NEAR EAST UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
DEPARTMENT OF MEDICAL MICROBIOLOGY
AND CLINICAL MICROBIOLOGY

CEFTAZIDIME RESISTANCE TO PSEUDOMONAS AND KLEBSIELLA STRAINS ISOLATED FROM CLINICAL SAMPLES

M.Sc. THESIS

AFAYI PRINSLEY NDIKAKA

Nicosia

February, 2023

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Supervisor:
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APPROVAL

After careful scrutiny of the thesis titled: **CEFTAZIDIME RESISTANCE TO PSEUDOMONAS AND KLEBSIELLA STRAINS ISOLATED FROM CLINICAL SAMPLES** submitted by **AFAYI PRINSLEY NDIKAKA**. It has met the unanimous consensus and in our combined opinion, it is fully adequate, in scope and in quality, as a thesis for the degree of Master Educational Sciences, and hereby recommended for approval and acceptance.

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DECLARATION

I, Afayi Prinsley Ndikaka, registration n◦: 20206677, in the department of medical microbiology and clinical microbiology, faculty of medicine near east university, hereby declare that, this work titled “ceftazidime resistance to Pseudomonas And Klebsiella Strains Isolated From Clinical Samples” is my original work. it has not been presented in any application for a degree or any academic pursuit. i have acknowledged all borrowed ideas nationally and internationally through citations.

AFAYI PRINSLEY NDIKAKA

08/02/2023

CERTIFICATION

This is to certify that this dissertation titled **“CEFATAZIDIME RESISTANCE TO SPEUDOMONAS AND KLEBSIELLA STRAINS ISOLATED FROM CLINICAL SAMPLES”,** is the original work of **AFAYI PRINSLEY NDIKAKA**. This work is submitted in partial fulfillment of the requirements for the award of a Master of Science Degree in MEDICAL MICROBIOLOGY AND CLINICAL MICROBIOLOGY in the FACULTY of MEDICIN, NEAR EAST UNIVERSITY.

Prof Dr NEDIM CAKIR

(Supervisor)

DEDICATION

The book is dedicated to my entire family and friends who have been by my side throughout my study.

AFAYI NDIKAKA PRINSLEY

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For his ongoing assistance, direction, and inspiration, I would like to acknowledge and convey my sincere gratitude to my thesis supervisor, Prof. Dr. Nedim Cakir (Chairman of the Department of Medical Microbiology and Clinical Microbiology/Near East University). I value all of his time, assistance, and suggestions. He continuously allowed me to write this paper on my own, but he gave me advice when he believed I needed it.

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AFAYI NDIKAKA PRINSLEY

ABSTRACT

Ceftazidime Resistance to Pseudomonas and Klebsiella Strains Isolated From Clinical samples

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OBJECTIVE: Infections caused by resistant gram-negative bacteria to antimicrobials occur at increasing rates. Therefore, routine screening of resistance patterns is crucial for treatment approaches using proper antibiotics. Nevertheless, there is not enough data with respect to antibiotic resistance profiles in North Cyprus. This study was conducted in order to investigate the resistance rates of *Pseudomonas aeruginosa,* and *Klebsiella pneumoniae* to ceftazidime from samples which were isolated from the Near East University (NEU) Hospital, North Cyprus.

METHOD: It was included in this study *P aeruginosa* and *K pneumoniae* which were isolated in the NEU Hospital Clinical Microbiology Laboratory between 2017 and 2022. Identification and susceptibility tests were performed by using the BD Phoenix 100 system (software version 6.01A). The antimicrobial susceptibility test results were determined according to the Clinical and Laboratory Standards Institute (CLSI) guidelines, and the resistance rates of bacterial isolates to antibiotics were examined retrospectively.

RESULTS AND CONCLUSION: In our study, we found ceftazidime resistance of *P aeruginosa* strains to be 24%. This ratio was higher for other cephalosporins (24% vs. 4%) than for Cefepime. In addition, resistance to ceftazidime was also much higher than piperacillin/tazobactam (24% vs 5%). In our study, imipenem resistance and ceftazidime resistance were very close to each other in Pseudomonas strains (24% vs 25%).

Klebsiella pneumonia strains showed the second highest resistance to ceftazidime (28%) among cephalosporins. However, ceftazidime were found to be more effective antibiotics than penicillin and ampicillin groups. We found that the most effective antibiotics against Klebsiella strains was piperacillin/tazobactam with 11% resistance rate.

Key word: pseudomonas,klebsıella, ceftazıdıme, resıstance

Özet

Klinik örneklerden izole edilen Pseudomonas ve Klebsiella izolatlarının seftazidime dirençleri

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Klinik Mikrobiyoloji Anabilim Dalı Yüksek Lisans öğrencisi

Şubat, 2023 sayfa, 60

Amaç: Antimikrobiyallere karşı dirençli gram negatif bakteriler ile oluşan enfeksiyonlar kliniklerde artan oranlarda sorun yaratmaktadır. Bu nedenle, uygun antibiyotikleri kullanarak tedavi yaklaşımları için rutin direnç kalıplarının bilinmesi gerekmektedir. Bununla birlikte, Kuzey Kıbrıs'ın Yakındoğu Üniversitesi (NEU) Hastanesi'nde izole edilen örneklerden *Pseudomonas aeruginosa* ve *Klebsiella pneumonia'*nın ceftazidime karşı direnç oranlarına ilişkin yeterli veri bulunmamaktadır. Bu çalışma, Kuzey Kıbrıs'ın NEU Hastanesi'nden izole edilen örneklerden *Pseudomonas aeruginosa* ve *Klebsiella pneumonia'*nın ceftazidime karşı direnç oranlarını araştırmak amacıyla yapılmıştır.

YÖNTEM: Bu çalışma, 2017 ile 2022 yılları arasında NEU Hastane Klinik Mikrobiyoloji Laboratuvarı'nda izole edilen *P. aeruginosa* ve *K. pneumoniae'yi* içermektedir. Mikrobiyal tanı ve duyarlılık testleri, Çalışmada retrospektif laboratuvar verileri kullanılmıştır. Belirtilen yıllar arasında tanı amacıyla BD Phoenix 100 sistemi (yazılım sürümü 6.01A) kullanılmıştır. Antimikrobiyal duyarlılık testi sonuçları, Klinik ve Laboratuvar Standartları Enstitüsü (CLSI) kılavuzlarına göre belirlenmiş ve antibiyotiklere karşı bakteri izolatlarının direnç oranları retrospektif olarak incelenmiştir.

SONUÇLAR: Çalışmamızda P aeruginosa suşlarının seftazidim dirençlerini %24 olarak saptadık. Bu oran diğer sefalosporinler içinde cephepimden daha yüksekti (24% vs 4%). Ek olarak seftazidime direnç piperasilin/tazobactamdan da çok daha yüksekti (24% vs 5%). Çalışmamızda imipenem direnci ile seftazidim direnci Pseudomonaslarda birbirine çok yakındı (24% vs 25%) Çalışmamızda P aeruginosaya en etkin antibiyotikler olarak piperacillin, meropenem ve cephepimi bulduk (%5,9 ve %4 respectively)

Klebsiella pneumonia suşları seflosporinler içinde en yüksek ikinci direnci ceftazidime gösterdiler. Ancak ceftazidim penisilin ve ampicillinden daha etkin antibiyotikler olarak saptandı. Sonuçlarımıza gore piperasilin tazobactam Klebsiella suşlarına karşı %11lik direnç ile en etkili antibiyotik olarak saptandı.

Anahtar kelime: pseudomonas,klebsıella, seftazıdıme, direnç

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LIST OF ABBREVIATIONS AND ACRONYMS

%	Percentage
R	Resistant
S	Sensitive
n	Total number of samples
EU	European Union
ECDC	European Centre for Disease Prevention and Control
HAIs	healthcare associated infections
EARS-Net	European Antimicrobial Resistance Surveillance Network
NEU	Near East University
ICU	Intensive Care Unit
TRNC	Turkey Republic of Northern Cyprus
EEA	European Economic Area
ESBL	Extended-spectrum B-lactamase
LPS	lipopolysaccharides
USA	United State of America
CRE	Carbapenem-Resistant Enterobacteriaceae
CDC	Centers for Disease Control and Prevention
PICU	Pediatric Intensive Care Unit
MDT	Multi-Drug Resistant
APACHE II	Acute Physiology and Chronic Health Evaluation II

CRP	C-Reactive Protein
IR	Imipenem-Resistant
KPC	*K pneumoniae* Carbapenemase
PDR	Pandrug-Resistant
BLI	B-Lactamase Inhibitors
EDTA	Ethylenediaminetetraacetic acid
PBPs	Penicillin-Binding Proteins
MHT	Modified Hodge Test
OPD	Outpatient Department
SPSS	Statistical Package for the Social Sciences
WHO	World Health Organization

CHAPTER ONE
INTRODUCTION

1.1 BACK GROUND OF STUDY

The rapid spread of antibiotic-resistant Gram-negative infections is a global issue that is having an increasing detrimental impact on health and the economy (Gandra et al., 2014). A recent study by the European Centre for Disease Prevention and Control (ECDC) showed that every year, infections brought on by bacteria resistant to antibiotics cause over 32,000 people to die. (Cassini et al., 2019). The majority of these multidrug-resistant infections are caused by healthcare associated infections (HAIs), and When drugs of last resort, such as carbapenems and colistin, become ineffective, the options for treatment decline (Potron et al., 2019).

According information obtained from the European Antimicrobial Resistance Surveillance Network (EARS-Net) from 2015, Greece has a high incidence of antibiotic-resistant bacteria infections among EU and EEA nations (Cassini et al., 2015). Infections with bacteria that were resistant to carbapenem or colistin accounted for the majority of this burden in 2015 (Albiger et al. 2015).

The Hellenic Center for Disease Control and Prevention (HCDCP) outline an average of 0.48 cases per 1000 patient-days and a crude 28-day death rate as part of Greece's nationwide effort to combat carbapenem-resistant Gram-negative bacteria in acute-care hospitals (Maltezou et al., 2014). According to the 2017 EARS-Net report, Pseudomonas aeruginosa (*P aeruginosa)* has a resistance rate of over 30% to at least three antimicrobial groups, including piperacillin/tazobactam, ceftazidime, fluoroquinolones, aminoglycosides, and carbapenems. Furthermore, for *Klebsiella pneumonia* (*K pneumoniae),* Greece is said to have the greatest rate due to drug resistance against carbapenems and fluoroquinolones (EARS-Net, 2017). The Enterobacteriaceae member *K pneumoniae* causes soft tissue infections, pneumonia, urinary tract infections, and septicemia that affect hospitalized and immune-compromised patients (Eftekhar et al., 2015). Large, multi-resistance plasmids, some of which may have devolved in various methods within the same institution, are frequently used to encode resistance (Bradford et al., 1994). Numerous strains of *K pneumoniae* and some Enterobacteriaceae are implicated in these epidemics, according to the result of the plasmid analysis of the isolates that caused them (Rice et al., 1990).

Uncertainty exists over the best course of treatment for ceftazidime-resistant *K pneumoniae* infections. The common occurrence of these resistance-encoding genes on multi-resistance plasmids severely restricts therapeutic options in many situations (Jacoby et al., 1991). Cefotaxime, Ceftizoxime, or Ceftriaxone are examples of other extended-spectrum cephalosporins that may show activity in vitro but lose effectiveness in vivo, most likely due to a substantial inoculum impact (Rice et al., 1990).

P aeruginosa is a bacillus bacterium that is Gram-negative and does not produce spores. It is present everywhere in nature, including in soil, water, and various kinds of flora (Nadeem et al. 2019). There are over 140 species in the *P aeruginosa* genus; only a small number of these diseases are infectious to humans, whereas the majority are mostly saprophytic and widespread in nature (Adedeji et al. 2007). Opportunistic pathogen *P aeruginosa* is linked to a number of nosocomial illnesses. *P aeruginosa* strains commonly cause pneumonia, urinary tract infections, skin infections, and soft tissue infections in bedridden individuals (Giamarellou, 2002). Due to its ability to develop resistance by acquiring or producing foreign resistance genes against multiple classes of antibiotics, *P aeruginosa* is generally inherently resistant to many antimicrobial agents (Mahesh *et al*. 2017).

A prominent cause of nosocomial and community-acquired diseases such bacteremia, pneumonia, and urinary tract infections is *P aeruginosa*. 2019 (Nadeem et al.). Numerous variables, such as the increasing prevalence of invasive operations, immunocompromised patients, and antimicrobial use, which has contributed to the rise of antibiotic-resistant organisms to enhanced relationship between this ubiquitous organism with disease. Patients in surgery wards, oncology division burn units, and critical care units frequently develop multi-resistant isolates that have a severe morbidity and fatality rate (Giamarellos-Bourboulis *et al.,* 2006). The existence of various innate and obtained elements of antimicrobial resistance makes it challenging to manage the outspread of these pathogens in hospital settings (Pawel *et al*. 2008). *P aeruginosa* is ranked as the fourth most prevalent nosocomial bacteria in hospitals, accounting for 10% of all disorders associated to healthcare (HCAIs). It can range from causing minor skin infections to leading to fatal sepsis, especially when these strains colonize vulnerable areas. (Amani *et al*. 2017).

Determine the risk factors, identify the resistant isolates, and implement preventative measures to limit infections brought on through antibiotic resistant Gram-negative bacteria. The selection of initial antibiotic treatment for antibiotic-resistant Gram-negative infections can be informed by the local patterns of antibiotic resistance (Cassini et al., 2019). Antimicrobial drug resistance has been extensively written about in the literature. *P aeruginosa* and *K pneumonia* resistance trends that occur North Cyprus are still unknown, nevertheless. The Near East University (N.E.U) Hospital in North Cyprus reported the presence of *P aeruginosa* and *K pneumoniae* isolates, and thus, these isolates were incorporated into this investigation. This led to the collection of information on the antibiotic resistance patterns of these important bacteria isolated from the Near East University Hospital for the purpose of this study.

1.2 STATEMENT OF PROBLEM

There are many different bacteria that cause illnesses in humans and lead to antimicrobial resistance when treatments are tried to treat them. Part of the most virulent bacteria linked to intensive care unit (ICU) acquired infections are still *P aeruginosa* and *k pneumonia*, which makes up about 11% to 29% of ICU isolates (Diekema et al., 1999). Examples of this bacterial include *P aeruginosa* and *K pneumoniae* to drugs such as ceftazidime. This is a huge concern to the health system and general public. The current cumulative resistance surveillance statistics, which are primarily drawn from ICU patients, are concerning. The top priority for healthcare providers in treating *P aeruginosa* and *K pneumoniae* infections is to effectively manage them and prevent the development of antibiotic resistance. In clinical applications mainly ceftazidime maybe the first drug of choice for empiric therapy. This occurs especially in ICU clinics.

In Tunisian hospitals, there is limited understanding of control strategies for antibiotic resistance, and individual efforts are being taken, especially for gram-negative bacteria. This study aimed to examine the impact of reducing the use of ceftazidime on gram-negative bacterial resistance, with a focus on *P aeruginosa*. Given the widespread use of ceftazidime, the study decided to investigate its restriction, which was being implemented for the first time in their ICU.

1.3 JUSTIFICATION

Several studies have proven that ceftazidime is the drug of drug of preference for *P aeruginosa* and *K pneumoniae* infections. Other studies have also shown that continuous use of ceftazidime in therapy, has resulted in antimicrobial resistance to this drug (of choice) In Turkey Republic of Northern Cyprus (TRNC). The aim of this study is to determine the incidence of *P aeruginosa* and *K pneumoniae* infections that are resistant to ceftazidime and to identify the associated risk factors in the Turkish Republic of Northern Cyprus (TRNC).

1.4 AIM OF THE STUDY

The purpose of this study was to investigate the incidence and identify specific risk factors related to drug resistance, as well as the prevalence of ceftazidime resistance in *P aeruginosa* and *K pneumoniae* isolates from clinical samples the Near East University.

1. Evaluate the prevalence of *K pneumoniae* and *P aeruginosa* ceftazidime other antibiotics.
2. Evaluate the Rick factors associated with development of antibiotics resistance.

1.5 INTENDED OUTCOME OF THESIS/SIGNIFICANCE

A shortage of knowledge exists regarding the most effective strategies for preventing outbreaks of colonization and infection caused by these strains. Restrictions on usage of ceftazidime seems to have helped one outbreak in a chronic care facility. This study will also provide information on risk factors of acquiring antimicrobial resistance.

1.6 LIMITATION OF STUDY

This research work is limited to the laboratory investigation no direct contact with patients to have an idea of their daily habits.

1.7 SCOPE OF STUDY

The study was carried out on isolates gotten from Near East University Teaching Hospital, aimed at investigating the prevalence of *P aeruginosa* and *K pneumoniae* resistance.

1.8 AREA OF STUDY

This research work was carried out in the microbiological laboratory of Near East University TRNC.

CHAPTER TWO

LITERATURE REVIEW

2.1 GENERAL INFORMATION

Worldwide, there have been alarming increases of incidence regarding harmful microorganisms which are resistant to various antibiotic treatments. Analysis of common subtypes (strains) of gram-negative bacteria that produce extended spectrum, namely *K pneumoniae* and *P aeruginosa*. B-lactamases are more frequently found in medical facilities all around the world (Jacoby et al., 1991). Intensive care unit therapy, longer hospitalization, prior antimicrobial treatment, and increasing general use of ceftazidime in hospital facilities are risk factors for the formation of these strains (Meyer et al., 1993).

The main determinants of the prevalence of resistant bacteria, antimicrobial selective pressures, fluctuate greatly throughout time and space. Monitoring resistance is therefore essential for empirically choosing the best antimicrobial medicines to treat infected patients. The best method for identifying minor changes in resistance is thought to be tracking temporal patterns of resistance (Morris et al., 2002). Tracking and monitoring for antibiotic resistant bacterial is also needed for gauging at the severity regarding issues in order to decide whether or not to take action (NNIS, 1999). Programs like Alexander, PROTEKT, SENTRY, and others (Van Beneden et al., 2003) are effective examples of research that's been done globally by gathering strains and having a reference laboratory test them. In an instance, it was thought that the Alexander project had given researchers a way to track changes in respiratory infections' susceptibility patterns on a global, regional, and national scale (Felmingham et al., 2005). The most accurate information may be obtained from these kinds of applications. They are, nevertheless, exceedingly expensive.

Analyzing data from routine susceptibility tests performed in hospitals may be constrained by inherent factors because of different methodology and interpretations, but it doesn't require a lot of additional resources (Cornaglia et al., 1997). In 1997, the KONSAR initiative was launched in Korea (Chong et al., 1997) based on a suggestion from the World Health Organization (WHO). The examination data from hospital-conducted tests and evaluating strains by the coordinating laboratory are two surveillance techniques that have been deployed. Analysis of the 2003 test results (lee et al., 2006)

showed a continued rise in vancomycin resistant to *Enterococcus faecium*, fluoroquinolone resistant to *K pneumoniae*, and imipenem-resistant Acinetobacter spp.

It is necessary to separate these two groups in the analysis because resistant rate of bacteria gained due to hospital environment (nosocomial infection) are typically greater than those of bacteria obtained in the community. However, it can sometimes be challenging to divide them apart effectively. Data were previously solely collected from hospitals for the KONSAR program, however, in 2003, information was also gathered from a private laboratory that analyzed a large number of samples primarily from primary care clinics across Korea (lee et. al., 2006).

Most significant among the strains of the gram-negative bacterium Klebsiella, along with the natural environment, it is frequently present in the mouth, skin, and gut microbiota is *K pneumoniae*. *K pneumoniae* is a bacterial, in people who are already weak (opportunistic pathogen), can result to nosocomial infections, such as sepsis, pneumonia, urinary tract infections, and soft tissue infections, are a major concern in healthcare and can have serious consequences. These infections can occur in patients who are receiving medical treatment in a hospital setting and can lead to severe illness and even death. They are considered serious because they are mostly a consequence of an antibiotic-resistant bacteria and Present challenges in treatment. (Guh et al., 2015). Extended-spectrum B-lactamase is one of the drug-resistance indicators that this particular bacterial is widely known for being able to amass and disseminate (ESBL). It is particularly challenging to treat severe illnesses generated by ESBL-producing *K pneumoniae* since these organisms are becoming more and more resistant to a variety of medicines and may be brought on by extremely complicated mechanisms (Harris et al., 1999). The pathogenicity of *K pneumoniae* is because a number of factors, including biofilms, adhesins, urease, LPS, and capsule. Despite testing, there are currently no effective vaccines in use (ECDC. 2017). This pathogen commonly selects resistance during anti pseudomonas therapy among initially susceptible isolates, leading to the establishment of resistance to several medications (Harris et al., 1999).

The one and most frequently observed organisms linked to hospital-acquired illnesses, especially in clients who are immunocompromised, is *P aeruginosa*, which is notorious for its adaptability and potential to develop resistance mechanisms to antibiotics. The most

effective alternative to treat actual infections brought on by these pathogens is beta-lactam antimicrobials. However, in patient isolates of *P aeruginosa* from South America, the formation of lactamases, such as cephalosporinases and carbapenemases, has received much attention and is the most potent mode of beta-lactams resistance described among Gram negative organisms globally Clarifying the elements present in atypical and/or ineffectively recognized phenotypes is essential given the significance of carbapenems for the remedy of infections brought on by *P aeruginosa*. Information about these processes raises the alarm for a change in the way antibacterial and sedative resistance are used, which has an impact on the treatment of illnesses brought on by these pathogens that are commonly treated with just polymyxins.

Community-acquired and hospital acquired disorders resulting from *P aeruginosa* are bacteremia and urinary tract infections. Patients with impaired immune systems, such as those with neutropenia or cancer patients, may require specific attention to prevent infections. These days, the prevalence of morbidity and death has increased due to strains of *P aeruginosa* that are antibiotic resistant (Nadeem *et al*., 2019). Numerous variables, such as the increasing prevalence of invasive operations, immunocompromised patients, and antimicrobial use, that helped with identification and emergence of resistant organisms, has contributed to enhance the relationship of this ubiquitous organism with disease. Patients in surgery wards, oncology burn units, and critical care units frequently display multi-resistant isolates, which include substantial morbidity and mortality. Due to the abundance of natural and acquired resistance factors resulting in antibiotic resistance, it is often challenging to contain the spread of this organism in healthcare environments. (Pawel *et al*., 2008). The fourth most frequent nosocomial pathogen, *P aeruginosa* is responsible for 10% of all diseases associated with healthcare (HCAIs). From minor cutaneous infections to fulminant sepsis, it can range in severity; indeed, the colonization of such strains in the fundamental framework might be fata (Amani *et al*., 2017).
According to a report from the European Antimicrobial Surveillance Network (EAS-Net) by 2015, the mean resistance levels of invasive *P aeruginosa* isolates to fluoroquinolones, piperacillin-tazobactam and carbapenems were almost 20%, while resistance to ceftazidime and aminoglycosides was around 13%. Ceftazidime and carbapenem resistance remained stable between 2011 and 2015, however piperacillin-

tazobactam resistance was shown to be growing there. All things considered, significant differences in resistance rates were seen among the various European nations, with the southern and eastern nations exhibiting higher resistance rates than the northern nations. A multicenter study revealed higher levels of resistance for piperacillin-tazobactam, ceftazidime, fluoroquinolones, and aminoglycosides using *P aeruginosa* isolates isolated from circulatory system infections from Spanish hospitals. Finally, carbapenem resistant bacterial was comparable to that described by EARS-Net (Ruiz-Garbajosa *et al*., 2017).

2.1 HISTORY OF PSEUDOMONAS AERUGINOSA AND KLEBSIELLA PNEUMONIAE

Carle Gessard, a French chemist and bacteriologist, made the initial discovery of *P aeruginosa* in 1882. In his experiment, Gessard identified the microorganism through analyzing how soluble it's pigments are in water, which displayed a blue green color under ultraviolet light. His study, entitled "On the Blue and Green Coloration that Appears on Bandages," was centered on this research. Carle Gessard followed up his experiment's results by giving the strand the scientific name *P aeruginosa*, identifying its pigment derivative, and formulating a Hypothesis that explained the harmful character and the contagious similarities it has with other organisms (Chen, Selena, 2018).

The first description of *K pneumoniae* was published in 1882 by Carl Friedlander. He concluded that the bacterium was an encapsulated bacillus after separating it from the lungs of patients who had died from pneumonia. The bacterium was initially referred to as Friedlander's bacillus and was later renamed Klebsiella in 1886 (Jondle et al., 2018).

2.1.1 General Microbiological Characteristics of *P aeruginosa* and *K pneumoniae*

P aeruginosa organisms flourish in wet areas for example water and soil. It is prevalent on vegetable and fresh fruits. Human colonization starts with the digestive system and later extends to moist skin region which include axilla and perineum. It grows in smooth, fluorescent green colonies at 42 °C and has a distinctive sweet (grape-like) smell that makes *P aeruginosa* simple to identify when cultured on solid media within the microbiology laboratory. Pseudomonas require little dietary needs as a whole. *P aeruginosa* frequently simply requires acetate and ammonia as means through which it

can obtain carbon and nitrogen, correspondingly; some are also able exploit a large range of ambient substances to get sustenance. Additionally, *P aeruginosa* has the ability to grow anaerobically and doesn't need fermentation; instead, it gets its energy from the oxidation of carbohydrates. Its ability to grow in unfavorable conditions is due to its adaptable nutritional needs. These microorganisms are challenging to get rid of from polluted locations, including theatres, hospital rooms, clinics, and medical equipment (Engleberg *et al*., 2007)

K pneumoniae, is a type of bacterium that falls under the Enterobacteriaceae family. This bacterium is gram-negative, meaning it does not hold the crystal violet dye used in the gram-staining procedure, which has a protective outer membrane or envelope. Additionally, it does not have the ability to move on its own. Its pathogenicity, or ability to cause disease, is influenced by various factors, including its ability to cause infection and resistance to drugs. The most important contributor to its virulence is its polysaccharide capsule, that shields it from the host's immune system and prevents it from being destroyed by the host's own defense mechanisms. Currently, there are 77 different types of Klebsiella that have been identified, and those without capsules are generally less harmful. *K pneumoniae* possess another virulence factor in the form of lipopolysaccharides (LPS) on their outer membrane. These LPS trigger an inflammatory response in the host organism, which can lead to severe complications such as sepsis and septic shock. Additionally, these bacteria have appendages called fimbriae that enable them to adhere to host cells, which aid in their ability to infect the host. Another virulence factor known as siderophores which are used by the organism to acquire iron from the host, which is necessary for the organism to infect and spread within the host. (Rønning et al., 2019).

K pneumoniae is a bacteria known for its high resistance rate to antibiotic, which is due to genetic modifications within the organism. Alexander Fleming first discovered that gram-negative bacteria, including, were resistant to beta-lactam medications. Further research revealed that *K pneumoniae* produces a beta-lactamase enzyme that breaks down the beta-lactam ring in drugs, rendering them ineffective. The emergence of extended-spectrum beta-lactamase (ESBL) producing was first reported in Europe in 1983 thereafter in USA in 1989. This type of resistance makes even third-generation cephalosporins

useless for treatment, leading to the use of carbapenems as an alternative. However, *K pneumoniae* is responsible for a significant proportion of carbapenem-resistant infections, with over 80% of the 9000 reported cases caused by carbapenem-resistant Enterobacteriaceae (CRE) being attributed to *K pneumoniae* in 2013, according to the Centers for Disease Control and Prevention (CDC). This resistance is linked to the increased production of ESBL enzymes, changes in the outer membrane and the upregulation of efflux pumps in the organism.

2.2 EPIDEMIOLOGY

Towards the end of the 19th century, following Louis Pasteur's achievement in developing sterile culture techniques, the distinct bacterial species *P aeruginosa* was first documented. The first research done on *P aeruginosa*, "On the Blue and Green Color of Bandages," was written by pharmacist Carle Gessard in the year 1882. This study was based on the unusual blue and green color of the bacteria, which was later found to be caused by a phenazine derivative called pyocyanine. This color is reflected in the ancient names such as *Bacillus pyocyaneus*, *P polycolor*, *Bakterium aeruginosa*, and *P pyocyaneus. P aeruginosa* was recognized as a pathogen by 1889, but its pathogenicity was initially questioned, and it was mostly considered as a source of potent antibiotic chemicals.

Prior to 1947, there were only 91 cases of *P aeruginosa*-related septicemia reported in the literature. Prior to that time, the bacterium was probably present in both the inanimate and animate surroundings, but it wasn't until the latter half of the 20th century that its relevance to be an infection to humans, particularly in hospitalized patients, became clear. Given that *P aeruginosa* is straightforward to culture and diagnose, it seems unusual that clinical microbiologists missed it. Since patient susceptibility has changed and also significance of *P aeruginosa* if being a hospital acquired infection has significantly changed, these developments in the life sciences and medical science are likely to be responsible (Botzenhart & Doring, 1993).

Gessard first isolated *P aeruginosa* from a green pus in 1882. Because of its widespread existence, *P aeruginosa* can play a significant role in many human infections. *P aeruginosa* is a highly adaptable bacterium that primarily inhabits soil environments,

yet it may also endure in water. Because of its variety of food sources, *P aeruginosa* can endure the degradation of toxic waste. A significant plant pathogen that affects lettuce, tomato, and tobacco plants is *P aeruginosa*.

In addition to sinks, showers, respiratory equipment, and fresh water habitats (streams, lakes, and rivers), it can also be found there (Fujitani et al., 2008*). P aeruginosa* is at times ingested by people obtaining through these different means. However, it doesn't adhere to healthy, undamaged epithelial tissue. Because of this, *P aeruginosa* may be present in normal intestinal flora, and in people with strong immune systems, it may not cause infection. (Engleberg *et al*., 2007).

Very recent study which involved 24,178 patients who had hospital-acquire infection, bloodstream illnesses that occurred in USA between 1995 and 2002, *P aeruginosa* was found to be the third most common cause of gram-negative infections, accounting for 4% of cases. The incidence of hospital-acquired infections among children in the pediatric intensive care unit (PICU) was 1.5 per 100 patient-days. The rate of nosocomial infections was highest among cardiac surgery patients, at 2.3 per 100 patient-days. The most common nosocomial infections were bacteremia (51.7%), lung infections (19.0%), and urinary tract infections (17.2%), which were linked to the use of invasive devices. The most frequently isolated organisms were P aeruginosa (24%) and coagulase-negative staphylococci (39%). *P aeruginosa* is linked with a range of infections that affect humans, including acute and chronic lung infections, burn sepsis, and newborn sepsis. This bacterium is a frequent opportunistic pathogen that can infect people who have weak host defenses, including those who have chronic neutropenias, neurtrophil function issues, hematologic cancers, HIV/AIDS, diabetes mellitus, and hematologic cancers. Additionally, patients with cystic fibrosis frequently have chronic lung illness (Fujitani *et al*., 2008).

Humans are primary reservoir to *K pneumoniae*. The organism is present in the feces of 5%–38% of the general population, and in the nasopharynx of 1%–6% of individuals. initially infection reservoirs are the patient's digestive tract and hospital staff members' hands. However, it has been discovered that people with Chinese ancestry and those who battle chronic alcoholism experience higher rates of colonization. Hospitalized patients have a much higher carrier prevalence for *K pneumoniae* than the general

population. One study found that hospitalized individuals had carriers in their stool at levels as high as 77%, and that the quantity of antibiotics given was inversely correlated with these carrier rates (Walter et al., 2018).

K pneumoniae can cause two types of pneumonia: community-acquired and hospital-acquired. While it is a rare infection, it is a common diagnosis for community-acquired pneumonia, accounting for 3-5% of cases in developed countries and up to 15% in less developed countries. Globally, it is linked with11.8% of nosocomial-pneumonia cases. Additionally, causes 8-12% of pneumonia cases in patients using a ventilator and 7% in those without. The mortality rate for people with alcoholism and septicemia caused by *K. pneumoniae* is high, ranging from 50-100%.

2.2.1 Antimicrobial Resistance

Multidrug resistance (MDR) has significantly increased recently and is now acknowledged as a major issue on a global scale (Potron et al., 2015). A number of research studies have looked into what factors may increase the likelihood of developing multi-drug resistant (MDR) strains of a particular microbe. For example, a case-control study done in South America Brazil compared 141 patients with MBL-producing strains to 27 clients without MBL-producing strains (Lucena *et al.*, 2015). The results of the multivariate analysis demonstrated that urinary tract infection and ICU stay were key factors in metallo-beta-lactamase infections. Additionally, strains of metallo-beta-lactamases were linked to an earlier start to the illness and a rapid progression to mortality. In Brazil, a two-year retrospective-research that started in 2010 examined over 50 intensive care unit patients infected by *P aeruginosa* (Matos et al., 2016). A significant proportion (37%) of patients had multidrug-resistant *P aeruginosa,* with 21% of the isolates testing positive for the blaSPM-1-like gene. Notably, MDR was more common in patients who spent a longer time in the hospital (87.1 days on average). Another research conducted in China found that more than half (54%) of patients with *P aeruginosa* infections had MDR strains. Factors that increased the risk of MDR included tracheal intubation (OR 2.21) and the use of carbapenems.

A study found that patients with multidrug-resistant strains of a certain microbe tended to have longer hospital stays and a higher mortality rate (Peng et al., 2014). Another

study, which looked at 63 cases of carbapenem-resistant *P. aeruginosa* bacteremia, found that the Acute Physiology and Chronic Health Evaluation II (APACHE II) score at the time of infection and the microbe's ability to form biofilm were both independently linked to a higher risk of death (Jeong et al., 2014). Additionally, another study discovered the APACHE II score to be a standalone indicator of colonization (DalBen *et al*., 2013).

A study was conducted in a group of immunocompromised individuals where 31 people who were colonized with a highly resistant form of *K pneumonia* were compared to 93 people without it. The study found that having a central venous catheter, a urinary catheter, a high level of a protein called C-reactive protein (CRP), and taking the antibiotic ciprofloxacin were all associated with increased risk of colonization by this microbe. (Willmann et al., 2014). Another study aimed to determine the factors that increase the risk of multidrug-resistant (MDR) *P aeruginosa* infections and the resulting mortality in a group of patients with hospital-acquired pneumonia caused by *P aeruginosa*. The study was conducted retrospectively and included patients from various locations worldwide. (Micek et al., 2015). 226 of the 740 individuals had MDR forms of the infection. Diminishing age, diabetes mellitus, and ICU confirmation were independent indications of MDR. In a study, it was found that multidrug-resistance (MDR) was independently associated with a higher death rate while hospitalized (44.7% compared to 31.7% for non-MDR, p=0.001). An observational study recently compared patients infected with metallo-beta-lactamase-mediated imipenem-resistant (IR) *P. aeruginosa* (Dad) with those who did not have it (Babu et al., 2014).

The researchers discovered that imipenem resistance itself, rather than metallo-beta-lactamase generation, was the most significant indicator of prognosis. The higher death rate seen in the IR-MBL-PA group resulted from factors such as underlying infections, Charlson score, and virulence. A second study, with 324 patients and 676 controls, examined the effect of resistance on health outcomes, mortality, and length of hospital stay (Barrasa-Villar et al., 2017). The study found that patients infected with a resistant pathogen had higher rates of overall mortality and death within 30 days of infection. Both 15.1% of cases and 19.7% of controls had Pseudomonas (the second most common Gram-negative bacteria after E.coli). A comprehensive audit and meta-analysis were conducted on patients with neutropenia to investigate the link between resistance

and death (Righi *et al*., 2017). Second analysis, conducted with 324 patients and 676 controls, evaluated the influence of resistance on health outcomes, mortality, and hospital stay length. (Barrasa-Villar et al., 2017).

The study found that patients infected with a resistant microbe had increased death rates from all causes and within 30 days of infection. Both 15.1% of cases and 19.7% of controls had Pseudomonas (the second most common Gram-negative bacteria after E. coli). An extensive audit and meta-analysis was conducted on patients with neutropenia to examine the connection between resistance and death (Riu *et al*., 2016). The results show that higher resistance is associated with a number of factors that increase the severity of infections, such as APACHE II score, underlying health conditions, intubation, and catheter use, and is also linked to increased mortality (Bassetti et al. 2018). Acquired antibiotic resistance determinants, including the *K pneumoniae* carbapenemase gene (KPC), have been linked to outbreaks in various regions of the world. (Paczosa & Mecsas, 2016). Currently, colistin is widely considered as the final resort against *K pneumoniae* that produces KPC, but reports of colistin-resistant Klebsiella strains are on the rise. (Tzouvelekis *et al*, 2012).

Bacteria display various antibiotic resistance mechanisms such as reduced permeability, efflux systems, antibiotic-inactivating enzymes, and target changes. For the most part some of these known resistance mechanisms can be seen in *P aeruginosa* and *K pneumoniae*, by intrinsic chromosomal or hereditary resistance factors affecting main antibiotic groups: -lactams, aminoglycosides, quinolones, and polymyxins. Penicillin with B-lactamase inhibitors (BLI) such as ticarcillin/piperacillin with clavulanic acid and tazobactam, monobactams (aztreonam), fosfomycin, and polymyxins are 8 categories of antibiotics mainly used for treating *P aeruginosa* infections. *P aeruginosa* strains are categorized as pandrug-resistant (PDR) if resistant to all antimicrobials, extensively drug-resistant (XDR) if resistant to all but one category, and multi-drug resistant (MDR) if resistance is shown to one agent in three categories (El Zowalaty et al., 2015). The quick rise of MDR, XDR, and PDR strains is due to changes in the regulation of resistance gene expression, mutations, alterations in membrane permeability, and horizontal acquisition of enzymes that deactivate antibiotics or modify targets. It is important to note that the

coexistence of these mechanisms gives rise to multi-resistance in various strains (Fujii *et al*., 2014).

The eCDC's 2016 report on *P aeruginosa* in Europe revealed that 33.9% of bacteria demonstrated resistance to at least of the antibiotic groups being tracked (piperacillin-tazobactam, fluoroquinolones, ceftazidime, aminoglycosides, and carbapenems). All antimicrobial groups revealed significant inter-country variability in this study, with southern and eastern Europe generally having higher rates of resistance than northern Europe. For example, in regards to carbapenem resistance, 25% to 50% of invasive isolates are resistant in countries like Romania, Slovakia, Romania, Croatia, and others. In countries like Slovakia, Romania, Croatia, and others, 25% to 50% of invasive strains are multi-resistant when considering resistance to three or more of the antimicrobials mentioned before (Bassetti *et al*. 2018).

2.2.2 Resistance Mechanism Of Quinolone

The discovery of the importance of quinolones as a therapy for various clinical symptoms dates back to 1962 with the creation of nalidixic acid by George Lesher at the Sterling-Winthrop Research Institute. Nalidixic acid was originally created as a byproduct of the manufacture of chloroquine and its roots can be traced back to the antimalarial drug chloroquine. Despite its creation, it took several years for nalidixic acid to receive approval for the treatment of urinary tract infections caused by Gram-negative bacteria due to its cytotoxic effects on the digestive tract and central nervous system and limited impact on Gram-positive bacteria. The original quinolones target Gram-negative bacteria. Their widespread use as antibiotics without proper regulations resulted to a fast rise in antimicribial resistance. Bacteria such as Acinetobacter spp., Campylobacter spp., Clostridium spp., *Escherichia coli, K pneumoniae, S aureus,* and *Streptococcus pneumoniae*, among others, have been studied and found to be resistant. There are seven bacterial pathogens that frequently are responsible for infections in humans are *K pneumoniae*, *Staphylococcus aureus*, and *P aeruginosa*. Information on these pathogens is gathered and reported by the ECDC.

A study carried out across Europe showed that the mean resistance percentage of four types of bacteria to quinolones in European countries in 2015 and 2018. The bacteria

include Acinetobacter spp., Escherichia coli, Klebsiella pneumoniae, and *P aeruginosa*. In general, mean resistance percentage increased from 2015 to 2018. However, there were countries with low resistance percentage, such as Belgium and Norway for Acinetobacter spp. in 2018 and Iceland for Klebsiella pneumoniae in 2018. On the other hand, countries with high resistance percentage included Greece for Acinetobacter spp. in 2015 and Slovakia for *P aeruginosa* in 2018. It is worth noting that Cyprus had the highest resistance percentage for Escherichia coli in both 2015 and 20

2.2.3 Resistance Mechanism Of Beta-Lactams

P aeruginosa wild-type strains have an inducible class C AmpC cephalosporinase that makes them resistant to first and second-generation cephalosporins, cephamycins, second and third-generation cefotaxime, ceftriaxone, carbapenem, ertapenem, and aminopenicillins with or without beta-lactamase inhibitors. Normally, the AmpC cephalosporinase expression is low, allowing carbapenems (imipenem, meropenem, doripenem), carboxypenicillin, ureidopenicillin, third-generation ceftazidime, fourth-generation cefepime, and aztreonam to remain effective. However, carbapenems are the only -lactam class that can have reduced sensitivity from induced or constitutive AmpC overexpression or point modification. The influence of AmpC in *P aeruginosa* extends to cefepime, which is different from the impact of AmpC in Enterobacteriaceae. Some strains of P aeruginosa can produce TEM (2b Bush functional group), PSE or CARB (2c Bush functional group) type class A serine lactamases. These enzymes primarily target carboxypenicillin and ureidopenicillin, and sometimes they are not affected by BLI. The resistance to cefepime, cefpirome and aztreonam by these lactamase-producing strains of *P aeruginosa* varies, while ceftazidime and carbapenem remain effective against them.

P aeruginosa has been found to produces different of class A serine ESBLs, including the PER, VEB, GES, and BEL kinds. Enzymes from the ESBL Enterobacteriaceae family, including TEM, SHV, and CTX-M, have been discovered in *P aeruginosa*, most likely resulting from horizontal gene transfer. These class A ESBL compounds exhibit a similar -lactam hydrolysis pattern with the development of resistance to carboxypenicillins, ureidopenicillins, C3G and C4G (ceftazidime, cefepime, and cefpirome), and aztreonam but not carbapenems despite having a low genetic character.

Moreover, the BLI compounds tazobactam and clavulanic acid inhibit these enzymes to varying degrees.

The presence of class D -lactamases, also known as oxacillinases or OXA-types enzymes, has been detected in *P aeruginosa*. These enzymes fall under the Bush functional group 2d. OXA-50, a naturally occurring oxacillinase in *P aeruginosa*, is the exception. Other OXA-type enzymes are introduced into *P aeruginosa* through the transfer of mobile genetic elements. These oxacillinases, such as OXA-1, OXA-2, and OXA-10, can break down cefepime and make carboxypenicillins and ureidopenicillins resistant. They often resist beta-lactamase inhibitors (BLI). The extended-spectrum oxacillinases, OXA-2 and OXA-10, have mutations that result in increased hydrolysis of ceftazidime, cefepime, and aztreonam. However, BLI can often inhibit their activity, making it difficult to detect them through standard laboratory methods. The wide spread of extended-spectrum oxacillinases among different bacterial species is made possible because they are commonly located on mobile genetic components such as integrons and plasmid.

Carbapenemase enzymes have been found in P *aeruginosa*, which are similar to Enterobacteriaceae. *P aeruginosa* produces class A KPC or GES-2 type and class B MBL carbapenemases. GES-2 evolved from GES-1 (an ESBL) through point mutation, while *P aeruginosa* acquired the KPC carbapenemase from Enterobacteriaceae. The most common type of carbapenemase found in *P aeruginosa* is the MBL, that has five forms including IMP, VIM, NDM, SPM, and GIM. IMP and VIM have multiple variations, while only one variation is found for the other types. Mostly these enzymes usually are stored using mobile genetic components like plasmids, integrons, and cassettes, which facilitate it's spread. MBLs are resistant to BLI and have a broad spectrum of action against all b-lactams, except monobactam and aztreonam. MBL activities often go undetected due to their link with other b-lactam resistance pathways, which are frequently tested for. Integrons are mobile genetic elements that carry MBL genes at the same time other resistance genes such as aminoglycoside-modifying enzymes during cell expansion. (Bassetti *et al*. 2018).

2.2.4 Resistance That Develops With Changes in Penicillin-Binding Proteins (PBP)

B-Lactam antibiotics like antipseudomonal penicillin, cephalosporins, monobactams, and carbapenems remain critical in treating life-threatening *P aeruginosa* infections in hospitals. In spite of the increasing prevalence of resistance to first-line antimicrobials, often linked to multidrug resistance (MDR) phenotypes, their effectiveness is declining. Main while with acquisition of potent exogenous -lactamases through horizontal gene transfer (HGT) is a growing threat, especially class B carbapenemases (MBLs or ESBLs), B-lactam resistance is still mostly driven by the selection of chromosomal mutations. These mutations lead to hyperproduction of the chromosomal cephalosporinase AmpC, causing resistance to penicillin, cephalosporin, and monobactam, or repression/inactivation of porin OprD, leading to carbapenem resistance.

B-lactam antibiotics, such as antipseudomonal penicillin, cephalosporins, monobactams, and carbapenems, remain essential to treat *P aeruginosa* nosocomial infections, but resistance to them is increasing. While the transfer of exogenous -lactamases, such as class B carbapenemases (MBLs or ESBLs), is a growing threat, resistance often arises from chromosomal mutations, such as the hyperproduction of AmpC and inactivation of OprD. Up-regulation of efflux pumps, like MexAB-OprM and MexXY-OprM, also contributes to resistance, making all B-lactams vulnerable. However, new derivatives undergoing clinical testing, like ceftolozane, show promise. PBPs, the targets of B-lactam antibiotics, are another potential resistance mechanism, but their role in resistance for most nosocomial pathogens is unclear. PBP mutations with low B-lactam affinity have been reported in some species, but only one clinical isolate supports their existence in Enterobacteriaceae.

The role of penicillin-binding proteins (PBPs) in imipenem resistance in Acinetobacter baumannii has received attention. Some studies have found reduced expression of PBP2 in imipenem-resistant strains, while others have found no connection between PBP expression and resistance. The involvement of PBPs in *P aeruginosa* B-lactam resistance is not well understood, with some research suggesting nonessential PBPs may play a role, but this has not been firmly established. To learn more, researchers studied pairs of *P aeruginosa* strains that developed pan-beta-lactam resistance in clients in the ICU. They

used an integrative approach to investigate the role of PBPs in resistance and identify conventional B-lactam resistance mechanisms by comparing the profiles of PBP-encoding genes, expression of AmpC, OprD, and efflux pumps, and PBP binding affinities for imipenem, ceftazidime, and ceftolozane in the resistant strains (Moyá *et al*., 2012).

2.2.5 Laboratory Diagnosis of Metallo-betalactamases

To detect MBL production in *P aeruginosa* and *K pneumoniae*, both phenotypic and molecular methods can be used. Molecular techniques such as PCR, DNA probes, cloning, and sequencing will accurately identify MBL genes, but are limited to reference labs. Phenotypic techniques rely on the ability of metal chelators like EDTA and thiol-based substances thatblock MBL action, and include the Modified Hodge Test (MHT), Double Disc Synergy Test (DDST), the Imipenem-EDTA combined disc diffusion test, and the MBL E test (Sachdeva *et al*., 2017).

Microbiology laboratories need to have a quick, easy, and affordable way to screen for isolates that produce MBL. Chelating agents reduce MBL activity. Clinical isolates producing MBL can be identified using DDSTs that involve a disc of ceftazidime and a disc of 2-mercaptopropionic acid (MPA) or a disc of imipenem and a disc of EDTA. To achieve the best results, however, the spacing between the two discs must occasionally be adjusted. Yong et al. reported that a disc of imipenem with added EDTA (750 mg) effectively detected MBL-producing clinical isolates of pseudomonas and Acinetobacter with high sensitivity. While the imipenem/EDTA disc method and the double E-test are useful for initial screening and unlikely to produce false negatives, Chu et al. warned against relying solely on them, as EDTA susceptibility is common in Gram-negative isolates, making it difficult to determine the effect of imipenem/EDTA and EDTA alone on the test organism. Lee et al. presented a modified Hodge test, which can screen for carbapenemase-producing Gram-negative bacilli, but unable to be distinguished between those that produce and those that don't. Despite various phenotypic techniques being documented, standardized recommendations for MBL screening by organizations such as CLSI (previously NCCLS) and international groups are currently lacking (Zowalaty *et al*., 2015).

2.2.6 Risk Factors of Antimicrobial Resistance

A 2018 study investigated the correlation between sociodemographic traits and antibiotic resistance of clinical isolates to ceftazidime and cefriaxone. Results showed no significant association between age, sex, specimen type, and hospital stay length, except for the source of the isolates. The study found that most urinary tract isolates were resistant to third-generation cephalosporins. (cefriaxone or cefazidime).

Table 2.1: Socio-demographic Characteristics Association with Resistance Pattern of Clinical Isolates (Fanta et al. 2018)

Characteristics		Ceftazidime			Ceftrazone		
		R	S	P-value	R	S	P-value
	≤19	23	20		23	20	0.06902
Age	20-64	80	61	0.07622	73	68	
	≥65	46	18		44	20	
Gender	female	56	38	0.69325	54	40	0.98642
	male	95	59		86	68	
Specimen Type	Sputum	30	21		26	25	
	urine	41	15		41	15	
	Wound swap	50	45	0.08527	45	50	0.01426
	stool	29	17		28	18	
	≤1 Days	35	27		30	32	
	2-3 Days	72	40		66	46	
Hospital Stay	4-6 Days	19	20	0.29227	21	18	0.35481
	≥7 Days	23	13		23	12	

CHAPTER THREE
MATERIALS AND METHODS

3.1 STUDY DESIGN

This study carried out on 100 samples for pseudomonas and 100 samples for klebsiella. This results where obtain from 2017 to 2022. The results obtain have been represented in tables bellow. The samples used for this study were aged from 20 to 70 years majority of the sample population were between 40-50yrs giving a percentage of a mean age of about 34years. This investigation was conducted at Near East University hospital-microbiology laboratory department and focused on both inpatient and outpatient visitors to the hospital. The study sample comprised 100 clinical specimens collected from patients in various departments including OPD, ICU, CCU, Emergency and various specialized wards (such as pulmonology, oncology, neurology, gastroenterology, and cardiology). Both young and old patients were represented, with specimens collected from individuals of both genders. The experiment isolated *P aeruginosa* and *K pneumoniae* from the specimen.

3.2. TOOLS AND EQUIPMENT

The tools used in this study are the *Antec* medical petri dish, automatic pipette, wire loop, test tubes, Surgical-field autoclave (model M50D), 1000mL conical flask, spatula, masking tape, pH meter, syringe, dispenser, measuring ruler, marker, test tube wrack, sterile swab stick, ERMA INC photoelectric calorimeter, cotton wool.

3.3. SPECIMENS COLLECTION

Between 2017 and 2022, the microbiology lab will collect clinical *K pneumonia* and *P aeruginosa* specimens. Until they were used, the isolated strains were stored in bacteria storage tubes -80°C.

3.4. SPECIMEN PROCESSING

Specimen obtained from PIMS are grown using Blood Agar and MacConkey agar plates, there after these samples are incubated between 24-48 hours at 37°C. Morphology

of colonies, appearance and features are examined applying standard microbiological techniques, including colony color, size, Gram stain and biochemical testing.

3.5. COLONY MORPHOLOGY

The culture morphology that characterizes of *K. pneumoniae* isolates were believed to include: lactose-fermenting color, mucoid consistency, moderate to large size, circular to irregular form, dome-shaped to convex or spreader elevation, and curled to lobated or entire margins. Main while culture characteristics for *P aeruginosa* observed as color (green pigment), oxidase positive, size and shape (flat and smooth colonies)

3.6. GRAM STAINING

1. Prepare a thin smear of the bacterial sample.
2. A drop of distilled water is added to the slide, Pick a bacterial colony and emulsify it in the drop to make the smear.
3. The smear is first dried using air after which it is fixed with heat the smear on a spirit lamp.
4. Flood the smear with crystal violet for 1 minute and rinse with distilled water.
5. Apply Lugol's iodine for a duration of 60 seconds, followed by rinsing with regular tap water.
6. Decolorize with a decolorizing solution.
7. Clean with distilled water and apply with safranin for 1 minute then finally apply a counterstain with safranin.
8. Remove the slide with distilled water, air-dry and examine under oil immersion to identify the bacteria.

3.7 QUALITY CONTROL

In some cases, known Gram-positive and Gram-negative bacteria are stained for comparison with test organisms.

3.8 BIOCHEMICAL TESTS

To identify the organisms, various biochemical tests were conducted, and an already identified control was down with each test.

Turbidity Standard Solution (0.5 McFarland Standards)

One of the methods used in this research involved the double disk synergy test, which was altered and plated using 10g of Imipenem disk, 0.5 molar EDTA, MHA, and 0.5 Mcfarland. A 20 mm measurement gap was started next to the Imipenem disk after the agar gel was applied to the Muller Hinton agar cultivated with the test microorganism. The petri dish was sealed, and a blank disk containing 20 L of 0.5m EDTA was inserted at the distance of the 20 mm interval. Incubation at 35 °C for 24 hours. One of the methods used in this research involved the double disk synergy test, which was altered and plated using 10g of Imipenem disk, 0.5 molar EDTA, MHA, and 0.5 Mcfarland. A 20 mm measurement gap was started next to the Imipenem disk after the agar gel was applied to the Muller Hinton agar cultivated with the test microorganism. The petri dish was sealed, and a blank disk containing 20 L of 0.5m EDTA was inserted at the distance of the 20 mm interval Incubation at 35 °C for 18 hours or overnight.

3.9 ANTIBIOTIC SENSITIVITY TEST

Preparation of inoculum

Three to four colonies of the test microorganism were selected using a sterile loop and transferred to a tube with normal saline. The inoculum density was compared to 0.5 McFarland. The suspension was used within 15 minutes. Image 4.6 shows a test for citrate (left) and indole (right). Test sample B, negative control A, positive control C, and 22 3.9.2 plate inoculation were performed using sterilized cotton swabs to create a lawn culture on the media plate. The swab was pressed against the tube wall to remove excess liquid and spun. The plate was inoculated 4-5 times with 60-degree rotation to evenly distribute the inoculum.

Figure3.1: K pneumonia colonies on MacConkey agar

Figure 3.2: *P aeruginosa* on MacConkey agar

Application of discs

The discs were carefully taken from the vial with sterile forceps. One by one, discs were gently placed on the agar plate while being softly pressed to establish complete contact with the agar surface. The petri dish lid was gently lifted from one side. Plates are then covered with lids after the discs were applied, and they were then inverted and kept at 36–37 degrees Celsius for 24 hours in an incubator.

Reading and interpretation of results

After incubation, the diameter that represents the inhibition zone (including the disc) is measured to the nearest millimeter with a ruler. By looking at a petri dish's reverse, measurements of the zones were taken. Results for all of the sensitivity plates were reported as sensitive, resistant, and intermediate sensitive.

Control strains used in the project: Escherichia coli ATCC 25922

3.10 STATISTICAL ANALYSIS

Data values were expressed as frequency, percentages, means, and ±SD. Chi-square test is used to evaluate relationships between variables. Findings were presented visually. SPSS version 25.00 was used for analysis with a significance level of 0.05.

CHAPER FOUR

RESULTS

4.1. SOCIO-DEMOGRAPHIC DATA

Table 4.1: Socio-Demographic Data of Patients

K pneumonia related (n=100)			*P aeruginosa* related (n=100)		
Age range	Gender		Age range	Gender	
	male	female		Male	Female
21-30	4	7	21-30	7	6
31-40	5	11	31-40	9	12
41-50	6	15	41-50	6	14
51-60		20	51-60	11	13
61-70	9	12	61-70	6	16

Table 4.2 Distribution of *K pneumonia* and *P aeruginosa* Isolates Based on Patient Samples (NEU Hospital 2017-2022)

specimen	*K pneumonia*	*P aeruginosa*
	n (%)	n (%)
Wound material	19 (19.0)	5 (4.4)
sputum	15 (15.0)	9 (8.8)
Cerebrospinal fluid	2 (2.0)	
Deep tracheal aspirate	17 (17.0)	8 (7.8)
Urine	30 (30.0)	60 (59.8)
Blood	8 (8.0)	12 (11.8)
catheter	5 (5.0)	5 (4.9)
Other	4 (4.0)	2 (2.5)
Total	100 (100)	100 (100)

Table 4.3: *P aeruginosa* Isolates' Rates Of Antimicrobial Resistance (NEU Hospital 2017-2022)

Antibiotic	Number of isolates tested	Resistance (%)
Piperacillin-tazobactam	99	5 (5)
Ticarcillin-clavulanic acid	76	11 (14.4)
Ceftazidime	100	24 (24.0)
cefepime	98	4 (4.0)
Aztreonam	97	35 (36.0)
imipenem	100	25 (25.0)
Meropenem	98	9 (9.1)
Colistin	80	14 (17.5)
Gentamicin	100	29 (29.0)
Amikacin	100	13 (13.0)
ciprofloxacin	89	35 (39.3)
Levofloxacin	78	20(25.6)
Norfloxacin	34	8 (23.5)

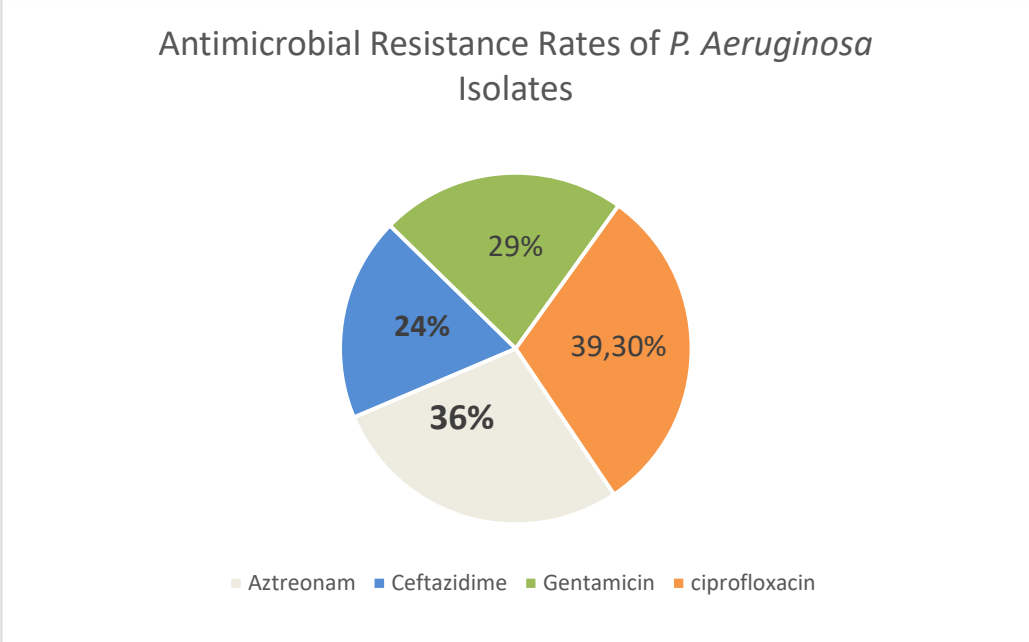

Figure 4.1: Antimicrobial Resistance Rates of *P. Aeruginosa* Isolates

Table 4.4: Antimicrobial Resistance Rates Of *K pneumoniae* Isolates (NEU Hospital 2017-2022).

Antibiotic	Number of isolates tested	Resistance (%)
Amoxicillin-clavulanic acid	57	15(29.7)
Ampicillin-sulbactam	99	37(37.3)
Piperacillin-tazobactam	99	11(11.1)
Ticarcillin-clavulanic acid	43	9(20.9)
Cefazolin	98	30(30.6)
Cefepime	99	25(25.2)
Ceftriaxone	66	17(25.7)
Cefoxitin	23	3(13.0)
Cefuroxime	79	27(34.1)
Ceftazidime	100	28(28.0)

Aztreonam	99	20((20.2)
Gentamicin	100	15(15.0)
Tetracycline	21	7(33.3)
Ciprofloxacin	64	15(23.4)

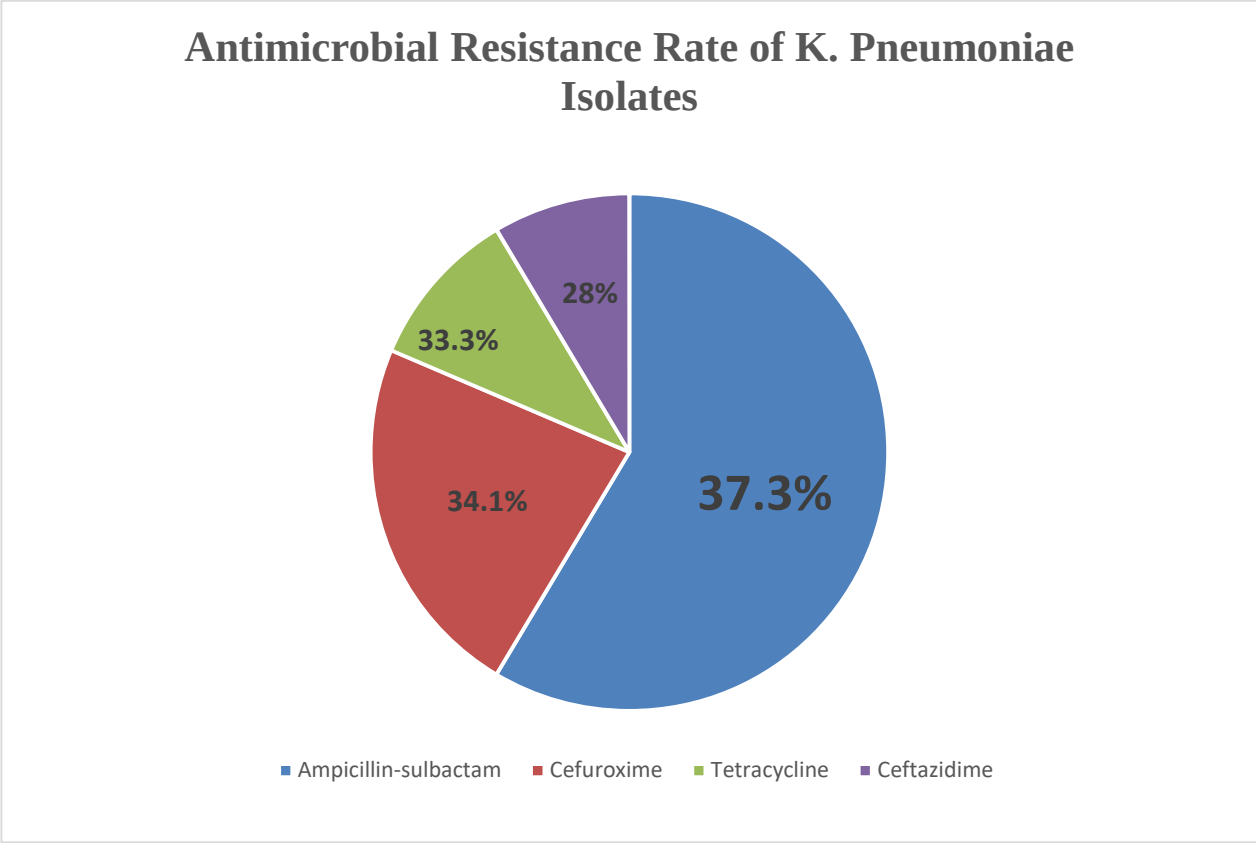

Figure 4.2: Antimicrobial Resistance Rate of *K. Pneumoniae* Isolates

Table 4.5: Risk factors of *P aeruginosa* and *K pneumoniea*

CHARACTERISTICS							
PSEUDOMONASSS					**KLEBSIELLA**		
		R	**S**	**P value**	**R**	**S**	**P value**
Age	≤30	7	12	0.199	4	11	0.08
	31-50	18	24		19	43	
	≥51	13	26		15	8	
sex	female	17	43	0.032	27	33	0.100
	male	12	28		14	26	
Type of specimen	sputum	6	9	0.751	3	6	0.111
	urine	10	20		25	35	
	Deep tracheal aspirate	6	11		1	4	
	catheter	2	3		1	4	
	Cerebrospinal fluid	0	2		0	0	
	Blood	2	6		4	8	
	Others	1	3		0	4	

Social demographic data of patient was evaluated in table 4.5 Above using chi square, a P-value not more than 0.01 and if below. 005 they are considered highly statistically significant. However, in the table above there is no value result of statistical significance.

In our study, we found ceftazidime resistance of P aeruginosa strains to be 24%. This ratio was higher for other cephalosporins (24% vs. 4%) than for Cephepix. In addition, resistance to ceftazidime was also much higher than piperacillin/tazobactam (24% vs 5%). In our study, imipenem resistance and ceftazizidime resistance were very close to each other in Pseudomonas (24% vs 25%).

K pneumonia strains showed the second highest resistance to ceftazidime (28%) among ceflosporins. However, ceftazidime were found to be more effective antibiotics than penicillin and ampicillin groups. We found that the most effective antibiotics against Klebsiella strains was piperacillin/tazobactam with 11% resistance rate.

CHAPTER FIVE
DISCUSSION, LIMITATIONS, CONCLUSION AND RECOMMENDATION

5.1 DISCUSSION

The rise in antibiotic-resistant bacterial strains is caused by of the widespread usage of broad range antibiotics. The majority of bacteria that cause widespread health issues, in this case the Pseudomonas and Klebsiella strain have high rates of resistance. More than half of the identified bacterial strains in this investigation were found to be resistant to cefriaxone or ceftazidime, which is consistent with World Health Organization reports from 2014 (W.H.O, 2000). It was also the aim of this research to assess risk factors of ceftazidime resistance to Pseudomonas and Klebsiella among samples from the Near East University Hospital. Resistance to other antimicrobial agents was also identified in this study. Socio-demographic information was insufficient to establish a statistically meaningful link between ceftazidime and the degree of Pseudomonas and Klebsiella strain resistance.

Based on a variety of parameters, the variations in drug resistance patterns between isolates were assessed. There were no statistically significant values found in this study with regard to that. This is Consistent with the study by Fanta et al. in 2018 (table 2.4). Additionally, no socio-demographic variable was discovered to be statistically significant in this investigation by Fanta et al. The specimens from which the strains were obtained, however, were an exception in the same investigation. This difference might be as a result of the study using a larger sample size and also as a result of different bacterial involved in the study. In another study caried out in India between 2000 and 2002 it was found that gender had result of 0.001 (L Savas et al., 2002) which was statistically significant which is in contrary to that obtained in this study. This might be because of the difference in sample size because the study in India had a longer duration allowing as many as possible agents to take part in the study there was a difference in the methods used between these studies.

In a study in Basel that evaluated the impact of “Socio-Demographic Factors On Knowledge And Practices Related To Antibiotics And Antibiotic Resistance In Vietnam, Using A Cross-Sectional Survey”. This study if was found out that age range and gender

had P- values of 0.126 and 0.903 for gender and age range respectively (Khanh et al., 2022) which are similar with results obtained in this study which were also statistically insignificant.

Most pathogens causing health problems have high resistance rates. One of the main goals of my research was to determine the resistance of pseudomonas and klebsiella strains to ceftazidime and other antimicrobials like Ceftriaxone. The results, as shown in tables 4.3 and 4.4, revealed that over half of the isolated bacterial strains were resistant to either cefriaxone or cefazidime.

In this study it was found that 24.0% of *P aeruginosa* were found to be resistant against ceftazidime these results are consistent with 23.7% resistant rate against ceftazidime obtained from blood isolate in a Spanish hospital, the sample sizes of this studies were also close (Ruiz-Garbajosa *et al*., 2017). These results differ from European results of 19.3% and 19.7 which was a comparison of 2015 and 2018 respectively obtained from “A comparison of European countries' resistance to quinolones in 2015 vs. 2018.” This difference may be narrowed down to the fact the sample sizes were different, method used or duration with which the research was carried out. The results obtained on *P aeruginosa* resistance (13.2%) against ceftazidime which was rank second most resistant bacteria after *Escherichia coli* in a commercial laboratory in Seoul which was carried out in 2004 (Kyungwon Lee et al., 2006).

In this study it was also found out that 29.0% of samples were resistant to Gentamicin which is slightly equal to 21.1% and 24.8 obtained from research conducted in the Spanish hospital from blood isolates and from respiratory isolate in an EU hospital (Ruiz-Garbajosa *et al*., 2017). These results are also exact with 28.6% resistant rate obtained by Savas in 2000 to 2002 researching on "Urinary Tract Infections in Healthcare Settings: Microorganisms, Antibiotic Resistance and Risk Factors" (Savas et al., 2002)
K pneumoniae was the second bacteria under investigation in this research and it was found out that 28.0% of samples were found to be resistance to ceftazidime. This is far more than10.7% resistant rate gotten from a study carried out in primary care clinics in outside of Seoul, this research however, was done for commercial purposes which might affect the method used (Kyungwon Lee et al., 2006). *K pneumoniae* was in investigation amongst other gram -negative bacteria like, *Escherichia coli*, *Staphylococcus aureus*,

Streptococcus, *P Aeruginosa*, etc this was a comparison between 2015 and 2018. This study was carried out in countries around Europe and a mean value was gotten. The mean value for *K pneumoniae* was 29.7% which is very close to results obtained in this study.

K pneumoniae was also investigated against Cefepime with a resistant rate of 25.0% which is far less than 73.8% (L Savas et al ,. 2002). Tetracycline had a resistant rate of 33.3% however, not all samples were tested with Tetracycline. Which is far more that resistant rate of 16.7% found in the USA in 2010 the difference might be due to location and time differences. (Guillerm V. et al,. 2013). Gentamicin, Ceftriaxone, and Aztreonam had resistant rate of 15.0%, 25.7 and 20.2% compared to 9.2%, 12.1%, and 22.2% in another study caried out in the USA. There is not a huge difference between the results (Guillerm V. et al., 2013).

5.2 CONCLUSSION

In this study it was concluded that isolate of *P aeruginosa* and *K pneumoniae* were increasing getting resistance against ceftazidime amongst many other antibiotics. It can also be concluded that factors such as age, sex, and type of specimen are not rick factors for acquiring resistance to these antibiotics. In our study, we can also conclude that imipenem resistance and ceftazidime resistance were very close to each other in Pseudomonas (24% vs 25%). *K pneumonia* strains showed the second highest resistance to ceftazidime (28%) among cephalosporins

5.3 LIMITATIONS

This study was written based on result obtained from the microbiology department of the Near East University Hospital which student could not have any interaction with patient or get patients to answer questions.

5.4 RECOMENDATIONS

Student should be given access to meet patients and be able to interact with patients on the topic they are working on. More studies on this topic should be carried out so as to assist clinicians.

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Ceftazidim resistance

ORİJİNALLİK RAPORU

%12	%8	%11	%
BENZERLİK ENDEKSİ	İNTERNET KAYNAKLARI	YAYINLAR	ÖĞRENCİ ÖDEVLERİ

BİRİNCİL KAYNAKLAR

1	serval.unil.ch İnternet Kaynağı	%4
2	Moya, B., A. Beceiro, G. Cabot, C. Juan, L. Zamorano, S. Alberti, and A. Oliver. "Pan--lactam Resistance Development in Pseudomonas aeruginosa Clinical Strains: Molecular Mechanisms, PBPs Profiles and Binding Affinities", Antimicrobial Agents and Chemotherapy, 2012. Yayın	%1
3	www.ncbi.nlm.nih.gov İnternet Kaynağı	%1
4	Georgios Feretzakis, Evangelos Loupelis, Aikaterini Sakagianni, Nikoletta Skarmoutsou et al. "A 2-Year Single-Centre Audit on Antibiotic Resistance of Pseudomonas aeruginosa, Acinetobacter baumannii and Klebsiella pneumoniae Strains from an Intensive Care Unit and Other Wards in a General Public Hospital in Greece", Antibiotics, 2019 Yayın	%1