same antibiotic which was in contrast to this study that all isolates of the bacteria were resistant to this drug and very low rate of susceptibility of *S.aureus* to this agent which was agreed with this study.

Likewise, Frazer, (2007) showed that TMX can be used as one of the treatment options for *Enterobacter spp.* due to its effect on the bacteria which was agreed with this study where TMX had an effect on (75%) of these isolates.

Moreover, Chambers, (2001) stated that the non susceptibility of some of the isolates to this antimicrobial agents may be due to reduced cell permeability and pumping it from the resistant bacterial cells, whereas, its effect by acting as antimetabolites which block the essential enzymes of the folate metabolism.

4.9.6. Susceptibility of Bacterial Isolates to Flouroquinolones :

The present study used two types of quinolones in vitro, sparfloxacin and lomefloxacin, where results in figure (4-4-I) showed the variable susceptibility rate of the tested isolates to sparfloxacin, and (74.2%) of *M.catarrhalis* isolates were susceptible to this antibiotic, (50%) of *H.influenzae* and *Enterobacter spp.*, and *Acinetobacter spp.* were susceptible to this antibiotic, the sensitivity of both *S.pneumoniae* and *K.pneumoniae* (66.6%) and (33.3%) respectively, *P.aeruginosa* isolates were fully (100%) susceptible to this agent with complete resistance to the single isolate, *S.aureus*, whereas, results in figure (4-4-J) showed the sensitivity of these isolates to lomefloxacin showed as follows ; *M.catarrhalis* at (55.5%) , (66.6%) for both *S.pneumoniae* and *K.pneumoniae*, the sensitivity of the isolates ;

H.influenzae, *P.aeruginosa*, and *Enterobacter spp.* (40%) , (50%) and (25%) respectively, whereas, *Acinetobacter spp.* and *S.aureus* showed complete resistance to this antimicrobial agents.

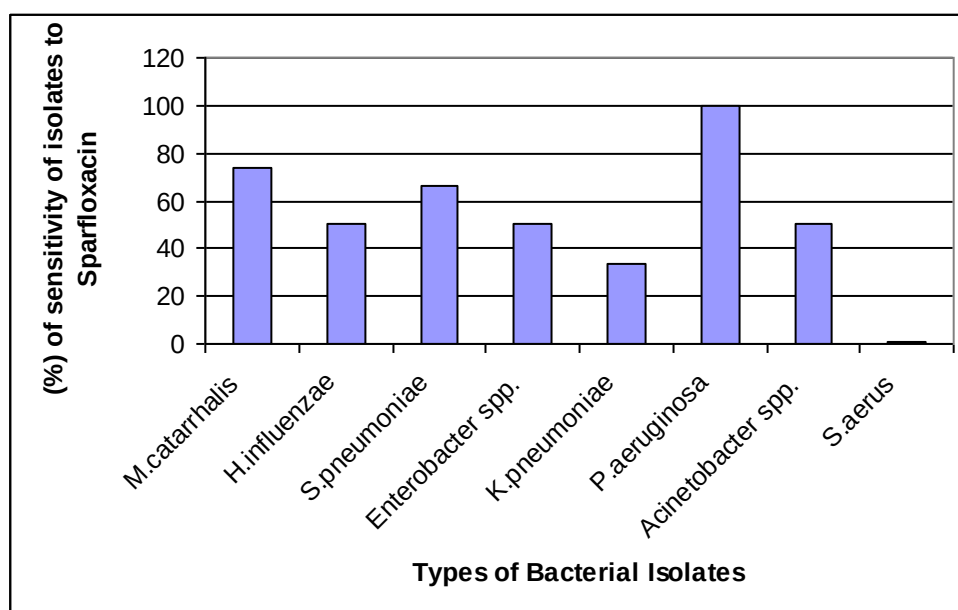


Figure (4-4-I) : The sensitivity of bacterial isolates to Sparfloxacin

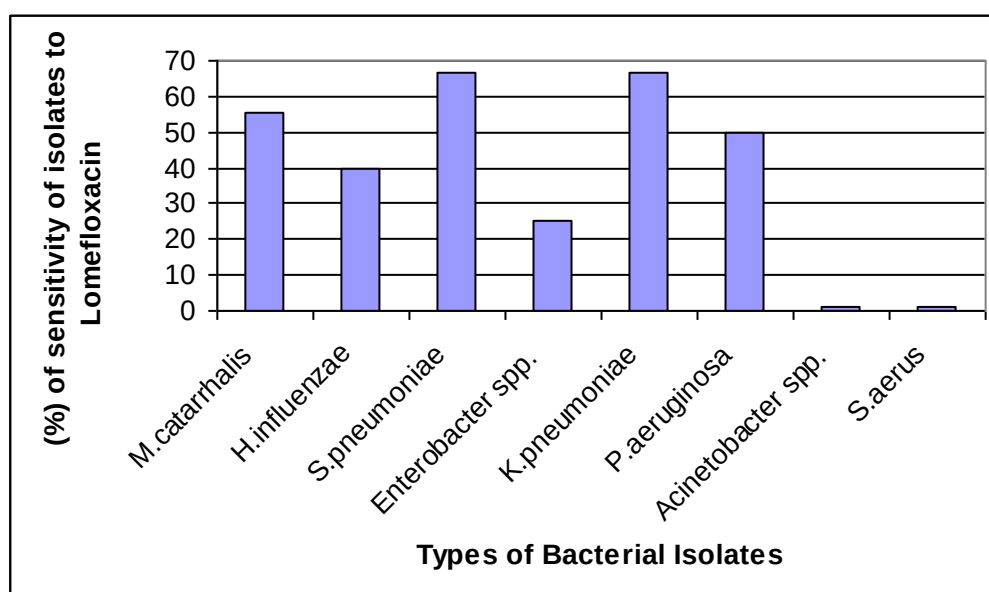


Figure (4-4-J) : The sensitivity of bacterial isolate to Lomefloxacin

The results in this study were agreed with Skerret, (2006) who observed that *M.catarrhalis* isolates were highly sensitive to flouroquinolones like sparfloxacin which was agreed with this study, on the same manner, Thornsberry *et al.*, (1999) stated that *H.influenzae* were sensitive at a high rate to flouroquinolones like; sparfloxacin and lomefloxacin, also, Jawad, (2007) showed that *S.pneumoniae* isolates were highly susceptible to quinolones (97%), and *K.pneumoniae* were sensitive to the flouroquinolones at a rate higher than the susceptibility of this isolate in this study.

Besides, the sensitivity of *P.aeruginosa* to quinolones like, sparfloxacin and lomefloxacin were agreed with Silbert *et al.*, (2001) who mentioned that this bacteria was sensitive at a rate of (70%), Koelman *et al.*, (2001) stated that the susceptibility of *Acinetobacter spp.* to quinolones may be due to that the bacteria is an integron negative. The single isolate of *S.aureus* does not reflect the antibiotic profile of susceptibility and only one isolate may be mutant type and become resistant to some antibiotics.

It has been stated by Patersen , (2006) that flouroquinolones have a good activity against *Enterobacter spp.* in vitro and the rate of resistance occurs mainly with extended B-lactamase producing organisms. Moreover, Nix, (1998) pointed flouroquinolones like, sparfloxacin and lomefloxacin acted by inhibition of the top isomerase IV and DNA gyrase and they prevent microbial DNA replication and transcription and these agents concentrated intracellularly in most tissues including

the bronchial mucosa, epithelial lining fluid, and sputum, which may enhance their effectiveness against these pathogens.

It has been shown by this study that all bacterial isolates showed a variable susceptibility to azithromycin at a rate (65%), followed by cefotaxime (58.8%), sparfloxacin was in the third place (53%), whereas, the rest of the antimicrobial agents exhibited lower rate of effect on the tested bacterial isolates in this study.

4.10. Effect of N-Acetyl cysteine (NAC) on Some Bacterial Isolates :

This study showed the effect of N-acetylcysteine (NAC) on 20 bacterial isolates, it was seen that all 8 isolates of *M.catarrhalis* had lost their growth when be grown in media containing NAC, only one isolate of *H.influenzae* tested had a growth in the presence of NAC on chocolate agar, whereas 80% of these isolates lost their growth also, all 3 isolates of *S.pneumoniae* had no growth in the presence of NAC, the single isolate of *K.pneumoniae* and *Acinetobacter* exhibited no growth with NAC, the single isolate of *P.aeruginosa* resisted NAC presence and showed growth on both blood and chocolate agars, whereas the single isolate of *V.streptococci* had no growth with NAC, and the study showed that (90%) of the isolates tested showed no growth with NAC, and only (10%) of the isolates had growth in vitro, as shown in table (4-8).

This study differs from the previous studies in that it studied the effect of NAC on 20 bacterial isolates in vitro after incubation of the previously prepared

Table (4-8) : The Effect of N-Acetyl cysteine (NAC) on Some Bacterial Isolates

Type of bacterial isolate	No.	Growth with NAC	No growth with NAC
<i>M.catarrhalis</i>	8	0	8
<i>H.influenzae</i>	5	1	4
<i>S.pneumoniae</i>	3	0	3
<i>P.aeruginosa</i>	1	1	0
<i>K.pneumoniae</i>	1	0	1
<i>Acinetobacter spp.</i>	1	0	1
<i>V.streptococci</i>	1	0	1
Total	20	2 (10%)	18 (90%)

sputum with NAC on brain heart infusion broth and the positive result being further recultured on ordinary media to compare the results of growth before and after addition of NAC.

Indeed, N-acetylcysteine (NAC), is used in the medical treatment of chronic bronchitis, cancer, and paracetamol intoxication (Riise *et al.*, 2000), and it is one of the smallest drug molecule in use and it has antibacterial properties, where the molecule is a thiol-containing antioxidant that disrupts disulfide bonds in mucous and competitively inhibits the amino acid cysteine utilization as shown by Naszal *et al.*,(2000),also, NAC exhibits direct and indirect antioxidant properties

and its free thiol group is capable of interacting with the electrophilic of reactive oxygen (RO), where this interaction with RO leads to the intermediate formation of NAC thiol with NAC disulphide as a major end product and NAC exerts an indirect antioxidant effect related to its role as a reduced glutathione precursor (GSH) (Auroma *et al.*, 1989).

Besides, Riise *et al.*, (1994) observed that the ratio of significant intrabronchial bacterial growth was lower in patients with chronic bronchitis who were treated with oral NAC medication than in patients without this therapy, and in contrast to the present study in which the NAC was as a chemical material in vitro to test its effect on some bacterial isolates recovered from COPD patients, whereas the study of Riise *et al.*, (2000) used NAC as a drug in vitro for some COPD patients and was shown by their study that the rate of positive culture was low with NAC use and NAC had a negative effect on bacterial adhesive capacity of *S.pneumoniae* and *H.influenzae* with negative cultures, whereas the cultures were positive without NAC treatment.

The current study showed that all 8 isolates of *M.catarrhalis* had a negative growth on both blood and chocolate agars, and this may be attributed to the fact that NAC can reduce the attachment of bacteria to pharyngeal epithelial cell and possibly by removing a layer of ruthenium red-positive extracellular material from the epithelial cells and the effect of NAC on bacteria and bacterial biofilms that showed a response to NAC which may facilitate efficient use of this material

as a biofilms inhibitor (Zheng *et al.*, 1999), likewise, it has been stated by Reinchenberger and Tamm, (2002) that NAC was previously used as a mucolytic compound in chronic bronchitis has also antioxidative effect and it influences intrabronchial bacterial colonization and addition of daily 600 mg NAC lead to a significantly higher bacterial eradication rate and treatment with NAC may reduce the number ,duration of acute exacerbation episodes and influence lung function.

On the same manner, Riise *et al.*, (2000) noticed that the presence of NAC during growth of *H.influenzae* and *S.pneumoniae* were significantly reduced their adherence ability to human oropharyngeal epithelial cells in vitro and the effects were dose dependant and NAC affects the adhesive capacity which lowered the bacterial binding for *H.influenzae* and *S.pneumoniae*, also , it had been shown by Malley *et al.*, (2004) that the production of pyocyanin enhances *P.aeruginosa* virulence, and many of pyocyanins have cytotoxic effects on human cells which result from it`s ability of redox cycle where pyocyanin directly accepts electrons from reduced nicotine amide dinucleotide (NADH) or (NADPH) with subsequent transfer to O₂ generating reactive oxygen(RO), reduced glutathione (GSH) which is an important cellular antioxidant that contributes to the regulation of redox-sensitive signaling system and loss of GSH could be due the to pyocyanin induced (H₂O₂) formation. However, overproduction of catalase can partially prevent the pyocyanin- mediated decline in cellular GSH, also pyocyanin oxidized other thiol-containing compounds, cysteine and NAC and thus GSH may enhance pyocyanin-

induced cytotoxicity by functioning as alternative source of reducing equivalents for pyocyanin redox cycling and this may appear the cause of the resistant *P.aeruginosa* to the effect of NAC.

Moreover, Olofsson *et al.*, (2003) pointed that the NAC reduced the adhesion of *K.pneumoniae* and it decreases the extracellular polysaccharides (EPS) production by the bacteria by changing its surface character and as a result of the exposure to NAC by changing the EPS, the bacteria become unable to form large microcolonies and only single cell or small microcolonies were formed which changed the texture of the biofilm formed, and the same mechanism was applied to *Acinetobacter spp.* which was also affected by NAC in vitro by affection of EPS which was decreased as a result of exposure to NAC and also NAC can reduce the adhesion of the bacteria and it was less resistant than the *K.pneumoniae*.

On the same manner, Zuin *et al.*, (2005) noticed that the given NAC as a drug may reduce symptoms of COPD, rate of exacerbation, and lung function decline in COPD and it may be due to the ability of NAC to reduce mucous viscosity and facilitate expectoration.

In addition, NAC reduced cigarette smoke-induced abnormalities in the polymorph nuclear leukocytes (PMN), alveolar macrophages and epithelial cells), and NAC could also render oropharyngeal bacteria aspirated from the oropharynx less able to adhere to mucous or epithelial cells in the lower airways as reported by Clancy *et al.*, (1995).

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