Sudan University of Science and Technology

College of Graduate Studies

Bacterial Etiologies of lower Respiratory Tract Infection among Patients in Intensive Care Unit in National Hospital Riyadh -Saudi Arabia

المسببات البكتيرية لعدوى الجهاز التنفسي السفلي لدى مرضى وحدة العناية المركزة بالمستشفى الوطني بالرياض ـ المملكة العربية السعودية

Dissertation Submitted In Partial Fulfillment for the Requirements of M.Sc. Degree in Medical Laboratory Science

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ا لآيـة

بسم الله الرحمن الرحيم

(اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ (1) خَلَقَ الْإِنْسَانَ مِنْ عَلَقٍ (2) اقْرَأْ وَرَبُّكَ الْأَكْرَمُ

(3) الَّذِي عَلَّمَ بِالْقَلَمِ (4) عَلَّمَ الْإِنْسَانَ مَا لَمْ يَعْلَمْ (5))

سوره العلق

DEDICATION

To:

My dear parents

Sisters and Brothers

My husband

And Friends

ACKNOWLEDGMENTS

First of all my thanks and eulogize were due to ALMIGHTY ALLAH,The beneficent and merciful, for giving me health and strength to accomplish this work.With great pleasure and respect, I would like to express my sincere gratitude to my supervisor Prof. Yousif Fadlalla for giving me the valuable help, encouragement, and guidance that have been indispensable in completing this work successfully. My appreciation and thanks were also due to anyone who helped me directly or indirectly in the preparation and revision of this study during the research work, my best regards to all without any exception.

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ABSTRACT

Lower respiratory tract infections (LRTI's) are the most frequent infections among patients in intensive care units. This a cross-sectional study aimed to detect the bacterial etiologies of lower respiratory tract infection among patients in intensive care unit in National Hospital -Riyadh - Saudi Arabia from January to December 2018. A total of 105 intensive care unit patients the specimens were collected (tracheal aspirate ,bronchial lavage and sputum) and inoculated on MacConkeys agar, blood agar and chocolate agar .Identification and susceptibility test were carried out by use vitek machine .Out of 105 ICU patients 82 (78%) were males and 23 (21.9) females with mean age 52.8 ± 21.6 SD. They were suffering from pneumonia 62(59%), acute bronchitis 4 (3.8%), lung abscess 19 (18%) and cystic fibrosis 14 (13%). From 105 lower respiratory specimens, 99 (94.3%) specimens showed growth and 6 (5.7%) showed no growth.

The study demonstrated that high frequency of growth in males and it was 76 (72.4%) and 23 (21.9%) of growth in females patients. High proportion of growth were in tracheal aspirate 93(88%), bronchial lavage 8 (7%), sputum 4 (3%). High frequency of growth was *Pseudomonas aeruginosa,* 75 (71.4%), *Acinetobacter baumannii* 69 (65.7%) *Klebsiella pneumoniae* 62 (59%), *Proteus mirabilis* 21 (20%), *E.coli* 12 (11.4%), *Providencia* 10 (9.5%), *Enterobacter aerogenes* 7(6.6%), *Staphylococcus aureus* 21(20%),*Serratia marcescens* 5(4.7%).Distribution of bacterial isolation according to type of specimens ,in tracheal aspirate specimen showed high proportion of *Pseudomonas aeruginosa* 67 (72%), in bronchial lavage specimen high frequency of *Klebsiella pneumoniae* 7 (87.5%) and high frequency of *Staphylococcus aureus* 3 (75%)in sputum specimen .The distribution of the bacterial isolates according to gender showed frequency of *Pseudomonas aeruginosa* in females patients 17(73.9%),followed by *Klebsiella pneumonia* 14(60.8%), *Proteus mirabilis*

9(39.1%), *Acinetobacter baumannici* 8(34.7%), *Staphylococcus aureus* 8(34.7%), *E.coli* 7(30.4%), *Enterobacter aerogenes* 4(17.3%), *Providencia* 4(17.3%), *Serratia marcescens* 3(13%),whereas *Acinetobacter baumannii* was predominant in males patients 61(74.3%) followed by *Pseudomonas aeruginosa* 58(70%), *Klebsiella pneumonia* 48(58%), *Staphylococcus aureus*13(15.8%) *Proteus mirabilis* 12(14.6%), *Providencia* 6 (7.3%), *E.coli* 5(6%), *Enterobacter aerogenes* 3(3.6%). *Serratia marcescens* 2 (2.4%).

The frequency of bacterial isolation according to ages groups showed highly frequency of *Klebsiella pneumoniae* 8(36%) in age group (20-30) years, in ages groups (31-40) , (41-50) years showed equal proportions in both *Klebsiella pneumonia* 8 (7.6%), *Acinetobacter baumannii* 10(9.5%),*Pseudomonas aeruginosa* 5(4.7%), *Proteus mirabilis* 7 (6.6%) and *Staphylococcus aureus* 6(5.7%), *E coli* 4 (3.8%) ,whereas *Acinetobacter baumannii* was predominant in age group (51-60) years 46 (43.8%) and *Pseudomonas aeruginosa* predominant in age group (>61) years 57 (54%). High percentage of *Pseudomonas aeruginosa* 49 (79%) and *Klebsiella pneumonia* 18(29%) in pneumonia patients *Acinetobacter baumannii* 14 (73%) in lung abscess patients, *Staphylococcus aureus* 8 (57%) in cystic fibrosis patients and *E.coli* 3(75%) in acute bronchitis patients. A single pathogen (*klebsiella pneumonia* ,*Acinetobacter baumannii, Pseudomonas aeruginosa, Proteus mirabilis, Staphylococcus aureus, E.coli,*)was isolated from 32(32.32%) patients and 67(66.76%)had mixed bacterial growth, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* were more common in co-infection.

المستخلص

تعد التهابات الجهاز التنفسي السفلي أكثر الإصابات شيوعًا بين المرضى في وحدات العناية المركزة.

تهدف هذه الدراسة المستعرضة إلى اكتشاف مسببات البكتريا لعدوى الجهاز التنفسي السفلي بين المرضى في وحدة العناية المركزة في المستشفى الوطني بالرياض – المملكة العربية السعودية في الفترة من يناير إلى ديسمبر ٢٠١٨. تم جمع ما مجموعه ١٠٥مريض في وحدة العناية المركزة تم جمع عينات (نضح القصبة الهوائية ، غسل الشعب الهوائية والبلغم) وتطعيمه على أجار الدم وأجار الشوكولاتة ، و ماكونكي. تم إجراء اختبار تحديد الحساسية والإصابة باستخدام آلة فايتك من بين ١٠٥ مرضى وحده العنايه المركزه (٧٨ %) ٨٢ ذكور و ٢٣ (٢١,٩) إناث مع متوسط العمر ٥٢,٨± ٢١٠٦ SD .

كانوا يعانون من الالتهاب الرئوي ٦٢ (٥٩ %) ، التهاب الشعب الهوائية الحاد٤ (٣,٨ %) ، خراج الرئة . ١٩ (١٨ %) والتليف الكيسي ١٤(١٣%) . من١٠٥ عينات تنفسية ، أظهرت٩٩(٩٤,٣%) نموًا و ٦ (٥,٧%). لم تظهر أي نمو

أظهرت الدراسة أن ارتفاع وتيرة نمو الذكور وكان ٦٧ (٧٢,٤%) و ٢٣ (٢١,٩%) من النمو في المرضى الإناث. وكانت نسبة عالية من النمو في نضح القصبة الهوائية 93(٨٨%) ، غسل الشعب الهوائية 8 (65.7٪) ٦٩ الراكده ٢، (٧١,٤%) ، ٧٥ الزائفه (٧ %) ، البلغم ٤ (٣%). كان ارتفاع وتيرة النمو كليبسيلا الرئوية ٦٢ (٥٩ %) ، بروتيوس ميرابيليس ٢١ (٢٠ %) ، إيكولي ١٢(١١,%) ، بروفيدنسيا ٠٠ ، السيرشيا (، المكورات العنقودية الذهبية 21 (٢٠%) 7 (٦,٦%) الانتيروباكتر (9.5 %) ، معويويات وزعت العزلة البكتيرية وفقًا لنوع العينات ، في عينة نضح القصبة الهوائية على نسبة عالية . (4.7٪)٥ ، في عينة غسل الشعب الهوائية عالية التردد من الكلبسيلة الرئوية 7 (٨٧,٤%) (٪) (٧٢ %) ٦ الزائفه من وارتفاع وتيرة المكورات العنقودية الذهبية٣ (٧٥ %) في عينة البلغم. توزيع العزلات البكتيرية وفقا لنوع الجنس أظهرت تواتر الزائفة الزنجارية في المرضى الإناث ١٧ (٧٣,٩%) ، تليها كليبسيلا الالتهاب المكورات العنقودية الذهبية 8 (٣٤,٧%) ، الراكده (٣٩,١%) ، البروتيوس٩ (الرئوي 14 (٦٠,٨% ، بروفيدنسيا ٤ (١٧,٣ %) ، (١٧,٣%) ٤ ، اينيروباكتر (٣٠,٤%) 7 ، الاسكريشيا القولونيه ((34.7 % ب كانت الغلبة للمرض عند الذكور الذكور ٦١(٧٤,٣%) سيراتيا مارسيسنس (١٣%) ، في حينالراكده تليها الزائفة الزنجارية 58 (70٪) ، كليبسيلا الالتهاب الرئوي 48 (58٪) ، المكورات العنقودية الذهبية

، (15.8٪) 13 بروتيوس ميرابيليس 12 (14.6٪) ، بروفيدنسيا 6 (7.3٪) ، كولاي 5 (6 ٪

(2.4٪) 2 سيرشيا .(3.6٪) 3 انتيوباكتر.

أظهر تواتر العزلة البكتيرية وفقًا للفئات العمرية تكرارًا كبيرًا لداء الكلبسيلة الرئوية 8 (36٪) في الفئة العمرية (٢٠–٣٠) عامًا ، في الفئات العمرية (٣١–٤٠) ، (٤١–٥٠) سنة وأظهرت نسبًا متساوية في ، (٤,٧٪) ، الزائفه٥ (٩,٥٪) ١٠ ، الراكده (كلا المجموعتين كليبسيلا الالتهاب الرئوي ٨ (٧,٦٪ ، بينما (٣,٨٪) 4 ، السكريشي القولونيه (والمكورات العنقودية الذهبية 6 (٥,٧٪ ٦,٦٪) 7 البروتيس كان البوماني سائدا في الفئة العمرية (٥١–٦٠) سنة ٤٦ (٤,٨٪) وسودوموناس آيروجينوسا الراكده و (٧٩٪) ٤٩ الزائفه الغالبة في الفئة العمرية 611< سنة ٥٧ (٥٤ ٪). نسبة عالية من بكتريا في مرضى خراج (73٪) 14 الراكده الكليبسيلاالتهاب الرئوي 18 (29٪) في مرضى الالتهاب الرئوي في مرضى التليف الكيسي و 6. 75 ٪) في مرضى التهاب (57٪) 8 االمكورات العنقوديه الرئه و ،البروتيس. الزائفه الشعب الهوائية الحاد. تم عزل ممرض واحد (الالتهاب الرئوي الكليبسيلا ، تاراكده من 32 (32.32 ٪) ، و 67 (66.76 ٪) كان لديهم نمو (، الاسكريشي القولونيه المكورات العنقوديه الزائفه و أكثر شيوعا في العدوى المشتركة بكتيري مختلط ،

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LIST OFABBREVIATION

AMC	Amoxicillin- clavulanic acid
AMK	Amikacin
ATM	Aztreonam
CAP	Community Acquird Pneumonia
CAZ	Ceftazidime
CEF	Cefepime
CIP	Ciprofloxacin
E	Erythromycin
FOX	Cfoxitin
GEN	Gentamicin
HAP	Hospital Acquired pneumonia
IMP	Imipenem
LNZ	Linezolid
LVX	Levofloxacin
MEM	Meropenem
MNO	Minocycline
MXF	Moxifloxacin
OXA	Oxacillin
QDA	Quinupristin/Dalforistin
SAM	Ampicillin-sul/bactam
TCC	Ticarcillin/clavulanic acid
TE	Tetracycline
TGC	Tigecycline
TOB	Tobramycin
TZP	Pipracillin/tazobactam
VAN	Vancomycin
VQP	Ventilated asociassion pneumonia

CHAPTER ONE

INTRODUCTION

CHAPTER ONE

INTRODUCTION

1. Introduction

Lower respiratory tract infection (LRTI) include those affecting the trachea, bronchi, bronchioles and lung parenchyma (Tom, *et al*.,2012).

The upper respiratory tract is heavily colonized by normal flora but the lower respiratory tract is sterile. (Abilo and Meseret ,2006). Acute lower respiratory infections include pneumonia infection of the lung alveoli, as well as infections affecting the airways such as acute bronchitis and bronchiolitis, influenza and whooping cough. They are leading causes of illness and death in children and adults across the world. The importance of lower respiratory infections may be underestimated (Gibson, *et al.,* 2013).The most clinically useful classification is Community acquired pneumonia, hospital-acquired pneumonia, pneumonia immunocompromised individuals, aspiration pneumonia, secondary to viral infection, ventilator associated (Tom, *et al*., 2011).

A number of factors have been suspected of or identified as increasing the risk for pneumonia or colonization of the lower respiratory tract by *Pseudomonas* an *Acinetobacter* and *Klebsiella* in the intensive care unit (ICU), including advanced age, chronic lung disease, immunosuppression, surgery, use of antimicrobial agents, presence of such invasive devices as endotracheal and gastric tubes, and type of respiratory equipment. However, there is little doubt that of all these factors, prolonged respiratory therapy with mechanical ventilation, and prior antimicrobial therapy are the most important. Because the only factor amenable to prevention in this setting is antimicrobial therapy, avoiding unnecessary antibiotics should be a high priority in the management of such patients. Crude mortality rates of 30% to 75% have been reported for nosocomial pneumonia caused by *Pseudomonas* and/or *Acinetobacter spp.,* with

the highest rates reported in ventilator-dependent patients. It is therefore the prognosis associated with this type of infection is considerably worse than that associated with infection caused by other Gram-negative or Gram-positive bacteria. Because bactericidal synergy against *Pseudomonas* and *Acinetobacter spp*. has been shown when carbenicillin and an aminoglycoside are combined, the use of an effective beta-lactam (piperacillin, ticarcillin, ceftazidime, or imipenem) and aminoglycoside combination remains the preferred therapeutic approach when possible. (Jean and Jean ,2000)

1.2 Rationale

Lower respiratory tract infection in ICU patient are among the most common bacterial infection worldwide. Bronchitis and pneumonia were most common in lower respiratory tract infections. (Macfarlane *et al.*, 2001).These infections are caused by a variety of pathogen bacteria. *Pseudomonas aeruginosa* and *Acinetobacter baumannii* being the most important ones. Because these organisms are cause opportunistic healthcare-associated infections in patients who are critically ill or immune compromised (John, and McGowan ,2006). Hospital acquired infection is an increasing problem in intensive care units, where the patients are more susceptible and the organisms often more resistant than in other environments. Between 5% and 15% of hospital in-patients develop an infection during their admission (Eggiman and Pittet, 2001).In addition, critically ill patients in an intensive care unit (ICU) are 5–10 times more likely to acquire a nosocomial infection than those in general wards (Weber, *et al.*, 1999).Lower respiratory tract infections (LRTI's) are the most frequent infections among patients in intensive care units. They are the most common hospital-acquired infections in ICU .This study will high light frequency of bacterial etiology of lower respiratory tract among intensive care unit patients.

1.3 Objectives:

1.3.1 General objective

To detect the bacterial etiologies of lower respiratory tract infection among patients in intensive care unit in National Hospital -Riyadh - Saudi Arabia

1.3.2 Specific Objective:

1- To isolate and identify bacterial etiologies of lower respiratory tract infection from specimens collected from ICU patients by conventional culture method and semi-automated vitek

2- To perform antimicrobial susceptibility tests to the isolated bacteria by vitek machine.

3-To detect the frequency of bacterial isolates among ICU patients with lower respiratory tract

CHAPTER TWO

LITERATURE REVIEW

CHAPTER TWO

Literature review

2.1 History

Lower respiratory tract infections (LRTIs) are the third most important cause of mortality globally and are responsible for more than 4 million deaths annually. (Chrysanthi, *et al*, .2012). Lower respiratory tract infections (LRTI) are the most frequent infections acquired on intensive care units (ICU) (Bonten, 2011).The two most common LRIs are bronchitis and pneumonia. (Sophie, 2007)

2.2 Anatomy of the lower respiratory tract (LRT)

The second half of the human airways, continuing after the larynx, including the trachea, bronchi, and the respiratory structures within the lungs – the, bronchioles, and alveoli.

2.2.1. Trachea

The trachea is the largest tube in the respiratory tract and consists of tracheal rings of hyaline cartilage. Beginning from the inferior end of the larynx, it is a flexible pipe-like structure primarily responsible for letting the inhaled air travel down the airways to reach the lungs. (Robert, *et al*.2013).

2.2.2 Bronchi

A bronchus is a passage or airway in the respiratory system that conducts air into the lungs. The first bronchi to branch from the trachea are the right main bronchus and the left main bronchus, also known as the primary bronchi. (Netter,2014)

2.2.3. Lung

The lungs are the largest organs in the lower respiratory tract. The lungs are suspended within the pleural cavity of the thorax (Matthias,*et al*. 2004).

2.2.4. Alveoli

Alveoli (alveolus), the last part of the lower respiratory tract, they are tiny air sacs in the lungs where gas exchange takes place (Matthias, *et al.*, 2004).

2.3 Acute Lower Respiratory Infections

Acute lower respiratory tract infections are a persistent and pervasive public health problem. They cause a greater burden of disease worldwide than human immunodeficiency virus infection, malaria, cancer, or heart attacks (Mizgerd , 2006). In the United States, they cause more disease and death than any other infection, and there has been little change in mortality due to respiratory tract infection for more than five decades. (Armstrong, *et al* .,1999).Lower respiratory tract infections (LRTI) are the most common hospital-acquired infections on ICUs. They have not only an impact on each patient's individual health but also result in a considerable financial burden for the healthcare system. (Leistner, *et al.*, 2013) .

2.4. Acute Bronchitis and Bronchiolitis

Acute bronchitis, also known as a chest cold, is short-term bronchitis – inflammation of the bronchi (large and medium-sized airways) of the lungs. (Albert,2010). Acute bronchitis is one of the most common diseases. About 5% of adults are affected and about 6% of children have at least one episode a year. (Wenzel and Fowler 3rd, 2006). Bronchiolitis is an infection of the lower airways , About 10% to 30% of children under the age of two years are affected by bronchiolitis at some point in time. (Friedman , *et al.*, 2014).It is the leading cause of hospitalizations in those less than one year of age in the United States. (Ralston, *et al.*, 2014).

2.5 Pneumonia

Pneumonia is an infection that inflames the air sacs in one or both lungs. The air sacs may fill with fluid or pus (purulent material).It causes symptoms for 3-4 weeks and is more common in very young children and elderly adults. Pneumonia is most commonly classified by where or how it was acquired as community-acquired pneumonia (CAP), which is contracted from coming into contact with the infection in daily life,hospital-acquired pneumonia (HAP), which is contracted after a period of time in hospital,ventilator-associated pneumonia (VAP), which is acquired after a procedure called endotracheal intubation, when a tube is inserted into the trachea to help a person breathe, pneumonia immune compromised individuals, aspiration pneumonia and secondary to viral infection (Harma , *et al*., 2007)   .

2.6 Community-acquired pneumonia (CAP)

Is a major respiratory disease with a high prevalence in the general population, clinical heterogeneity and variable severity. Community-acquired pneumonia (CAP) is one of the most common infectious diseases and is an important cause of mortality and morbidity worldwide (Jain, *et al*., 2015).The incidence of CAP in the UK increased by 34% between 1997 and 2005, (Trotter, *et al.,* 2008).The rates of serious complications of CAP, such as admissions to intensive care and complicated parapneumonic effusions (CPE), are also on the increase. (Grijalva , *et al*., 2011). Some forms of CAP can be prevented by vaccination. (José and Brown,2017).

2.5.2. Hospital-acquired pneumonia (HAP) or nosocomial pneumonia

Pneumonia that occurs 48 h or more after hospital admission, and which was not incubating at the time of admission, is defined as hospital-acquired pneumonia (HAP). It is thus distinguished from community-acquired pneumonia. It is usually caused by a bacterial infection, rather than a virus. HAP is the second

most common nosocomial infection (after urinary tract infections) and accounts for 15–20% of the total. It is the most common cause of death among nosocomial infections and is the primary cause of death in intensive care units (Gerald, *et al*.,2004).

2.5.3. Ventilator-associated pneumonia (VAP)

Is a sub-type of hospital-acquired pneumonia (HAP).It is a type of lung infection that occurs in people who are on mechanical ventilation breathing machines in hospitals. As such, VAP typically affects critically ill persons that are in an intensive care unit (ICU)(Michetti, *et al., 2012*).VAP is a major source of increased illness and death. Persons with VAP have increased lengths of ICU hospitalization and have up to a 20–30% death rate (Cook, 2000).

2.5.4. Pneumonia in Immune compromised Patients

Pneumonia is the most common infectious disease for the immune compromised host because the lungs could be the portal of entry for a wide range of pathogens via respiration. .The spectrum of potential pathogens known to cause pulmonary infections in immune compromised individuals has grown as a result of intensified immune suppression, prolonged patient survival, the emergence of antimicrobial-resistant pathogens, and improved diagnostic assays. Immune compromised hosts are defined by susceptibility to infection with organisms of little virulence in normal individuals or with increased severity of common infections (Fishman and Rubin, 1998).

2.5.5. Aspiration pneumonia

Is a type of lung infection that is due to a relatively large amount of material from the stomach or mouth entering the lungs. You can also aspirate food that travels back up from your stomach to your esophagus. All of these things may carry bacteria that affect your lungs. Complications may include lung abscess, some include chemical induced inflammation of the lungs as a subtype, which

occurs from acidic but non-infectious stomach contents entering the lungs. Infection can be due to a variety of bacteria (DiBardino and Wunderink ,2015).

2.5.6. Secondary pneumonia to viral

Seasonal and pandemic influenza are frequently complicated by bacterial infections, causing additional hospitalization and mortality. Secondary bacterial respiratory infection can be subdivided into combined viral/bacterial pneumonia and post-influenza pneumonia, which differ in their pathogenesis. During combined viral/bacterial infection, the virus, the bacterium and the host interact with each other. Post-influenza pneumonia may, at least in part,be due to resolution of inflammation caused by the primary viral infection (Van der Sluijs ,2010).Bacterial respiratory infection during influenza virus infection can be divided into combined viral/bacterial pneumonia or secondary bacterial infection following influenza. Secondary bacterial infections following influenza are more easily recognized clinically compared to combined viral/bacterial pneumonia since these bacterial infections tend to occur during the recovery phase from influenza (Madhi and Klugman, 2004).

2.6. Necrotizing Pneumonia

Necrotizing pneumonia is a rare and severe complication of bacterial community-acquired pneumonia (CAP). Lying on a spectrum between lung abscess and pulmonary gangrene (Surgical management of acute necrotizing lung infections.(Reimel *et al*., 2006).Necrotizing pneumonia is characterized by pulmonary inflammation with consolidation, peripheral necrosis and multiple small cavities (Surgical management of lung gangrene. (Krishnadasan *etal.,2000)*. Compromise of the bronchial and pulmonary vascular supply has the potential for devitalization of lung parenchyma. The lack of blood supply to the underperfused areas impedes delivery of antibiotics, allowing for uncontrolled infection and further destruction of lung tissue (Hammond JM, *et al*., 1993) .

2.7. Cystic Fibrosis

Cystic fibrosis (CF) is the most common autosomal genetic disease. It is an inherited disease that causes thickened mucus to form in the lungs, pancreas and other organs. In the lungs, this mucus blocks the airways, causing lung damage and making it hard to breathe. This disease affects persons without

distinction of age or sex but can be asymptomatic in a great number of cases. (chaparro.C, *et al*., 2001).

2.8. Chronic Pneumonia

Chronic pneumonia is an inflammation of the lungs that persists for an extended period of time, without a sudden onset. People can develop chronic pneumonia at any age.Pneumonia affects approximately 450 million people globally (7% of the population) and results in about four million deaths per year (Ruuskanen O,*et al* .,2011)

2.9. Nosocomial infections in the intensive care unit

Nosocomial infection in the intensive care unit (ICU) is associated with increased mortality, morbidity and length of stay. The clinical spectrum of lower respiratory tract infections (LRTI) potentially affecting patients managed in the intensive care unit (ICU) includes different diseases with peculiar epidemiological, clinical and microbiological aspects. Different scenarios might be identified by physicians dealing with nosocomial respiratory infections. On one hand, some hospitalized patients may develop nosocomial pneumonia outside the ICU, but then be transferred to the ICU because of the development of organ failure and the need for critical care support: these patients are considered to have non-ICU-acquired nosocomial pneumonia requiring ICU admission (Micek*, et al.,* 2015., Ranzani, *et al.,*2014*)* .On the other hand, the clinical course of patients already admitted to the ICU, for a variety of reasons, may be complicated by the occurrence of a LRTI: these patients are considered to have an ICU-acquired pneumonia (ICUAP). ICUAP may affect patients who

are undergoing mechanical ventilation or during spontaneous breathing (Giunta, *et al*., 2013).

2.10 Predicting nosocomial lower respiratory tract infections by a risk index based system

Healthcare associated infection (HAI) represents a major public health problem from all around the world (Angela, *et al*., 2013).Patients with HAI might have prolonged hospital stays and have high morbidity and mortality, thus adding economic burden on the healthcare system.(Alp and Damani,2015).Pneumonia and other lower respiratory tract infections (LRTIs) were the most common type of HAIs. According to a large multicenter epidemiological survey from China, 8,739 (59.55%) of 14,674 HAIs cases belonged to LRTI (Chunhui, *et al*. 2014).

2.11. Symptoms

Symptoms of lower respiratory tract infections vary and depend on the severity of the infection, Less severe infections can have symptoms similar to the common cold, including a stuffed up or a runny nose, a dry cough,a low fever, a mild sore throat ,dull headache. In more severe infections, symptoms can include shortness of breath, weakness, fever, coughing and fatigue. (Sophie ,2007)

2.12. Bacteria cause pneumonias

The most common agents of nosocomial pneumonias are aerobic Gram-negative bacilli that rarely cause pneumonia in healthy individuals. It is a common infectious disease that is found in intensive care unit (ICU), which occurs in 8~38% of patients who underwent mechanical ventilation. (Safdar, *et al*., 2005).The incidence of pneumonia has been known to be higher in ICU patients than in general ward patients, and even 3~10-fold higher in patients who underwent mechanical ventilation (Bell, *et al.,* 1983). *Pseudomonas aeruginosa , Escherichia coli, Enterobacter aeruginosa, Proteus mirabilis*, and *Klebsiella*

pneumoniae species , *Acinetobacter baumannii* are often identified, and Gram-positive bacteria such as *Staphylococcus aureus* (Kollef, *et al.,* 2005).As the type of causative pathogens and the rate of drug-resistant, pathogens may vary depending on region and hospital. *Acinetobacter baumannii* is a typically short, almost round, rod-shaped (coccobacillus) Gram-negative bacterium. It can be an opportunistic pathogen in humans, affecting people with compromised immune systems, and is becoming increasingly important as a hospital-derived (nosocomial) infection.While other species of the genus *Acinetobacter* are often found in soil samples (leading to the common misconception that *A. baumannii* is a soil organism, too), it is almost exclusively isolated from hospital environments (Lin .M, Lan and Chung-Yu,2014). Although occasionally it has been found in environmental soil and water samples. (Antunes,*et al.,* 2014). *A,baumannii* has also been identified as an ESKAPE pathogen (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species), a group of pathogens with a high rate of antibiotic resistance that are responsible for the majority of nosocomial infections. Colloquially, *A. baumannii* is referred to as "Iraqibacter" due to its seemingly sudden emergence in military treatment facilities during the Iraq War {Rice, 2008)   .

Pseudomonas aeruginosa is a common encapsulated, Gram-negative, rod-shaped bacterium that can cause disease in plants and animals, including humans. A species of considerable medical importance, *P. aeruginosa* is a multidrug resistant pathogen recognized for its ubiquity, its intrinsically advanced antibiotic resistance mechanisms, and its association with serious illnesses – hospital-acquired infections such as ventilator-associated pneumonia and various sepsis syndromes. The organism is considered opportunistic as serious infection often occurs during existing diseases or conditions – most notably cystic fibrosis and traumatic burns. It generally affects the immunocompromised but can also infect the immunocompetent as in hot tub

folliculitis. Treatment of *P.aeruginosa* infections can be difficult due to its natural resistance to antibiotics. When more advanced antibiotic drug regimens are needed adverse effects may result. (Balcht and Smith, 1994). It uses a wide range of organic material for food; in animals, its versatility enables the organism to infect damaged tissues or those with reduced immunity. The symptoms of such infections are generalized inflammation and sepsis. If such colonizations occur in critical body organs, such as the lungs, the urinary tract, and kidneys, the results can be fatal. Because it thrives on moist surfaces, this bacterium is also found on and in medical equipment, including catheters, (Itah and Essien, 2005).*P.* causing cross-infections in hospitals and clinics. *aeruginosa* is not extremely virulent in comparison with other major pathogenic bacterial species – for example *Staphylococcus aureus* and *Streptococcus pyogenes* – though *P. aeruginosa* is capable of extensive colonization, and can aggregate into enduring biofilms.(Høiby, *et al.,* 2010). *Pseudomonas aeruginosa* is primarily a nosocomial pathogen and it is represents the single most frequently isolated pathogen in patients with nosocomial pneumonia and burn-wound infections.(Allan ,*et al.,*1984).

Klebsiella pneumoniae is a Gram-negative, non-motile, encapsulated, lactose-fermenting, facultative anaerobic, rod-shaped bacterium. It appears as a mucoid lactose fermenter on MacConkey agar.Although found in the normal flora of the mouth, skin, and intestines, it can cause destructive changes to human and animal lungs if aspirated, specifically to the alveoli resulting in bloody, brownish or yellow colored jelly like sputum. (Ryan, K and Ray, CG,2004). *Klebsiella* possesses beta-lactamase giving it resistance to ampicillin, many strains have acquired an extended-spectrum beta-lactamase with additional resistance to carbenicillin, amoxicillin, and ceftazidime (Sanchez *et al*.,(2013).

Infections by K1 and K2 capsular serotype, the mucoid phenotype, and aerobactin production were important determinants of virulence. (Victor.*et al*., 2007) .

Escherichia coli is a Gram-negative, rod-shaped, facultative anaerobe and non sporulating coliform bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms (endotherms). (Tenaillon ,*et al* .,2010), *E coli* respiratory tract infections are uncommon and are almost always associated with *E coli* UTI. No virulence factors have been implicated. *E coli* pneumonia may also result from microaspiration of upper airway secretions that have been previously colonized with this organism in severely ill patients; hence, it is a cause of nosocomial_pneumonia. *E coli* pneumonia may also be community-acquired in patients who have underlying disease such as diabetes mellitus, alcoholism, chronic obstructive pulmonary disease, and *E coli* UTI. *E coli* pneumonia usually manifests as a bronchopneumonia of the lower lobes and may be complicated by empyema (Pitout and Laupland ,2008). Fimbrial adhesins, outer membrane proteins and some toxins produced by *E. coli*, that can directly interact with the epithelial cells of the intestinal, respiratory and urinary tracts. They are consider as virulence factor (Jacques,2013).

Proteus mirabilis is a Gram-negative, facultative anaerobic, rodshaped bacterium. *P. mirabilis* causes 90% of all *Proteus* infections in humans. It is widely distributed in soil and water (Chen., *et al*., 2012). *Proteus* species are most commonly found in the human intestinal tract as part of normal human intestinal flora, *Proteus* is also found in multiple environmental habitats, including long-term care facilities and hospitals. In hospital settings, it is not unusual for gram-negative bacilli to colonize both the skin and oral mucosa of both patients and hospital personnel. Infection primarily occurs from these reservoirs. *Proteus* species are not the most common cause of nosocomial infections.(Williams,G and Stickler D,2008). Fimbriae and other adhesions, iron and zinc acquisition, proteases and toxins, swarming growth, biofilm formation, and regulation of pathogenesis through

DNA binding proteins all this consider as Virulence Factors (Schaffer, J and Pearson, M.,2015).   .

Enterobacter is a genus of common Gram-negative, facultatively anaerobic, rod-shaped, non-spore-forming bacteria of the family Enterobacteriaceae. It is the type genus of the order Enterobacterales. Several strains of these bacteria are pathogenic and cause opportunistic in immunocompromised (usually hospitalized) hosts and in those who are on mechanical ventilation. The urinary and respiratory tracts are the most common sites of infection. (Cabral, 2010).

Serratia is genus of Gram-negative, facultativelyanaerobic, rod-shaped bacteria . They are do not produce spores. The most common and pathogenic of the species in the genus, *S. marcescens*, is normally the only pathogen and usually causes nosocomial infections. *S. marcescens* is typically found in showers, toilet bowls, and around wetted tiles. It is an opportunistic human pathogen. *S. marcescens* is thought to be transmitted through hand-to-hand transmission by hospital personnel. In the hospital, *Serratia* species tend to colonize the respiratory and urinary tracts. (Kämpfer, *et al*., 2016).

Providencia stuartii is a Gram negative bacillus that is commonly found in soil, water, and sewage. it is motile via flagella, non-sporulating.It can also grow in anaerobic conditions. *Providencia stuartii* is the most common *Providencia* species capable of causing human infections. It is an opportunistic pathogen seen in patients with severe burns or long-term indwelling urinary cathetres(Chi-Hung, *et al*., 2008).

Staphylococcus aureus is a Gram-positive, round-shaped bacterium that is a member of the Firmicutes, and it is a usual member of the microbiota of the body, frequently found in the upper respiratory tract and on the skin.It is a facultative anaerobe that can grow without the need for oxygen. Although *S. aureus* usually acts as a commensal of the human microbiota it can also become an opportunistic pathogen, Pathogenic strains often promote infections by

producing virulencefactors such as potent protein toxins, and the expression of a cell-surface protein that binds and inactivates antibodies. (Masalha, *et al* .,2001) .An estimated 20% to 30% of the human population are long-term carriers *S. aureus* which can be found as part of the normal skin flora, in the nostrils and as a normal inhabitant of the lower reproductive tract of women (Kluytmans *et al*.,1997).

2.13.Pathogenesis

The lung are constantly exposed to particulate material and microbes that are present in the upper airways and by micro aspiration tract. The lower air way usually remains sterile because of the pulmonary defense mechanism. The development of pneumonia indicates either defect in host defenses exposure to a particularly virulent microorganism or overwhelming inoculums.(Kanoh and Rubin,2010).

2.14. Virulence factors for colonization or infection with multidrug-resistant

Multidrug-resistant bacteria include prolonged length of hospital stay, exposure to an intensive care unit (ICU), receipt of mechanical ventilation, colonization pressure, exposure to antimicrobial agents, recent surgery, invasive procedures, and underlying severity of illness.(Fournier and Richet, 2006).

The opportunistic bacterial pathogens responsible for most cases of pneumonia can cause a range of local and invasive infections. Bacterial colonization (or carriage) in the upper airway is the prerequisite of all these infections. Successful colonizers must attach to the epithelial lining, grow on the nutrient-limited mucosal surface, evade the host immune response, and transmit to a susceptible host. (Steven and Jeffrey,2015).Widespread environmental contamination is often demonstrated, and outbreaks of infection have been traced to respiratory care equipment, wound care procedures, humidifiers, and patient care items. (Bernards, 2004).

2.15. Laboratory diagnosis

Diagnosis is usually made clinically. Chest radiographs showing new consolidations or infiltrates are definitive in helping to establish a diagnosis of pneumonia. When alveolar sacs fill with inflammatory cells and fluid, the chest radiograph will show consolidated well-defined densities that are unilateral (inhalation or aspiration pneumonia), bilateral (hematogenous spread to lungs).Specimens can be sputum, tracheal aspirate, bronchial lavage .Culture of blood samples for bacteria. Serology to detection of antibodies to the capsule of *Streptococcus pneumoniae*).Polymerase chain reaction (PCR) performed on sputum samples to rapidly determine the cause of the pneumonia (Bartlett, *et al.*, 2000).

2.16. Treatment

Antibiotics can be given to patients with acute exacerbations of chronic bronchitis. The indications for treatment are increased dyspnoea, and an increase in the volume or purulence of the sputum. The treatment of bacterial pneumonia is selected by considering the age of the patient, the severity of the illness and the presence of underlying disease. Amoxycillin and doxycycline are suitable for many of the lower respiratory tract infections seen in general practice.An important consideration in the treatment of a patient with a lower respiratory tract infection is to decide if an antibiotic is required at all. Many infections are viral and symptomatic treatment only is required. If an antibiotic is required, the choice of drug will depend on the site of infection, the severity of illness, the age of the patient, the presence of any other underlying diseases, history of drug reactions and the likely compliance of the patient.(Keryn Christiansen,1996).

2.17 .Prevention and control

Washing hands regularly with soap and warm water, and thoroughly, particularly after touching nose or mouth, and before handling food. Sneezing and coughing into tissues. This will help prevent the virus-containing droplets

from the nose and mouth entering the air where they can infect others. Throw away used tissues immediately, then wash hands. Do not share cups or kitchen utensils with others. Vaccines are available to prevent two of the most common and most deadly causes of lower respiratory tract infections: pneumococcal disease and influenza. Pneumococcal polysaccharide vaccine prevents pneumococcal bacteremia; influenza vaccines prevent influenza as well as several complications of influenza (Whitney and Harper,2004),

2.18. Previous studies

The study was conducted for the period of 3 years from January 2010 to December 2012 . The LRT specimens from 230 patients admitted in a NICU during the study period were processed Out of the 230 LRT specimens evaluated, 198 (86.08%) were culture positive. A total of 254 pathogens were recovered with a predominance of Gram-negative isolates ($n =$ 243; 96.05%) *Pseudomonas aeruginosa* was the most dominant pathogen followed by *Klebsiella pneumoniae*. (Trupti , G.*et al* .,2013) . Other study , one hundred tracheal aspirate from one hundred ICU admitted patients were enrolled. Among them 60 were male and 40 were female .their average age was between 40-60 years ,,most common pathogen was *Acinetobacter baumannii* (25%).the other organisms *was Pseudomonas aeruginosa* (15%), *klebsiella pneumoniae* (11%) *E.col* (14%)(Lamya Hoque *et al*.,2013).

There is another study which includes 201 patients (1285 patient days) admitted over a period of one-and-a-half years. A total of 77 infections were identified in 67 patients (33.5%).The most high infections was pneumonia (53%).The most commonly   identified   organisms   were   *Acinetobacter   baumannii* (34.8%), *Pseudomonas   aeruginosa* (23.9%) and *Escherichia   coli* (15.2%) (*Agarwal R et al*.,2006).

There were study conducted in Medical College Hospital in North Kerala, India by(Syed .*et al*.,2013).Among the 1750 respiratory samples, 298(17.03%) were

found to be positive for bacterial isolates. 227 samples (76.17%) from among males and 71 samples (23.83%) from among females were culture positive, thus showing a male sex predilection .The highest isolation rate was observed in the 61-80 years (50%) age group . *Klebsiella pneumonia* (41.95%) was found to be the predominant organism which was isolated, followed by *Pseudomonas aeruginosa* (26.84%).

Other study conducted by (Maduakor u .*et al*., 2017).The mean age of the patients was 42.6 ± 16.8 years. Of the total 954 sputum samples, 431 (45.2%) were positive for micro-organisms. A single, unique pathogen was recovered in 415 patients (96.3%), and 16 (3.7%) were polymicrobial. The most predominant single pathogen was *Klebsiella* pneumoniae, 215 (49.9%), and the most prevalent bacterial combination was *Klebsiella* spp and *Pseudomonas aeruginosa*, 6 (1.4%). Antimicrobial susceptibility testing shows that most isolates of *K pneumoniae* were susceptible to imipenem (94.8%). Among the bacteria, *Escherichia coli* (13.3%) ranked highest, followed by *P aeruginosa* (12.5%), and the least was *Staphylococcus aureus* (2.1%).

Other study consisted of 426 patients with LTRIs from mid and far western region of Nepal between September 2011 and July 2014. Among the isolated Gram-positive organisms, *Streptococcus pneumonia* (n = 30, 51.7%) was the most predominant pathogen, followed by *Staphylococcus aureus* (n = 28, 48.3%). Among the isolated Gram-negative organisms, *Pseudomonas aeruginosa* (n = 71, 35.32%) was the most predominant pathogen, followed by *Haemophilus influenzae* (n = 68, 33.83%), *Klebsiella pneumonia* (n = 36, 17.19%), and *Escherichia coli* (n = 26, 12.94%). The pattern of resistance varied regarding the bacteria species, and there were multi-resistant isolates.(Salman Khan.*et al* ., 2015).The study conducted by (Shrestha S, *et al*., 2011) .A total of100 specimens including sputum and ET secration collected from patients diagnosed of nosocomial lower respiratory tract infection .among 113 bacterial isolate,109 were gram negative and 4 were gram positive,majority were

Pseudomonas aeruginosa (37.2%) followed by *Acinetobacter baumannii* (31.9%) *Klebsiella pneumonia* (21.2%) *Escherichia coli* (6.2%) and *Staphylococcus aureus* (3.5%)

CHAPTER THREE

MATERIALS AND METHODS

CHAPTER THREE

MATERIALS AND MERHODS

3.   Materials and Methods

3.1   Study design

This was descriptive cross sectional study

3.2   Study area

This study was carried out in National Hospital in Riyadh Saudi Arabia.

3.3   Study duration

The study was carried out from January to December 2018

3.4   Study population

Adult patients who were admitted to ICU

3.5   Inclusion criteria

Adult ICU patients suffering from lower respiratory infection, mechanical ventilator patients .

3.6   Exclusion criteria

Inpatients, outpatients, children , any patients not admitted to ICU, were excluded.

3.7   Ethical consideration

Approval to conduct this study was taken from the hospital authority and verbal consent was taken from the patients after being informed by the purpose of the study.

3.8   Sample size

A total of (n= 105) Subjects of ICU adult patients.

3.9   Sampling techniques

This study based on non-probability conventional sampling technique.

3.10 Data collection

Non self administered questionnaire (appendex 1) which include age, sex, other disease , catheterization ,mechanical ventilation ,sample type

3.11 Processing of the specimens

3.11.1 Specimen collection

The sample were collected from the patient including sputum ,tracheal aspirate and bronco alveolar lavage .A sputum sample (deep respiratory secretions, not saliva), usually collected first thing in the morning; sometimes, depending on the infection, up to 3 sputum samples were be collected over successive days. Samples were collected in wide mouth container. Broncho alveolar lavage (BAL) fluid was obtained by bronchoscopy.The collection of tracheal aspirate by the traditional technique was performed according to standard procedure.

3.11.2 Transportation of specimens

Following the microbiological collection the samples were transported to the microbiology laboratory, in a time not exceeding 30 minutes.

3.11.3 Checking the validity of specimens

Only samples with less than 25 epithelial cells per field (100 x magnification) on a Gram stained slide of a direct smear were considered.

3.11.4 Inoculation of specimens

By microliter-disposable plastic loop inoculate the sample on a chocolate agar plate, a sheep blood agar plate, and a MacConkey agar plate (Saudi prepared medical laboratory delivers). Incubated all plates overnight at 35℃, Chocolate agar plate, a sheep blood agar plate are incubated overnight in a 5% CO_2 atmosphere. Colonies were then enumeration the bacterial count by (cfu/mL). Microorganisms with counts $> 10^4$ cfu/mL were submitted for identification and antimicrobial susceptibility testing. If no growth was detected on any plate, the incubation was extended for 24 hrs (Lambotte, *et al*., 2002).

3.11.5 Gram's stain (QCA Qulmica clinica aplicada S.A)

Using sterile slides for each single colony isolated. One drop of distilled water was added on the sterile slide .By the loop a single colony was taken and mixed with DW on the slide circular .The smear was let to dry or by exposure to flame three quick times then stained by crystal violet as basic stain for 1 min, washed ,iodine added for 1 min, washed then decolorized by acetone alcohol(95% Ethanol) and washed immediately with clean water then neutral red was added as counter stain for 2 minutes , finally washed and examined under the microscope using x100 oil emersion . The result were Gram negative bacilli or Gram positive cocci

3.11.6. Identification of the isolation

The isolation were identify using vitek 2 machine (bioMerieux, Inc.,USA) (appendix 2).The VITEK 2 is an automated microbiology system utilizing growth-based Technology. The system is available in three formats (VITEK 2 compact,VITEK 2, and VITEK 2 XL) that differ in increasing levels of capacity and automation. Figure 3 shows the VITEK 2 compact system. All three systems accommodate the same colorimetric reagent cards that are incubated and interpreted automatically.

3.11.6.1. Procedure and Interpretation:

3.11.6.2. Suspension Preparation and Inoculation

A sterile swab or applicator stick was used to transfer a sufficient number of colonies of a pure culture and to suspend the microorganism in 3.0 mL of sterile saline (aqueous 0.45% to 0.50% NaCl, pH 4.5 to 7.0) in a clear plastic test tube. The turbidity was adjusted accordingly (see Table 3.1) and measured using a turbidity meter called the Densi ChekTM

Table 3.1: The uses of different turbidity STDS.

Product	McFarland turbidity concentration
Gram Negative	0.50-0.63 McF
Gram Positive	0.50-0.63 McF
Yeast	1.80-2.20 McF

The reagent cards (appendex 2) have 64 wells that can each contain an individual test substrate. Substrates measure various metabolic activities such as acidification, alkalinization, enzyme hydrolysis, and growth in the presence of inhibitory substances. An optically clear film present on both sides of the card allows for the appropriate level of oxygen transmission while maintaining a sealed vessel that prevents contact with the organism-substrate admixtures. Each card has a pre-inserted transfer tube used for inoculation (described below). Cards have bar codes that contain information on product type, lot number, expiration date, and a unique identifier that can be linked to the sample either before or after loading the card onto the system. Identification cards, which has biochemical test, were inoculated with microorganism suspensions using an integrated vacuum apparatus. A test tube containing the microorganism suspension was placed into a special rack (cassette) and the identification card was placed in the neighboring slot while inserting the transfer tube into the corresponding suspension tube. The filled cassette was placed into a vacuum chamber station. After the vacuum was applied and air was re-introduced into the station, the organism suspension was forced through the transfer tube into micro-channels that fill all the test wells . Inoculated cards were passed by a mechanism, which cuts off the transfer tube and seals the card prior to loading into the carousel incubator .The carousel has a capacity of 60 test cards were incubated on-line at $35.5 + 1.0$oC. As the carousel rotates, each test card moves into the reading position every 15 minutes .A mechanical device called the Reader Head, conveys the test card through the optical station. After the reading

cycle ,the test card returns to its slot in the carousel where it continues to incubate until its next read cycle. Data was collected at 15-minute intervals during the entire incubation period.

3.11.6.3. Reading and interpretation

A transmittance optical system allows interpretation of test reactions using different wavelengths in the visible spectrum. During incubation, each test reaction was read every 15 minutes to measure either turbidity or colored products of substrate metabolism. Calculations were performed on raw data and compared to thresholds to determine reactions for each test. On the VITEK 2 Compact, test reaction results appear as "+"," –", "(–)" or "(+)". Reactions that appear in parentheses were indicative of weak reactions that were too close to the test threshold. The databases of the VITEK 2 identification products were constructed with large strain sets of well-characterized microorganisms tested under various culture conditions. These strains were derived from a variety of clinical and industrial sources as well as from public (e.g., ATCC) and university culture collections. Test data from an unknown organism were compared to the respective database to determine a quantitative value for proximity to each of the database taxa. Each of the composite values was compared to the others to determine if the data were sufficiently unique or close to one or more of the other database taxa. If a unique identification pattern was not recognized, a list of possible organisms is given, or the strain is determined to be outside the scope of the database. An unknown biopattern was compared to the database of reactions for each taxon, and a numerical probability calculation is performed. Various qualitative levels of identification were assigned based on the numerical probability calculation.In rare cases, Mixed Taxa Identifications occurs when the biopattern was representative of a collective taxon and generates a genus-level, group-level, or slashline identification. Supplemental tests may be used to delineate representative species or subspecies of these collective taxa. In the case of low discrimination

identifications, two or three choices are listed in the order of their probability calculations. All taxa appearing in a low discrimination identification are viable choices and should only be ruled out after additional testing and/or observation.

Table 3. 2. Identification Levels.

ID Message confidence	Choices	% Probability	Comments
Excellent	1	96 to 99	N/A
Very good	1	93 to 95	N/A
Good	1	89 to 92	N/A
Acceptable	1	85 to 82	N/A
Lowe Discrimination	2 to3	Sum of choices=100 after resolution to one choice ,percent probability reflects the number associated with the selected choice	2 to 3 taxa exhibit the same biopattern separate by supplemental testing
Unidentified organism	>3 or 0	N/A	Either >3 taxa exhibit the same biopattren or Very atypical biopattern Does not correspond to any taxon in the database Check gram stain and purity

The GN card was used for the automated identification of 135 taxa of the most significant fermenting and non-fermenting Gram-negative bacilli. It was based

on established biochemical methods and newly developed substrates measuring carbon source utilization, enzymatic activities, and resistance. There were 47 biochemical tests and one negative control well. Final identification results were available in approximately 10 hours or less. The reference identification was determined with api® 20 E and api 20 NE identification kits.

The GP card was used for the automated identification of 115 taxa of the most significant non-spore-forming Gram-positive bacteria (primarily cocci). It was based on established biochemical methods and newly developed substrates. There were 43 biochemical tests measuring carbon source utilization, enzymatic activities and resistance. Final identification results awere available in approximately eight hours or less.

3.11.6.4. Reading and interpretation of susceptibility testing

The vitek system read the turbidity of suspension and the result observe and record in minimum inhibitory concentration. It was based on around 18-22 antibiotic and each one of this antibiotic has from 3 to 4 different concentration ,this feature was very important to get minimum inhibitory concentration (MIC) and the result interpreted as sensitive (S) or resist (R)

3.11.6.5. Quality control of vitek 2

Weekly QC for identification by using ATCC Strains

3.14 Statistical analysis

Data were analyzed using Statistical package for the social sciences (SPSS) version 23 program.

CHAPTER FOUR

RESULTS

CHAPTER FOUR
RESULTS

4. RESULTS

A total of 105 ICU adult patient were enrolled in this study ,among these 99 patients (94.3%) showed growth and 6 patient (5.7%) gave no growth illustrated in table 4.1.Out of 105 ICU patients 82 were males and 23 females

Table 4.1: Bacterial growth results

	Frequency	Percent
Growth	99	94.3%
No growth	6	5.7%
Total	105	100.0%

High proportion of sample type were in tracheal aspirate (TA) 93(88%), bronchial lavage (BAL) 8(7%),sputum 4 (3%) illustrated in table 4.2

Table 4.2 : Distribution of lower respiratory tract specimens

Type of specimens	Frequency	Percent
Tracheal aspirate	93	88.6%
Sputum	4	3.8%
Bronchial lavage	8	7.6%
Total	105	100

High percentage of lower respiratory infection were in pneumonia 62(59%), acute bronchitis 4 (3.8%), lung abscess 19 (18%) and cystic fibrosis 14 (13%) as showed in table 4.3

Table 4.3: Distribution of patients according to disease

Disease	Frequency	Precent
Pneumonia	62	59 %
Acute bronchitis	4	3.8%
Cystic fibrosis	14	13 %
Lung abscess	19	18%

Distribution of organisms according to disease as follow ,In pneumonia patients was high percentage of *Pseudomonas aeruginosa* 49 (79%)*,* in lung abscess patients showed high frequency of *Acinetobacter baumannii* 14 (73.6%), *Staph. aureus* 8 (57%) in cystic fibrosis patients and *E.coli* 3(75%) in acute bronchitis patients.

High percentage of growth in ICU male patients were 76 (72.4%) and in female
ICU patient were 23 (21.9%) table 4.4

Table 4.4 : distribution of bacterial growth according to gender

Gender		Growth		Total
		Growth	no growth	
Male	Count	76	6	82
	% of Total	72.4%	5.7%	78.1%
Female	Count	23	0	23
	% of Total	21.9%	0.0%	21.9%
Total	Count	99	6	105
	% of Total	94.3%	5.7%	100.0%

The distribution of bacterial isolation according to type of specimen was showed in table 4.5.In tracheal aspirate specimen show high frequency of *Pseudomonas aeruginosa* 67 (72%)followed by *Acinetobacter baumannii* 61 (65.5%), in bronchial lavage specimens the predominant organisms was *Klebsiella pneumonia* 7 (87.5%) and in sputum specimens showed high frequency of *Staphylococcus aureus* 3 (75%) in sputum samples.

Table 4.5 Distribution of bacterial isolation according to type of specimen

organisms	Tracheal aspirate	Sputum	Bronchial lavage	Total
A. baumannii	61(65.5%)	2(50%)	6(75%)	69
P. aeruginosa	67(72%)	2(50%)	6(75%)	75
K. pneumonia	54(58%)	1(25%)	7(87.5%)	62
E. coli	9(9.6%)	0	3(37.5%)	12
p. mirabilis	17(18.2)	0	4(50%)	21
p. stuartii	8(8.6%)	0	2(25%)	10
Ent. aerogenes	5(5.3%)	0	2(25%)	7
S.marcescens	5(5.3%)	0	0	5
Staph. Aureus	15(16.1%)	3(75%)	3(37.5%)	21
Total	241	8	33	282

A single pathogen (*K. pneumonia, A. baumannii , P. aeruginosa, p. stuartii, Staph. aureus , E.coli, S.marcescens*) was demonstrated in 32 (32.32%) patients and 67 (66.76%) had mixed bacterial isolate, *P. aeruginosa* and *A. baumannii* were more common in co-infection

Table 4.6: Distrubution of single or mixed isolation and bacteria

	Frequency	Percent
Single isolation	32	32.32%
Mixed isolation	67	66.76%

The predominant isolates of LRTIs infections were *P.aeruginosa* (71.4%) then *A.baumannii* (65.7%), *K.peuomonia* (59%), *Proteus* (20%),*Staph.aureus* (20%) and *E.coli* (11.4%) as showed in table 4.7

Table 4.7: Frequency of bacterial isolate

Organism	Frequency	Percent
Acinetobacter baumannii	69	65.7 %
Pseudomonas aeruginosa	75	71,4 %
Enterobacter aerogenes	7	6.6 %
Escherichia coli	12	11.4
Klebsiella pneumonia,	62	59 %
Serratia marcescens	5	4.7 %
Providencia stuartii	10	9.5 %
Proteus mirabilis	21	20 %
Staph. aureus	21	20 %
Total	282	268.3%

Total growth in tracheal aspirate sample were 89(84.8%), bronchial lavage 7 (6.7%) and sputum 3 (2.9%) as illustrated in table 4.8

Table 4.8 : Distribution of type of specimens according to bacterial growth

Type of specimens		Growth		Total
		Growth	no growth	
tracheal aspirate	Count	89	4	93
	% of Total	84.8%	3.8%	88.6%
Sputum	Count	3	1	4
	% of Total	2.9%	1.0%	3.8%
broncholavage	Count	7	1	8
	% of Total	6.7%	1.0%	7.6%
Total	Count	99	6	105
	% of Total	94.3%	5.7%	100.0%

Out of 105 patients ,102 (97%) under mechanical ventilation and 101 (96%) had NG tube.

Age group (20-30) years were high frequency of *K. pneumonia* 38(36%)
Age group (31-40) years and (41-50) years were equal frequency of *K.*
pneumonia 8(7.6%), *A. baumannii* 10 (9.5%), *P.aeruginosa* 5 (4.7%), *P.*
mirabilis 7(6.6%) *and Staph. aureus* 6(5.7%) *E.coli* 4(3%.8)
Age group (51-60) years were high frequency of *Acinetobacter* 46 (43.8%)
Age group (> 61) years were high frequency of *Pseudomonas* 57 (54%).The
highest isolation rate was observed in (> 61) years age group with males.
Showed in table 4.9

Table 4.9 Distribution of bacterial isolation according to age

Organisms	Age/years					
	20-30	31-40	41-50	51-60	>61	Total
A. baumannii	2(1.9%)	10(9.5%)	10(9.5%)	46(43.8%)	1(0.9%)	69
P. aeruginosa	6(5.7%)	5(4.7%)	5(4.7%)	2(1.9%)	57(54%)	75
K. pneumonia	38(36.1%)	8(7.6%)	8(7.6%)	5(4.7%)	3(2.8%)	62
E. coli	3(2.8%)	4(3%.8)	4(3%.8)	1(0.9%)	0	12
P. mirabilis	0	7(6.6%)	7(6.6%)	5(4.7%)	2(1.9%)	21
P. stuartii	0	2(1.9%)	2(1.9%)	4(3%.8)	2(1.9%)	10
Ent. aerogenes	2(1.9%)	0	0	3(2.8%)	2(1.9%)	7
S.marcescens	1(0.9%)	0	0	3(2.8%)	1(0.9%)	5
Staph. aureus	4(3%.8)	6(5.7%)	6(5.7%)	5(4.7%)	0	21
Total	56	42	42	74	68	282

High proportion was *Pseudomonas aeruginosa* 17(73.9%)in females patients
whereas in males patients predominant was *Acinetobacter baumannii*
61(74.3%) **as shwed in table 4 .12**

Table 4.10.Distribution of bacterial isolation according to gender

Organism	Male	female
A. baumannii	61(74.3%)	8(34.7%)
P. aeruginosa	58(70%)	17(73.9%)
K. pneumonia	48(58%)	14(60.8%)
E. coli	5(6.0%)	7(30.4%)
P. mirabilis	12(14.6%)	9(39.1%)
P. stuartii	6(7.3%)	4(17.3%)
Ent. aerogenes	3(3.6%)	4(17.3%)
S.marcescens	2(2.4%)	3(13%)
Staph. aureus	13(15.8%)	8(34.7%)

Distribution of organisms according to disease as follow ,In pneumonia patients was high percentage of *Pseudomonas aeruginosa* 49 (79%), in lung abscess patients showed high frequency of *Acinetobacter baumannii* 14 (73.6%), *Staph. aureus* 8 (57%) in cystic fibrosis patients and *E.coli* 3(75%) in acute bronchitis patients.

 P. aeruginosa was the predominant organism causing nosocomial LRTI

Antimicrobial testing were performed for isolate bacteria from ICU patients. Gram negative bacteria showed high susceptibility to Gentamycin 55%Cefepime 42.9%, Meropenem 39.7%and Tobramicin39.3% .For *Staph. aureus*, it was highly susceptible to Tigecyclin 100%, Gentamicin 95.2%,Amikacin95.2% .the frequency of multidrug resistance showed in *K. pneumonia* wihe Trimethoprim 83.9% Ciprofloxacin77.4%,Amikacin67.7% as showed intable 4.11

Table 4.11:Antimicrobial susceptibility pattern of bacterial isolation

Organisms	Antibiotic		AMK		AMC		IPM		SAM		FEP		FOX		CAZ		CIP		GEN	
	NO.		S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
Acinetobacter	69	NO.					6	63	16	53.	6	63			5	64	5	64	22	47
		%					8.69	91.3	23.2	75.3	8.69	91.3			7.24	92.7	7.24	92.7	31.9	67.9
Serratia marcescens	5	NO	5								5				3	2	5		5	
		%	100								100				60	40	100		100	
Enterobacter aerogenes	7	NO	5	2			4	3			5	2			2	5	5	2	5	2
		%	71.4	28.6			57.1	42.8			71.4	28.6			28.6	71.4	71.4	28.6	57.1	24.8
Escherichia coli	12	NO			5	7	11	1	2	10	12		8	4	10	2	9	3	6	6
		%	100		41.7	57.5	91.7	8.3	16.7	83.3	100		70	30	83	17	75	25	50	50
Klebsiella	62	NO	20	42	3	59	22	40	6	56	14	48	4	58	8	54	14	48	34	28
		%	32.2	67.7	4.83	95.1	35.6	64.3	9.67	90.3	22.6	47.4	6.45	93.6	12.9	87	22	77.4	54.8	45
Pseudomonas	75	NO	61	14			44	31			59	16			51	24		21	73	2
		%	81.3	18.6			58.7	41.3			78.7	21.3			68	32	72	28	96.9	3
Providencia	10	NO	8	2			4	6			6	4			4	6	10		4	6
		%	80	20			40	60			60	40			40	60	10		40	60
Proteus mirabilis	21	NO	17	4					7	14	14	7					6	15	8	13
		%	81	19					33.3	66.6	66.7	33.3					28.6	71.4	38.1	61.9

Organism			TE		CIP		GEN		QDA		LVX		CM		E		VA		LNZ	
Staph. Aureus	21	NO	20	1	15	6	20	1	21		17	4	81	3	81	3	81	3	21	1
		%	95.2	4.7	71.4	28.5	95.2	4.7	100		81	19	85.7	14.2	85.7	14.2	85.7	14.2	95.2	4.7

Organisms	Antibiotic	LVX		MEM		MNO		ATM		TZP		TCC		TGC		TOB		SXT	
	NO.	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
Acinetobacter	69 NO.	5	64	6	63	9	60			3	66	6	63	45	24	20	49	19	50
	%	7.24	92.7	8.69	91.3	13.1	86.9			4.14	95.6	8.69	91.3	65.2	43.7	29	71	27.5	72.4
Serratia marcescens	5 NO	3	2	3	2	1	4	4	1			4	1	3	2	2	3	5	
	%	60	40	60	40	20	80	80	20			80	20	60	40	40	60	100	
Enterobacter Aerogenes	7 NO	6	1	4	3	2	5	2	5	3	4	3	4	7		4	3	4	3
	%	85.7	14.2	57.1	42.8	28.6	71.4	28.6	71.4	42.9	57	42.9	57	100		57.1	42.8	57.1	42.8
Escherichia coli	12 NO	4	8	12		4	8	2	10	9	3	4	8	12		4	8	5	7
	%	33.4	66.6	100		33.4	66.6	16.7	83.3	75	25	33.4	66.6	100		33.3	66.7	41.7	58.3
Klebsiella	62 NO	8	54	17	45	11	51	7	55			6	56	34	28	8	54	10	52
	%	12.9	87	27.4	27.5	17.7	82.3	11.3	88.7			9,97	90.3	54.8	45	12.9	87	16.1	83.9
Pseudomonas	75 NO	46	29	43	32					52	23	54	21			61	14		
	%	61.3	38.6	57.3	42.6					69.3	30.6	72	28			81.3	18.6		
Providencia	NO	10		8	2			5	5	6	4	9	1			3	6	4	6
	10 %	100		80	20			50	50	60	40	90	10			30	60	40	60
Proteus mirabilis	21 NO	8	13	19	2			12	9	19	2	19	2			9	12	6	15
	%	38.1	61.9	90.5	9.5			57.1	42.8	90.5	9.5	90.5	9.5			42.9	57.1	28.6	71.4

Organism		LVX		CM		E		VA		LNZ		MYF		TGC		OX		SXT	
Staph. Aureus	21 NO	17	4	18	3	18	3	18	3	20	1	15	6	21		17	4	19	2
	%	80.9	19	85.7	14.2	85.7	14.2	85.7	14.2	95.2	4.7	71.4	28.5	100		80.9	19	90.4	9.5

No.=Number of isolates , AMK=Amikacin , AMC=Amoxicillin/Clavulanic Acid , AMP=Ampicillin , SAM=Ampicillin/Sulbactam , FEP=Cefepime , FOX=Cefoxitin , CAZ=Ceftazidime , CEP=Cefalotin ,

CIP=Ciprofloxacin GEN=Gentamicin , IPM=Imipenem , LVX=Levofloxacin , MEM=Meropenem ,   MNO=Minocycline ,ATM=Aztreonam,TZP=Piperacillin/Tazobactam ,

TCC=Ticarcillin/Clavulanic Acid ,   TGC=Tigecycline , TOB=Tobramycin , SXT=Trimethoprim/Sulfamethoxazole ,   E=Erythromycin. LNZ=Linezolid

,MXF=Moxifloxacin , VA=Vancomycin , OXA=Oxacillin , QDA=Quinupristin/Dalfopristin ,TE=Tetracycl

CHAPTER FIVE

DISUSSION, CONCLUSION AND RECOMMENDATION

CHAPTER FIVE

DISUSSION, CONCLUSION

AND RECOMMENDATION

5.1 DISCUSSION

Lower respiratory tract infections (LRTI) are the most frequent infections acquired on intensive care units (ICU) (Bonten, 2011),

A total of 105 patient in ICU, out of 105 specimens 93(88%) tracheal aspirate, 8 (7.6%) bronchial lavage, 4(3.8%) sputum, 99 (94.3%) were cultured positive, whereas 6 (5.7%) specimens showed no growth. It is similar to study conduct by Trupti B, *et al.,* (2013) in tertiary care center from Central India. LRT specimens of 230 patients who were admitted to NICU were evaluated. Out of 230 LRT specimens (27 (11.7%) sputum, 75 (32.6%) suction tip, 89 (38%) tracheal, 32 (13.9%) ,bronchial 7 (3.1%) ,198 (86.08%) were cultured positive, whereas 32 (13.91%) specimens showed no growth. They were found high frequency of tracheal aspirate specimen and high proportion of positive cultured among ICU patient.   .

In this study High percentage of lower respiratory tract infection was in pneumonia 62(59%). It is similar to study conducted by Muhammad I *et al*., 2016 in Shifa International Hospital Islamabad ¨They were found high percentage of pneumonia among ICU patients˙ Out of 164 ICU patients, 88 (53.6%) patients diagnosed as pneumonia

In this study, male patients were 82 (78.1%) and female patients were 23 (21.9%). High percentage of growth in male patients 76 (72.4%) and in female patients 23

(21.9%). Similar finding were reported by Syed .A, *et al* .2013 in Medical College Hospital in North Kerala, India. Among the 1750 respiratory samples, 298(17.03%) were found to be positive for bacterial isolates. 227 samples (76.17%) from among males and 71 samples (23.83%) from among females were cultured positive.

In this study, high frequency of *Pseudomonas aeruginosa* 67 (72%) and *Acinetobacter baumannii* 60 (64.5%) in tracheal aspirate specimen, high frequency of *Klebsiella pneumoniae* 7 (87.5%) in bronchial lavage specimen and high frequency of *Staphylococcus aureus* 3 (75%) in sputum samples. The predominant isolates of LRTIs infections were *P.aeruginosa* 75 (71.4%) then *A.baumannii* 69 (65.7%) ,*K.peuomonia* 62 (59%) and *E.coli* 12 (11.4%)

Different finding were observed by Shymaa A *et al*., (2016) in Menoufia Governorate, Egypt. Among the 763 LRTIs suspected patients (503 males and 260 females) 256 cases from ICUs patients. Prevalence of organisms which isolated from ICU patient were *Klebsiella* spp. (38.4%), *E.coli*,(32.5%) *Pseudomonas* spp.(60%) , *Acinetobacter* spp. (70.8%). The most common organism isolated from sputum was *Klebsiella* spp. (60.6%), from TTA was *Acinetobacter* spp. and *Citrobacter* (50%),from BAL was *Citrobacter.* (25%).This difference may be due to hospital environment, sample size, low value of hygiene, antibiotic used, technique which used to collected samples.

In this study, A single pathogen was demonstrated in 32 (32.32%) patients and 67 (66.76%) had mixed bacterial isolate of 105 patients. On other study conducted by Trupti, *et al*., (2013) they disagreed with my study who found single pathogen was in 150 (75.75%) patients and 48 (24.24%) had mixed bacterial etiology out of 230 patients .This variation may be due to multidrug resistance organisms, technique that was used to collected samples, patients with other infections beside lower respiratory tract infection.

In this study high proportion of *Pseudomonas aeruginosa* 49 (79%) in pneumonia patients. This finding was disagreed with Lamya H, *et al.,*(2013) in Islamic Bank Center Hospital, Dhaka Bangladesh.They disagreed with my study,100 pneumonia ICU patients were enrolled. Among them 60 were male and 40 were female. Among of them the most common pathogen was *Acinetobacter baumannii* (48%) *Pseudomonas aeruginosa* (21%). Thise variation may be due to antibiotic witch used , patient has other disease.

In this study high proportion was *Pseudomonas aeruginosa* 17 (73.9%) in females patients whereas in males patients predominant was *Acinetobacter baumannii* 61(74.3%) .Different finding were observe by Tuhina *et al.*, (2018) in Intensive Care Unit of a Tertiary Care Hospital, Varanasi, India, among a total of 2984 samples 993 samples positive growth and were identified as pathogens. Male patients predominated over female counterparts in the ratio 1.7:1. *Acinetobacter* spp. (42.9%) were the most common organism isolated from meal patients followed by *Klebsiella* spp. (15.10%), from female patients. The variation of result of female patient may be due to technique witch use to collected this sample, hospital environment. This variation may be due to antibiotic used ,hospital environment.

5.2 CONCLUSION:

From the previous results, we can conclude that Lower respiratory tract infection in ICU patient is among the most common bacterial infection in National Hospital Themostpredominantorganismswere *Klebseilla pneumoniae , Pseudomonas aeruginosa* and *Acinetobacter* spp, which are known as nosocomial infection and multidrug resistance organisms. Tobramicin, Gentamicin and Cefepime represent higher sensitivity for Gram- Gram-negative bacilli. About 55% of isolation sensitive to Gentamicin. highly proportion of antimicronial resistance was found in *klebsiella pneumonia*. For *Staph. aureus*, it was highly susceptible to Tigecyclin 100%, Gentamicin 95.2%.

5.3 RECOMMENDATIONS:

 Future research efforts should also aim to improve our ability to diagnose and exclude infection in the ICU setting to avoid administering unnecessary antibiotics to patients without true pulmonary infection. More studies with larger sample size are needed to verify this result. Nosocomial LRTI remain a problem mainly on ICUs. Patients at risk should be monitored with extra care. Hospital management should therefore implement control measurements to keep the incidence of ICU-acquired LRTI as low as possible. Use of advanced techniques like PCR to verify these results.

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APPENDICES

Appendex 1

Questionnaire

Name

Sex

Patient under mechanical ventilator   yes ☐   No ☐

Patient had NG tube   ☐   ☐

Specimen type

☐ trachealaspi.   ☐ sputum   ☐ bronchial

Result
Isolated organism
Susceptibility testing
Sensitive to
Resist to

Appendex 2

Figure 3.1 VITEK 2 Compact Instrument and Workstation
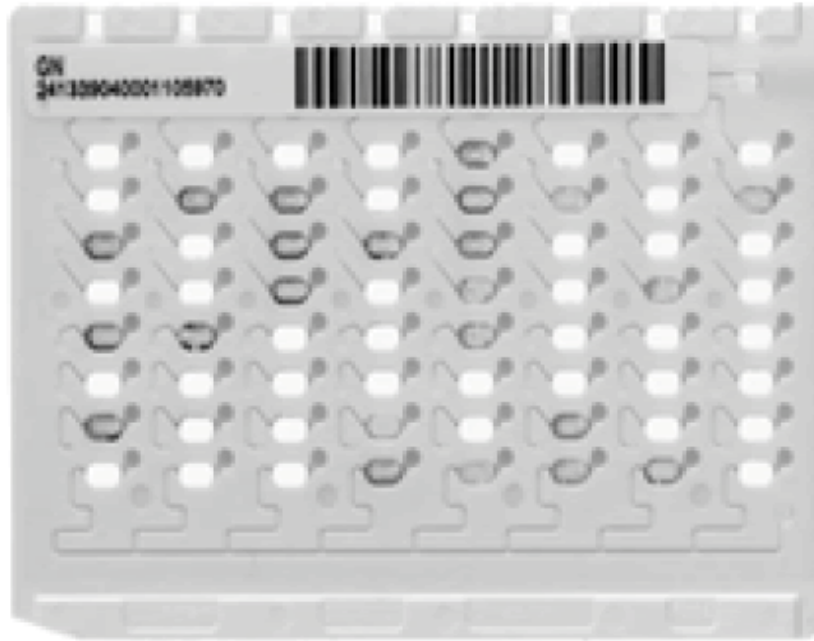
Figure 2: VITEK 2 GN colorimetric identification card.

Figure 3.2 colorimetric identification card