NEAR EAST UNIVERSITY

INSTITUTE OF GRADUATE STUDIES

DEPARTMENT OF MEDICAL MICROBIOLOGY AND CLINICAL MICROBIOLOGY

CARBAPENEM-RESISTANT *KLEBSIELLA.SPP* COLONIZATION AMONG INTENSIVE CARE UNIT (ICU) ADMITTED PATIENTS AT NEAR EAST UNIVERSITY HOSPITAL

M.Sc. THESIS

Hedaya Othman Mohammed Hassan

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Supervisor

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Approval

We certify that we have read the thesis submitted by **Hedaya Othman Mohammed HASSAN** titled **"Carbapenem-Resistant Klebsiella. spp colonization among intensive care unit admitted patients at Near East University Hospital"** and that in our combined opinion it is fully adequate, in scope, and in quality, as a thesis for the degree of Master of Medical and Clinical Microbiology.

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Declaration

In this capacity, I declare that this thesis was written in accordance with the academic standards and ethical principles of the Institute of Graduate Studies at Near East University. Moreover, as required by these rules and conduct, I have cited and referenced all non-original sources of information and data in accordance with these guidelines.

Hedaya Othman Mohammed Hassan

Acknowledgments

In the name of Allah, Most Gracious, Most Merciful Praise be to Allah, the Lord of the Worlds. First of all, I would like to express my gratitude to Almighty Allah for providing me with the opportunity, determination, and strength to complete my research and write my thesis. He is indeed near. Over the course of my life, and especially during the course of my research,

I'd like to express my heartfelt gratitude, appreciation, and admiration to my thesis advisor, Assoc. Prof. Meryem Guvener, for her help, guidance, and encouragement. I greatly appreciate her time, effort, and ideas. While she allowed me to do the majority of the work on my thesis, she provided guidance when she felt I needed it.

Additionally, I'd like to express my appreciation to the academic staff of Near East University's Department of Medical Microbiology and Clinical Microbiology for their assistance. Furthermore, I would also thank the staff of the Microbiology Laboratory at the Near East University Hospital for allowing us to use their tools during this project.

Finally, I would like to thank my benevolent and supportive father, my lovely mother, my greatest brothers and sisters. Without the encouragement and support of my family members, I would not be where I am today. Thank you so much for everything. In honor of their valiant sacrifices, this thesis is dedicated to them.

Abstract

Carbapenem resistance *Klebsiella spp* Colonization Among ICU admitted Patients at Near East University Hospital

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February 2022, 61 pages

Klebsiella pneumonia is a gram-negative bacillus member of the *Enterobacterteasea* family that can be found in nature commensally (soil, water, sewage) and on the mucus membranes of animals and humans. *Klebsiella* is classified as an enteric flora member in humans, which allows the bacteria to colonize the gastrointestinal canal and cause serious infection, particularly in critically ill patients in the ICU. Many factors may affect the ability of this bacteria to colonize the human gut, including bacterial factors and patient status. *Klebsiella pneumonia* has some virulence factors that help in colonizing and invading the patient's intestinal tract. These bacteria are naturally resistant to penicillin and have a high affinity for acquiring resistant genes. That made the infection difficult to treat. Carbapenem antimicrobial agents are considered the first line of treatment for β-lactam resistant bacteria. Unfortunately, *Klebsiella pneumonia* bacteria has become resistant to carbapenem. This is a big problem for the healthcare system. Patients who are in intensive care units are more likely to get nosocomial infections caused by carbapenem-resistant *Klebsiella pneumonia* (CRKP). The presence of patients in the ICU, the broad-spectrum antibiotics, long-term antibiotic treatment, and the immune systems of the patients are factors that affect the resistance and colonization\infection of *Klebsiella spp*. in the ICU. The aim of this thesis study was to investigate the colonization of CRKP into the intestines of intensive care patients at Near East University Hospital. Thirty-eight (43.7%) out of eighty-seven isolates were found to be CRKP by using the TSI test, the Citrate test, Simone test, and disc diffusion test for IMP, MEM, and ETP. There is no correlation between the mean age of patients and the presence of carbapenem-resistant *Klebsiella pneumonia* (P = 0.325). Also, there is no correlation between the gender of patients and the presence of carbapenem-resistant *Klebsiella pneumonia* (P = 0.339).

Keywords: *Klebsiella pneumonia*, colonization, carbapenem-resistant, ICU infections, nosocomial infection.

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List of Abbreviations

CRKP: carbapenem resistant *K. pneumonia*

ICU: Intensive care unit

LPS: lipopolysaccharide

NI: nosocomial infection

HCWs: healthcare workers

UTI: urinary tract infection

CDC: Centers for Disease Control and Prevention

CRE: carbapenem-resistant Enterobacteriaceae

ECDC: European Center for Disease Prevention and Control

EUCAST: European Committee on Antimicrobial Susceptibility Testing

CP: capsular polysaccharide

Cp-Kpn: carbapenemase-producing *Klebsiella pneumonia*

HAI: healthcare-associated infections

CPE: carbapenem producing *Enterobacteriaceae*

CPKP: carbapenemase-producing *Klebsiella pneumonia*

IMVIC: indole, methyl red, voges- proskauer, citrate test

SHV-1: sulfhydryl variable

MDR: multi-drug resistant

ABC: ATP-binding cassette family

MFS: major facilitator superfamily

MATE: multidrug and toxin extrusion

SMR: small multidrug resistance

RND: resistance-nodulation-cell division

OMPs: outer membrane proteins

PBPs: penicillin-binding proteins

EDTA: ethylenediaminetetraacetic acid

ESBL: extended spectrum beta lactamase

MBL: metallo-β-lactamases

NDM: new delhi metallo-β-lactamases

OXA: oxacillinase

CROs: carbapenem-resistant organisms

CR-GNB: carbapenem-resistant gram-negative bacteria

IC: immunochromatographic assay

MICs: minimum inhibitory concentrations

PCR: polymerase chain reaction

MHT: modified hodge test

EMB: eosin methylene blue agar

TSI: triple sugar iron test

SIM: sulphide indole motility medium

IMP: imipenem

MEM: meropenem

ETP: ertapenem

NHSN: National Health care and safety network

TRNC: Turkish Republic of Northern Cyprus

CR-hvKP: carbapenem-resistant, hypervirulent *Klebsiella pneumonia*

CHAPTER I

INTRODUCTIONS AND AIMS

One of the challenges that intensive care units (ICU) face is the high incidence of nosocomial infections (NI). Considerable morbidity and mortality are linked to these infections among critically ill patients, who also experience longer hospital stays and higher expenses as a result(Zaragoza et al., 2014).

Nosocomial infections in humans are frequently caused by *Klebsiella spp.* Urethral tract infections, pneumonia, septicemia, and soft tissue infections are all prompted by *Klebsiella pneumonia*, the much more prevalent *Klebsiella species*(Podschun & Ullmann, 1998). *Klebsiella* belongs to the *Enterobacteriaceae* family, a gram-negative bacterium. cause hospital-acquired infections, particularly in immunocompromised patients in intensive care units(Zhao et al., 2019).

The surface of the water, sewage, and plants are all places where *Klebsiella spp*. can be found.; they can also be found on the mucosa of the humans, horses, and pigs, where they colonize. Like *Enterobacter and Citrobacter, Klebsiella* is frequent in humans but not in the environment, unlike *Shigella* spp. or *E. coli*(Bagley et al., 1978). Several types of *klebsiella* are able to colonize the human intestine and cause diseases.*K. pneumoniae*, *K. edwardsii* and *K. ozaen*ae , *K. aerogene*(Cooke et al., 1979).

K. pneumonia is found in the nasopharynx and the intestines of humans as a saprophyte. The carrier rates vary widely from one study to the next. Stool samples have a detection rate of 5 to 38%, while nasopharynx samples have a detection rate of 1 to 6%. *Klebsiella spp*. are rarely found on human skin, where

the poor growth conditions for gram-negative bacteria make them only transient members of the flora(Podschun & Ullmann, 1998).

The urinary and respiratory tracts are the most commonly affected by nosocomial *Klebsiella* infections. Because of the differences in host defense mechanisms, it is expected that the virulence factors found in *Klebsiella* strains that cause UTIs will be different from those found in strains isolated from pulmonary sources of patients with pneumonia(Podschun & Ullmann, 1998).

Indeed, in 2013, the CDC declared carbapenem-resistant *Enterobacteriaceae* (CRE), of which *Klebsiella* species are the most prevalent, an urgent threat(Martin et al., 2018). *Klebsiella* species has emerged as a clinically significant bacteria as a result of increased antibiotic resistance and the natural tendency to acquire antibiotic resistance and initiate serious outcomes(Al Bshabshe et al., 2020). Furthermore, Antibacterial therapy and excessive use of antibiotics are frequently blamed for the emergence of multiresistant *Klebsiella* strains in hospitals(Podschun & Ullmann, 1998).

There are some factors contributing to the development of carbapenem-resistant *klebsiella*. infections: A longer length of time in the hospital, admission into the intensive care unit (ICU), a prior hospitalization, an increase in ICU days spent, transplant recipients, steroids used, central vein catheters used, machines used, tracheostomies present, and previous antibiotic use are all factors to consider(Liu et al., 2018). The carbapenemase family of *Klebsiella pneumoniae* is the most prevalent causative mechanism enzymatic of carbapenem resistance in humans (KPC)(van Duin, 2017).

Medically, clinical practitioners face a challenge from carbapenemase-producing *Klebsiella pneumoniae* strains (Cp-Kpn) because they are becoming more common in hospitals and are resistant to antibiotics(Reyes et al., 2019).

Carbapenem-resistant *Klebsiella* infection has been rapidly emerging as a life-threatening nosocomial disease, especially in the intensive care units(ICU). The aim of this study is to look into the colonization of *Klebsiella*. *spp* in the guts of the patients that are admitted to the ICU and their susceptibility to carbapenems at Near East University Hospital in Northern Cyprus.

CHAPTER II

LITERATURE REVIEW

Klebsiella, a gram-negative bacterium, is a major public health threat, is responsible for a considerable amount of fatalities and illnesses around the world. It is recognized to be an opportunistic pathogen, capable of causing a wide range of infections in both the healthcare setting and the community at large, including urinary tract infections, pneumonia, sepsis, meningitis, and pyogenic liver abscesses. Highly resistant *Klebsiella* species and the emergence of different antibiotic resistance phenotypes are major concerns and particularly worrisome to scientists. *Klebsiella pneumonia* and *Klebsiella* oxytoca are the most widespread organisms that assume responsibility for human *Klebsiella* diseases(Saxenborn et al., 2021).

Hospital-acquired infections caused by *Klebsiella pneumonia* are common among patients with compromised immune systems, particularly those in the intensive care unit (ICU). Multidrug-resistant *K. pneumoniae* infections are usually treated with a carbapenem, a first-line treatment. However, *K. pneumoniae* carbapenem-resistant (CRKP) is a public health concern because it can cause fatal infections with a mortality rate of 33.24%–50.6%(Sun et al., 2019).

2. General information

2.1. Intensive Care Unit Infection.

The terms "nosocomial infection" or "healthcare-associated infections" (HAI) refer to illnesses that patients acquire while receiving medical treatment but weren't present when they were admitted to the hospital(Sikora & Zahra, 2022). Patients in the ICU, where their immune systems have been compromised and they are subjected to more intrusive monitoring, are particularly vulnerable to

infection, despite the fact that everyone in the hospital is at risk of infection(Lewis et al., 2019).

Life-threatening diseases or trauma requiring intensive care are treated in intensive care units (ICU). All ICU patients are at risk for infection and death from nosocomial diseases. Treatment is required regardless of the outcome of the infection (Girou et al., 1998)

Healthcare systems around the world are under threat from the emergence a nd spread of multidrug-resistant organisms. with methicillin-resistant Staph aureus, vancomycin-resistant *Enterococci*, and carbapenemase-producing *Enterobacteriaceae* (CPE), mainly *Klebsiella* (CPKP)(Nekkab et al., 2017). One of the most common *Enterobacteriaceae* capable of producing carbapenemase (CPE) and a significant contributor to high-mortality infections in hospitals, Carbapenemase-producing *Klebsiella pneumonia*, has been linked to an increased mortality rate in new meta-analyses(Brink, 2019). To avoid the spread of nosocomial infections, proper control measures must be implemented(Fu & Wang, 2016).

2.2. Colonization

Many enteric pathogens are prevented from colonization when the intestinal microbiota is present. Microbiota-produced substances appear to inhibit pathogen growth, nutrient depletion by microbiota growth and microbiota-induced activation of innate and adaptive immune responses appear to play a role(Stecher & Hardt, 2011).

In order to prevent nosocomial infections such as those of the circulatory and urinary tracts, as well as those associated with the ventilator, it is essential to prevent carbapenem-resistant Gram-negative bacilli from colonizing the digestive tract. Colonized patients can be found early and accurately, which can help stop

the spread of these organisms(Abdalhamid et al., 2016). The human gastrointestinal tract is capable of dynamically colonizing multiple CRKP strains with a variety of plasmids. A major origin of *K. pneumoniae* disease, particularly among the critically ill, is thought to be the gastrointestinal system(Sun et al., 2019).

Hospital-acquired infections caused by *Klebsiella pneumonia* are common among patients with compromised immune systems, particularly those in the intensive care unit (ICU)(Sun et al., 2019).

2.3 General information about *Klebsiella* spp.

2.3.1 History

Klebsiella is a gram-negative bacteria genus. in the *Enterobacteriaceae* family. However, after being described by Carl Friedlander, the *Klebsiella* bacillus went by the name of the Edwin Klebs bacillus for many years before being given its current name in honor of Edwin Klebs(1834–1913), a German microbiologist who lived in the late nineteenth century(Ristuccia & Cunha, 1984a).

2.3.2 Morphology and biochemical characteristics

The *Klebsiella* genus is a gram-negative nontitle, non-spore-forming encapsulated rod, 0.3 to 1.5 nm in diameter and 0.5 to 5.0 nm in length **(Figure 1)**. They differ from other members of the *Enterobacteriaceae* family in that they tend to be shorter and thicker. The most common shapes are straight rods with slightly pointed or rounded ends, and it is possible to find them arranged in singles, pairs, or short chains, It's common to see diplobacillus forms in vivo(Ristuccia & Cunha, 1984a).

Because *Klebsiella* belongs to the *Enterobacteriaceae* family, it can grow in a standard lab medium, These species can grow aerobically or anaerobically (facultative anaerobe) with an optimal growth temperature ranging from 35°C to 37°C and an optimal PH of 7.2(Ristuccia & Cunha, 1984b). Biochemical reactions are used to identify and distinguish *Klebsiella* species("Antimicrobial Susceptibility, Biochemical Characterization and Molecular Typing of Biofield Treated Klebsiella Pneumoniae," 2015).

These are just some of the general biochemical traits of this species: lactose fermenter, mucoid colony, indole are negative, methyl red is negative, Voges-Proskauer is positive, and citrate is positive (IMVIC-++) **(Figure 2)**, so ,as members of *Enterobacteriaceae*, *Klebsiella spp*., they are chrome oxidase negative and catalase-positive (Eguchi et al., 1987).

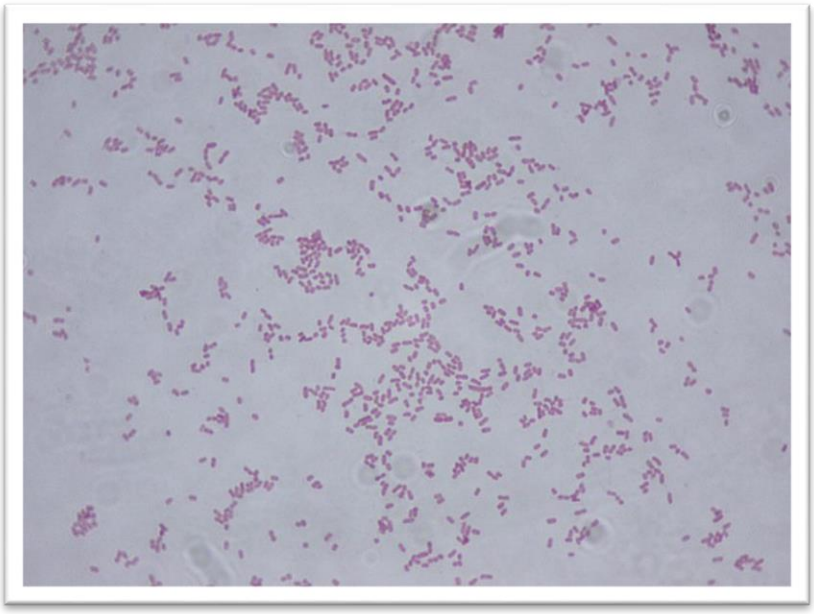

Figure 1. Gram stain of *Klebsiella oxytoca*(Högenauer et al., 2006).

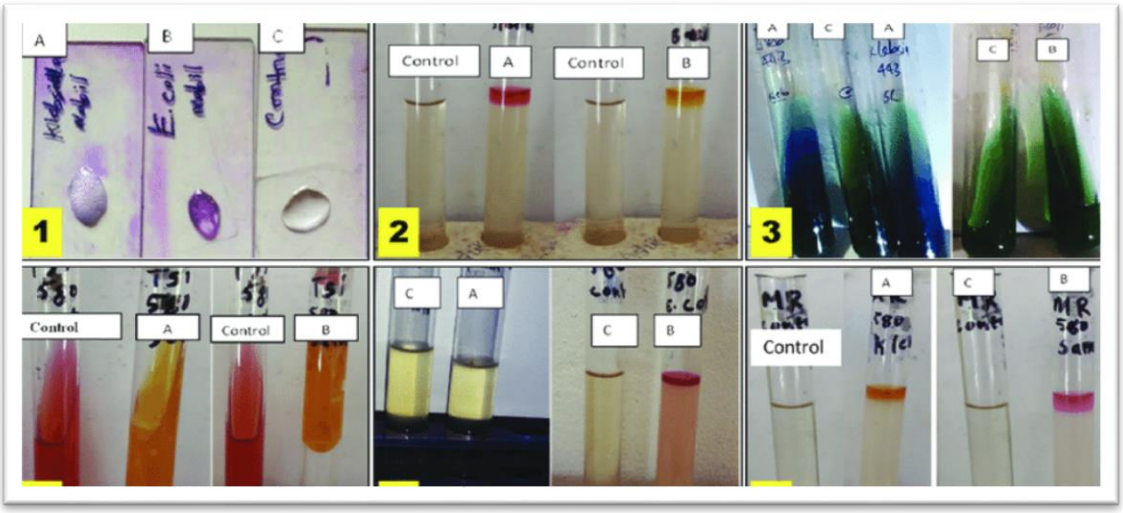

Figure 2. *Klebsiella spp*. identification biochemical tests. (1) Catalase test; (A) represents *Klebsiella* spp., (B) represents *E. coli*, and (C) represents the control. (2) Results of the Voges-Proskauer test; (A) *Klebsiella* spp. is positive, (B) *E. coli* is negative. (3) Simmon's citrate test; (A) is *Klebsiella* sp. positive, (B) is *E. coli* negative, and (C) is control. (4) TSI test; (A) *Klebsiella* sp., (B) *E. coli*, (5) Indole test; (A) *Klebsiella* spp., (B) *E. coli*, (C) control (A) *Klebsiella* spp. is negative, (B) *E. coli is positive, and (C) is the control(Salauddin et al., 2019).*

2.3.3 Epidemiology

Human carriers of *Klebsiella spp*. in the nasopharynx and the gastrointestinal tract are thought to be the main source of the spread of *Klebsiella* infection, with the highest rate of carriers in the respiratory tract(Casewell & Phillips, 1978). There is evidence to suggest that rather than healthcare delivery, the high incidence of hospital-acquired *Klebsiella* colonization appears to be linked to antibiotic use, this occurred most frequently in patients taking antibiotics, particularly broad-spectrum or more than one antibiotic synergically(Pollack et

al., 1972). In addition, the widespread use of antimicrobial therapy is also associated with an increased prevalence of the presence of *Klebsiella* pathogens resistant to antibacterial drugs (Selden, 1971).

The hands of hospital staff and patients' gastrointestinal tracts serve as the primary reservoirs for the transmission of *Klebsiella* in the hospital environment(Podschun & Ullmann, 1998).

Carbapenemase is primarily mediated by plasmids in bacteria. Carbapenem-resistant *K. pneumoniae* originated from classical *K. pneumoniae* strains that had acquired various blaKPC-encoding plasmids (CRKP), Since these plasmids contain a wide variety of transposable elements, they've been able to undergo frequent genetic transposition, leading to plasmid mergers and acquisitions and improved adaptability for the bacterial host, As a result, the remaining therapeutic options will be seriously impacted(Yang et al., 2021).

2.4 Resistance mechanism

2.4.1 Natural (intrinsic) Resistance

Genes in bacterial genomes that could produce a resistance phenotype are known as "intrinsic" resistance(Davies & Davies, 2010). Intrinsic antibiotic resistance is a naturally occurring phenomenon, In all bacteria, resistance to antibiotics has always been there, even before antibiotics were used to treat illnesses(Cox & Wright, 2013). A *K. pneumoniae* strain that makes an enzyme called the sulfhydryl variable penicillinase (SHV-1) is innately resistant to ampicillin, carbenicillin, and ticarcillin because it makes the enzyme on its own chromosomes(Piperaki et al., 2017).

2.4.2 Acquired Resistance

To fully comprehend how *Klebsiella* species acquire resistant genes, a deep look inside the genome material of *Klebsiella* species is necessary. *K. pneumoniae* was known to be an important source of infection risk in patients who were in the hospital with *K. pneumoniae* colonization in their digestive tracts. The estimated number of genes in the core genome of *K. pneumoniae*, which is present in more than 95% of isolates, is currently around 2,000. The accessory genome refers to the genes that differ between isolates. This includes plasmid-encoded genes as well as chromosomal genes. Because *K. pneumoniae* genomes generally contain genes ranging between 5,000 and 6,000 genes, accessory genes make up the majority of the genome. As well as these, the accessory genome contains genes that code for antibiotic-resistant enzymes and mechanisms. However, conjugative plasmids carrying the blaKPC–2 genes from a *K. pneumoniae* isolate can transfer resistance to carbapenems(Martin & Bachman, 2018).

2.4.2.1 Resistance developed by inactivation of antibiotics

Bacteria can produce enzymes that are able to decrease the avidity of drugs. Acetylation, phosphorylation, and adenylation are three of the most common biochemical reactions that enzymes perform(Munita & Arias, 2016). Resistance to antibacterial drugs, including aminoglycosides, beta-lactams (penicillin and cephalosporins), and chloramphenicol is mainly the result of enzyme inactivation or the formation of inactive derivatives by hydrolysis or other enzymatic means **(figure 3)**. Bacteria like *Klebsiella spp*. are capable of producing enzymes that inactivate antibiotics upon contact(Davies, 1994). For example, *K.pneumoniae* produces a carbapenem-hydrolyzing β-lactamase(class A β-lactamase, KPC-1)(Yigit et al., 2001).

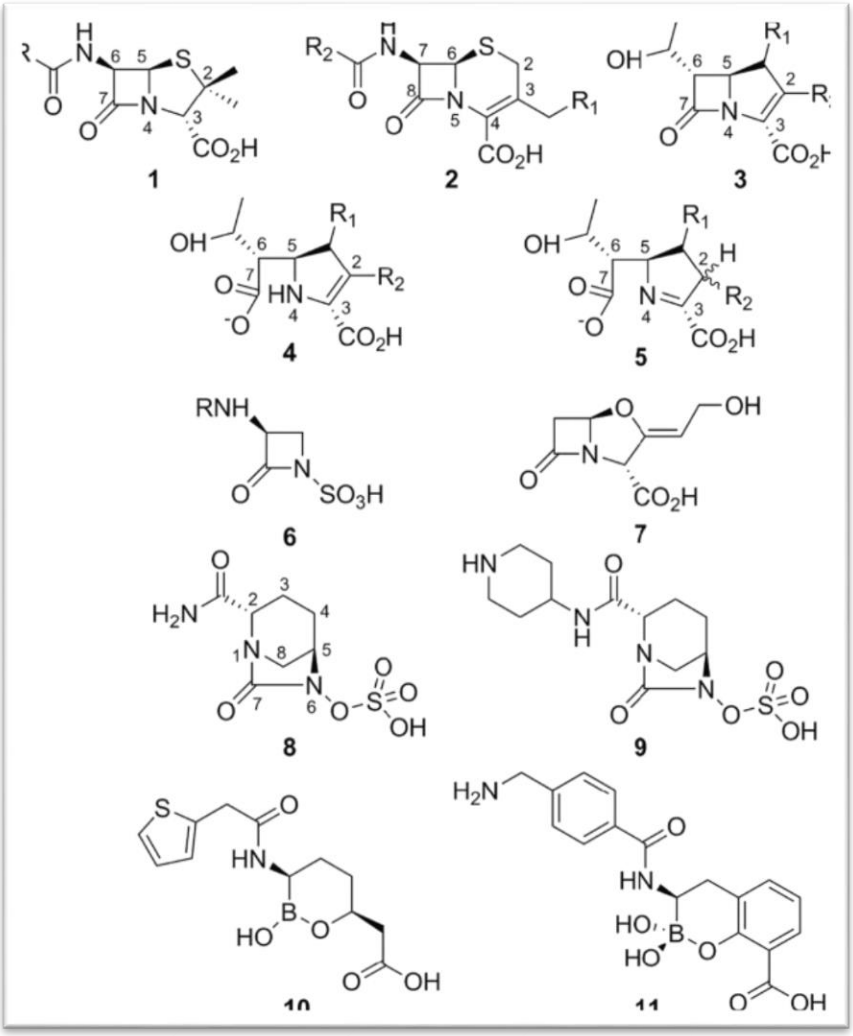

Figure 3. Constituent structures of beta-lactams and beta-lactamase inhibitors Hydrolyzed carbapenem (2Δ-pyrroline form). 5. carbapenem (1Δ-pyrroline) hydrolyzed form)(Tooke et al., 2019).

2.4.2.2 Resistance caused by changing the target molecule

As a common mechanism of resistance **(Figure 4)**, bacteria can alter their target sites for antibiotics. The target site of an antimicrobial agent can change by a spontaneous mutation, or a bacteria can gain drug resistance genes from another bacteria across genetic manipulation (conjugation, transduction, or transformation), which is how bacteria get their resistance genes(Lambert, 2005). Consider the following: Fluoroquinolone resistance results from changes in the primary site of action in gram-negative bacteria (DNA gyrase), a drug that targets nucleic acid synthesis(C Reygaert & Department of Biomedical Sciences, Oakland University William Beaumont School of Medicine, Rochester, MI, USA, 2018).

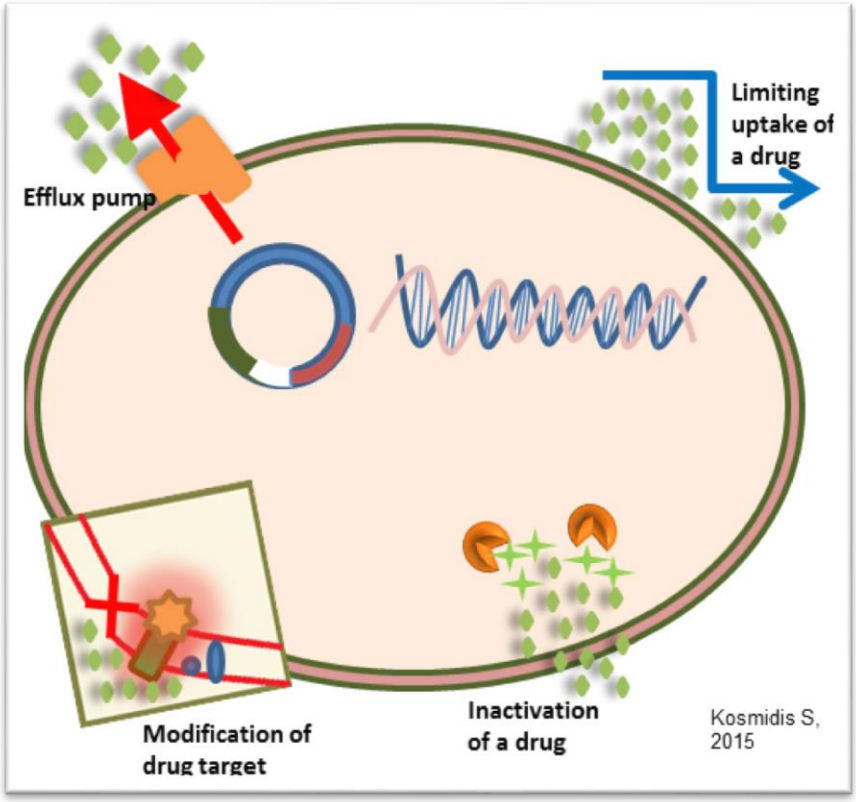

Figure 4. common antimicrobial resistance mechanisms(C Reygaert & Department of Biomedical Sciences, Oakland University William Beaumont School of Medicine, Rochester, MI, USA, 2018).

2.4.2.3 Change of cell wall permeability and resistance with active pump systems

Gram-negative bacteria have an outer hydrophobic membrane (LPS) that allows the bacteria to resist antimicrobial agents by changing the permeability of the cell wall. *Enterobacteria* such as *Klebsiella*, *Enterobacter*, *Serratia*, and *Salmonella* are the most common. Hydrophilic molecules can only pass through the aquatic pores of this outer hydrophobic membrane. A monomer component serves as an aqueous channel in the transmembrane pores. Pore diameters range from 1 to 1.2 nanometers. Changes in the number of pores or how well they work make it more difficult for antibiotics to

get into the cell(Delcour, 2009).In addition, there is a synergistic relationship between the permeability barrier and the active efflux pump in the intracellular penetration of bacterial cells. In order to protect bacteria from antibiotics, the outer membrane (OM) and efflux pump select compounds based on their particular properties(Krishnamoorthy et al., 2017).

Efflux pumps transport a broad variety of substances from the bacterial cell in order to remove potentially harmful substances (multi-drug [MDR] efflux pumps). Efflux pump genes are chromosomally encoded in bacteria. When a suitable substrate or environmental stimuli are present, some expressions are innate, regardless of environmental influences, while others are turned on or highly expressed (high-level resistance usually occurs by a mutation that alters the transport channel). Bacterial efflux pumps are categorized into five main groups based on their configuration and power source **(Figure 5)**, ABC (ATP-binding cassette family), MFS (major facilitator superfamily), MATE (multidrug and toxin extrusion) family, SMR (small multidrug resistance) family, and RND (resistance-nodulation-cell division) family. In gram-negative bacteria, efflux pumps can be found in all five families, with the RND family having the greatest clinical impact(C Reygaert & Department of Biomedical Sciences, Oakland University William Beaumont School of Medicine, Rochester, MI, USA, 2018). *K. pneumoniae* AcrRAB is an RND-based tripartite efflux pump, contributing to the phenotype of multidrug resistance(Padilla et al., 2010).

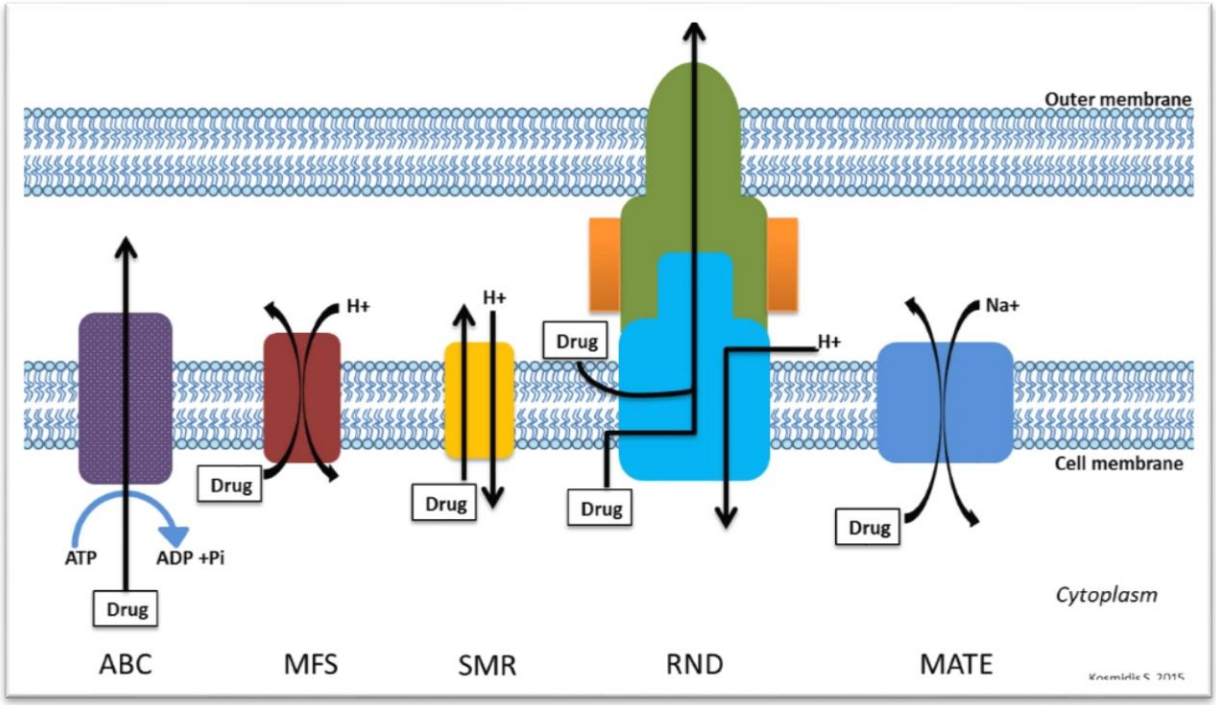

figure 5. five main efflux pump families (C Reygaert & Department of Biomedical Sciences, Oakland University William Beaumont School of Medicine, Rochester, MI, USA, 2018)**.**

2.5 Carbapenem and the mechanism of resistance

2.5.1 Carbapenems

In 1976, the first beta-lactamase inhibitors were discovered, which were naturally occurring by *Streptomyces clavuligerus*, a gram-positive bacterium, called Olivanic acids (compound 1), which have a "carbapenem main structure". The olivanic acids were no longer used because of their chemical instability and limited ability to get inside the bacteria's cells. Soon after, two new beta-lactamase inhibitors were discovered: clavulanic acid (compound 2) from *Streptomyces clavuligerus*, the first -lactamase inhibitor to enter clinical use, and thienamycin (compound 3) from *Streptomyces cattleya*. When Thienamycin was introduced, it became known as the first carbapenem. It would later be used as the patterning compound for all carbapenems. Carbapenems are β-lactam antibiotics that have a wide range of activity. Patients who have infections that have become life-threatening or who are thought to have antibiotic-resistant bacteria often get these as "last-line agents"(Papp-Wallace et al., 2011).

Carbapenems (imipenem, meropenem, or doripenem) can be used to treat both gram-positive and gram-negative bacteria, as well as anaerobic bacteria, but they are ineffective upon *Enterococcus faecium*, methicillin-resistant *Staphylococcus aureus*, and *Stenotrophomonas maltophilia*. Ertapenem's spectrum of activity is narrower than that of imipenem, meropenem, and doripenem because it lacks the ability to treat *Pseudomonas aeruginosa* and *Enterococcus spp*. Ertapenem, which has a half-life of about 4 hours, can be given once a day because it has a longer half-life than imipenem or meropenem(Zhanel et al., 2007).

Outer membrane proteins (OMPs), also referred to as porins, allow carbapenems to enter gram-negative bacteria. Carbapenems acylate PBPs after passing through the periplasmic space, and then peptide cross-linking and other peptidase reactions can be inhibited by this compound. leading to PBPs being inhibited. Carbapenems are capable of binding to a variety of PBPs. When PBPs are inhibited, autolysis continues because

cell wall formation is a versatile (three-dimensional) process in which formation and cell lysis occur simultaneously. As the peptidoglycan starts to break down, the cell eventually ruptures as a result of osmotic pressure(Papp-Wallace et al., 2011).

2.5.2. Carbapenem resistance mechanisms

Many types of gram-negative bacteria (e.g., *Pseudomonas spp*., *Acinetobacter spp*., and *Stenotrophomonas spp*.), and also the *Enterobacteriaceae* (e.g., *Klebsiella spp*., *Escherichia coli*), while also gram-positive bacteria (e.g., *Staphylococcus spp*., *Streptococcus spp*., *Nocardia spp*.), are capable of resisting the majority of currently useful carbapenems, which is a major public health issue. Some bacteria, like *Klebsiella pneumoniae*, *P. aeruginosa*, and *A. baumannii*, can resist carbapenems by a combination of resistant mechanisms. in which beta-lactamases, efflux pumps, and mutations affecting porins and PBPs expression and/or function are among the methods used(Papp-Wallace et al., 2011).

Different authors have classified beta-lactamases in various ways. Ambler's classification is currently the most commonly used **(figure 6)**. Ambler proposed four classifications for beta-lactamases: A, B, C, and D. These are Ambler's proposed classifications of beta-lactamases. An A and D are serine beta-lactamases, class B Metallo-beta-lactamases, and class C cephalosporinases. Since the 1980s, this classification has been used to distinguish beta-lactamases that cannot be suppressed by the compound EDTA (ethylenediaminetertraacetic acid) Serino-beta-lactamases, and those that could, Metallo-beta-lactamases. Carbapenemases are discovered in groups A, B, and D, so classes A and D are serine-carbapenemases, while class B is Metallo-carbapenemases(Quispe Pari et al., 2018).

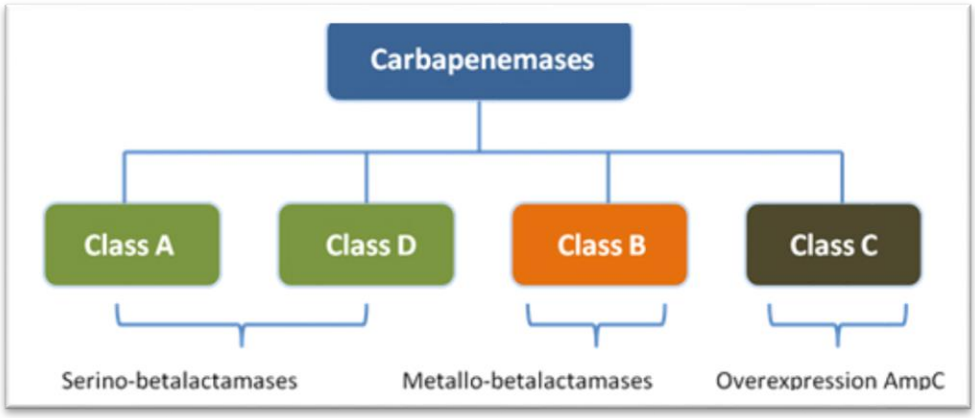

Figure 6. Ambler classification of carbapenemases(Quispe Pari et al., 2018).

Resistance to carbapenem is linked to two key mechanisms in K. pneumonia (Cr-KPN). The capacity to create beta-lactamases capable of hydrolyzing cephalosporins, like AmpC cephalosporinase e.g., DHA-1 and CMY-2, or ESBL, e.g., CTX-M-2, in combined effect with reduced membrane permeability in the cell wall, is the first. Mechanism number two promotes resistance by producing beta-lactamases, which are responsible for hydrolyzing most beta-lactam antimicrobial drugs, including carbapenems. If you look at the Ambler classification, it falls into one of three categories: group A (*K. pneumoniae* carbapenemase KPC), group B, or Metallo-β-lactamases (MBL) (New Delhi Metallo-β-lactamases, NDM), and group D oxacillinases (OXA-48-like carbapenemases), Furthermore, KPC-Kp has the capability to survive in human reservoirs and form biofilms that protect against hospital sanitization guidelines(Reyes et al., 2019).

Carbapenemases of class A alter the distance between active site compounds, leading to an alteration of the active site's overall shape. Class B β-lactamases require Zn2+ cations, which have been classified into three groups based on sequence alignments and structural analysis: B1, B2, and B3. Hydrolysis of carbapenems occurs

in all three groups. Class D β-lactamases hydrolyze β-lactams differently than class A and C enzymes(Papp-Wallace et al., 2011). In addition to inhibiting PBPs, OXA-48 enzymes are slow substrates for most serine-lactases, which hydrolyze the amide bond of a β-lactam to prevent it from reaching the PBP(Papp-Wallace et al., 2019).

2.5.3. Determination of Carbapenem resistance

Many clinical laboratories face a significant challenge in detecting carbapenem-resistant organisms (CROs). There are numerous Techniques that are phenotypic (observable type) and non-phenotypic for identifying carbapenem-resistant organisms. CR-GNB detection methods have evolved over the past decade. Phenotypic methods are used to detect carbapenemase enzyme activity, including growth-based viability assays, rapid colorimetric analysis, immunochromatographic, and molecular-based techniques(Al-Zahrani, 2018).

A significant number of carbapenemase-producing isolates have minimum inhibitory concentrations (MICs) that are close to the breakpoints of susceptibility, thus camouflaging the presence of resistance, especially in automated systems. Because of this, as a first-line screening method, breakpoints for disc diffusion susceptibility are necessary. We can categorize the carbapenemase detection tests into three broad categories: screening, confirmatory, and molecular (PCR, Sequencing). The screening category includes the disc diffusion technique, the Epsilometer test (E-test), as well as automated antibiotic sensitivity testing systems. The confirmatory category includes the MHT (Modified Hodge test), boronic acid disc test, Impregnated disc test with ethylene diamine tetraacetic acid (EDTA), and the 2-mercaptopropionic acid disc test(Asthana et al., 2014).

Disc diffusion is a phenotyping-based tool for estimating susceptibility patterns. **(Figure 7)**. To determine if an organism is resistant to an antibiotic, However, one of the best indicators for carbapenemase activity is ertapenem(Al-Zahrani, 2018).

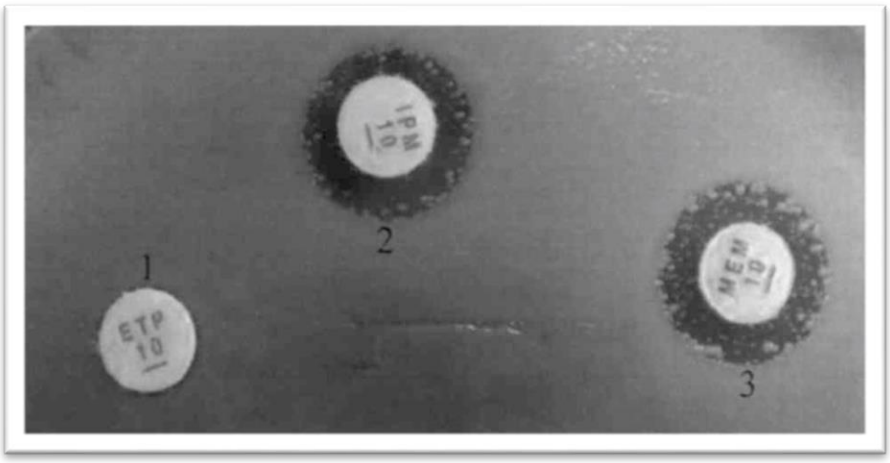

Figure 7. detecting carbapenem-resistant (Ye et al., 2017).

CHAPTER III

MATERIALS AND METHODS

3.1 Tools and Equipment

In this prospective study, we used rectal swabs **(Figure 8),** blood agar media, and Eosin methylene blue agar (EMB) for culturing the swabs. For antibiotic susceptibility and carbapenem-resistant identification, Mueller Hinton agar was used. For *Klebsiella spp*. identification, Triple Sugar Iron media (TSI), Simmons Citrate media, and Sulphide Indole Motility medium (SIM) were used. A 35-degree autoclave was used to incubate samples. A McFarland turbidity of 0.5 was measured by the densitometer **(Figure 9)** and antibiotic discs of Imipenem (10µg), Meropenem (10µg), and Ertapenem (10µg) were used.

Figure 8. Sterile swab.

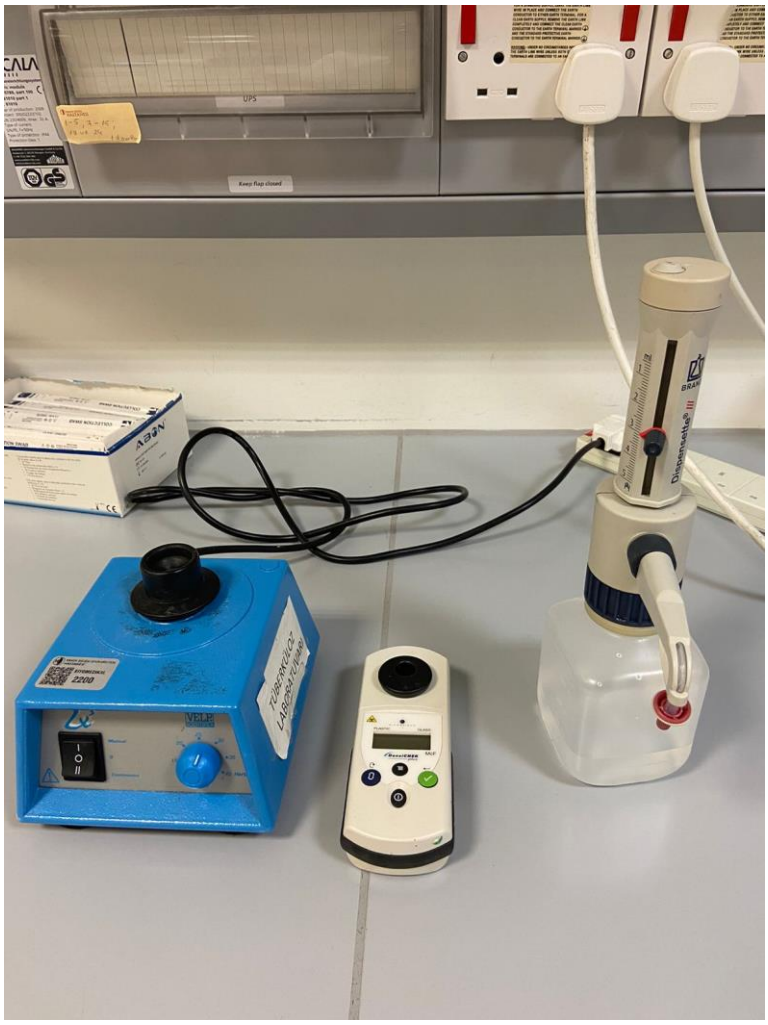

Figure 9. McFarland Densitometer 0.5 at NEU microbiology lab.

3.2. Study Group

Intensive care unit admissions at Near East University Hospital included all patients (ICU) who were checked with a rectal swab from January 2022 to March 2022. Ethical approval was obtained.

3.3. Culture of the Samples

A total of 87 clinical samples from the ICU had been cultured using conventional methods. A clinical sample from each patient was simultaneously cultivated on blood agar (Becton, Dickinson) and Eosin Methylene Blue Agar EMB (Becton, Dickinson), which were prepared in accordance with the directions provided by the manufacturer. The samples were placed in the incubator aerobically for 24 hours at 37 degrees Celsius. For the preparation: BBL™ Blood Agar Base (Infusion Agar), BD: in 1000 ml of purified water, 40 g of the powder was dissolved, thoroughly mixed, and autoclaved at 121 °C for 15 minutes. After being cooled down, 5% sterile defibrinated blood was added. BD BBL™ Eosin Methylene Blue Media, Levine: 37.4 gram of powder in 1000 ml of water, mixed well, and autoclaved at 121 °C for 15 min.

3.4. Identification of the *Klebsiella spp.*

We observed colony morphology and growth on both blood and EMB agar media after 24 hours of incubation. Three different biochemical tests were used to identify and distinguish *Klebsiella* spp.

3.4.1. Citrate Test

BBL™ Simmons citrate agar BD was prepared according to the company's instructions: 42.2 gram of the powdered agar was dissolved into 1 L of distilled water, mixed thoroughly, then autoclaved for 15 minutes at 121 °C, and used to identify Klebsiella spp. as they were able to utilize citrate as a carbon source. Using wire loops, we stabbed a few colonies from the culture plates into the citrate media tube. and incubated them aerobically for 24 hours at 35°C. The result was interpreted based on the colour change of the bromothymol blue pH indicator.

3.4.2. TSI Test

Another method for identifying *Klebsiella* was used. The TSI media had been prepared according to the manufacturer's instructions (cm0277 oxoid triple sugar iron agar), 65 g of powder was suspended in 1L of pure water and autoclaved for 15 minutes at 121 °C. We took small amounts of colonies from the growing culture media by inoculum needle. The bacterial colonies were stabbed into the TSI media tubes by an inoculum needle and streaked the surface of the agar media before removing the inoculum needle from the test tube **(Figure 10)**, covered loosely by cotton, and incubated aerobically at 35 °C for 24 hours. We interpreted the results after the incubation period based on the colour change of the media after *Klebsiella pneumonia* fermented the sugar and produced a small amount of gas.

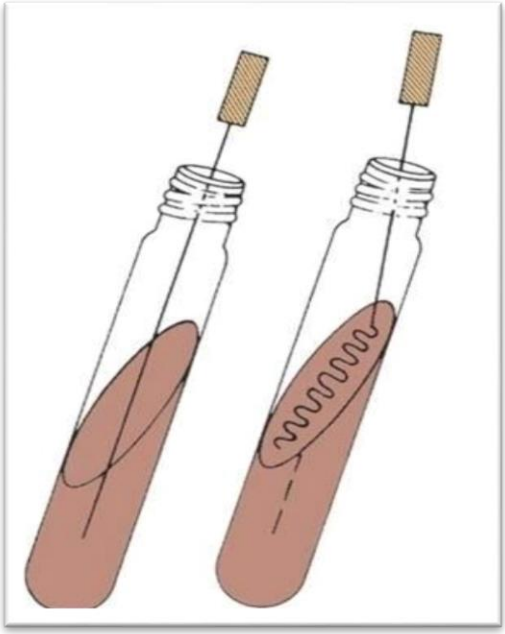

Figure 10. Inoculation of a Triple Sugar Iron agar tube.

3.4.3. SIM Test

Klebsiella spp. were recognized and distinguished using the Merck KGaA SIM medium. After being prepared in accordance with company instructions by adding 30 g of agar powder to 1 L of purified water, mixing until completely dissolved, and autoclaved at 121 °C for 15 minutes. We touched the colony of bacteria with a straight needle and stabbed it into the SIM medium tube. It was then incubated aerobically for 24 hours at 35 °C. We interpreted the results depending on the presence of bacterial growth at the site where it was stabbed and the indole negatively.

3.5. Determination of Carbapenem Resistance

Klebsiella. *pneumonia* carbapenem resistance was determined using a phenotyping method called disc-diffusion.

3.5.1. Disc Diffusion Method

The disc diffusion method was used to test carbapenem susceptibility (Imipenem, Meropenem, Ertapenem). BD Mueller Hinton agar was used, and the agar plates were prepared according to the manufacturer's instructions by dissolving 21g of the powder in 1L of purified water and autoclaving at 121 °C for 15 minutes. By using sterile cotton swabs, bacterial colonies were inoculated in saline to achieve a suspension of 0.5 McFarland turbidity, and then the agar plates were inoculated with the *K. pneumonia* suspension by using sterile cotton swabs. Antimicrobial-containing discs of Imipenem (10µg), Meropenem (10µg), and Ertapenem (10µg) were added to the plates, which were then incubated at 35 °C aerobically for 24hr **(Figure 11).** We used a ruler to measure the inhibition zone around antimicrobial-containing discs. To interpret the results, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines were used after incubation(Giske et al., 2021).

Figure 11. IMP, MEM, and ETP susceptibility on M.H agar at NEU microbiology lab.

3.6. Statistical Analysis

The Pearson's x2 test or Fisher's exact test were used to compare two proportions. SPSS (Statistical Package for the Social Sciences) Demo Ver 22 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis, and a p-value < 0.05 was considered statistically significant.

CHAPTER IV

RESULTS

In our study, rectal swab samples were collected from 87 admitted patients in the intensive care units and investigated for carbapenem-resistant *Klebsiella pneumoniae* (*K. pneumoniae*) strains. Our patients were divided into 57 (65.5%) males and 30 (34.5%) females, with an average age of 70.98±14.11 years (between 26-93 years). *Klebsiella pneumoniae* was found in 38 (43.7%) of these patients. All of these strains (100%) were found to be carbapenem-resistant strains. The resistance profiles and antibiotic zone diameters of the strains to carbapenem group antibiotics are shown in **(Table 1)**. Accordingly, it was determined that *K. pneumoniae* strains showed resistance to IPM 100% (38/38), MEM 71.1% (27/38), and ETP 86.8% (33/38). The mean of IPM, MEM, and ETP zone diameters were 16.87±7.42, 16.11±10.05, 7.74±12.10, respectively.

Table 1. Carbapenem resistance of *K. pneumoniae* strains

No	IPM zone diameter	IPM	MEM zone diameter	MEM	ETP zone diameter	ETP
1	30	Resistant	35	Sensitive	29	Sensitive
2	30	Resistant	32	Sensitive	33	Sensitive
3	30	Resistant	45	Sensitive	40	Sensitive
4	35	Resistant	37	Sensitive	30	Sensitive
5	21	Resistant	22	Sensitive	30	Sensitive
6	12	Resistant	15	Resistant	8	Resistant
7	25	Resistant	30	Sensitive	23	Resistant
8	30	Resistant	29	Sensitive	20	Resistant
9	31	Resistant	33	Sensitive	20	Resistant
10	20	Resistant	23	Sensitive	20	Resistant
11	23	Resistant	22	Sensitive	19	Resistant
12	25	Resistant	23	Sensitive	10	Resistant
13	18	Resistant	12	Resistant	0	Resistant
14	12	Resistant	9	Resistant	0	Resistant
15	13	Resistant	11	Resistant	0	Resistant
16	9	Resistant	3	Resistant	0	Resistant
17	15	Resistant	12	Resistant	0	Resistant
18	14	Resistant	10	Resistant	0	Resistant
19	10	Resistant	4	Resistant	0	Resistant
20	11	Resistant	10	Resistant	0	Resistant
21	10	Resistant	10	Resistant	0	Resistant
22	15	Resistant	13	Resistant	0	Resistant
23	10	Resistant	11	Resistant	0	Resistant
24	11	Resistant	15	Resistant	10	Resistant
25	16	Resistant	11	Resistant	0	Resistant
26	11	Resistant	10	Resistant	0	Resistant
27	10	Resistant	8	Resistant	0	Resistant
28	12	Resistant	10	Resistant	0	Resistant
29	12	Resistant	10	Resistant	0	Resistant
30	15	Resistant	11	Resistant	0	Resistant
31	14	Resistant	12	Resistant	0	Resistant
32	10	Resistant	9	Resistant	0	Resistant
33	13	Resistant	10	Resistant	0	Resistant
34	15	Resistant	12	Resistant	0	Resistant
35	11	Resistant	10	Resistant	2	Resistant
36	12	Resistant	11	Resistant	0	Resistant
37	15	Resistant	10	Resistant	0	Resistant
38	15	Resistant	12	Resistant	0	Resistant

*IPM: Imipenem; MEM: Meropenem; ETP: Ertapenem

There was no correlation between the presence of carbapenem-resistant *K. pneumoniae* in rectal swab samples of intensive care patients and gender (p=0.339). Again, there was no evidence of a statistically significant relationship between the patients with carbapenem-resistant *K. pneumoniae* strains and the mean age of the others (p=0.235).

CHAPTER V

DISCUSSION

The occurrence of carbapenem-resistant *Klebsiella pneumonia* hospital infections (CRKP)is one of the world's most serious health issues(Karampatakis et al., 2016). For a long time now, carbapenems have been considered the last-ditch option for treating infections and diseases promoted by multidrug-resistant gram-negative bacteria. That's why The introduction and spread of ESBL and carbapenemase genes in K. pneumonia isolates create a substantial public health risk(Effah et al., 2020). According to the Centers for Disease Control and Prevention's (CDC) National Healthcare Safety Network (NHSN), *Klebsiella spp*. is one of the most commonly reported pathogens responsible for healthcare-associated infections (HAIs)(Weiner-Lastinger et al., 2020, pp. 2015–2017). Because of the high prevalence of CRKP, infection control measures will be strictly enforced. It is becoming increasingly important to find new ways to combat bacterial resistance(Karruli et al., 2019).

New reports on the distribution of carbapenem-resistant *Klebsiella pneumoniae* strains isolated are being received from around the world. Carbapenem-resistant *Klebsiella* species outbreaks have been increasing in frequency around the world, including in Turkey(Dizbay et al., 2014). Carbapenem-resistant *K. pneumoniae* colonization and infection have been reported in Southeast Asia. According to the National Surveillance of Antimicrobial Resistance in Malaysia, the rate of *K. pneumonia* that was resistant to carbapenem rose from 0.5% in 2010 to 1.6% in 2014. In addition to Morocco, Italy, and India, all three countries they've grown tremendously in recent years. According to previous studies, it's possible that patients already have these strains when they come to the hospital, either from another hospital they were sent to or from the community. In the community, they may have gotten their strain from a previous healthcare encounter or by being around reservoirs or relatives who have these strains(Saharman et al., 2020).

Another European study found that *Klebsiella* strains that are resistant to antibiotics not only colonize patients but can also infect patients and cause serious infections. There were a number of invasive infections that were also common, including pneumonia, surgical site infections, and bacteremia. As a result of these resistant microbes, CRKP has also been linked to invasive infections like bloodstream infections and high mortality rates. An additional issue is an emergence of carbapenem-resistant isolates from individuals who were not in intensive care units (ICUs) and dissemination through routine clinics, collective outpatient units such as healthcare settings, haemodialysis, etc.(Candevir Ulu et al., 2017).

Prior research looked into the factors that could lead to multidrug-resistant bacteria colonization or infection. Being in the intensive care unit increases the likelihood of acquiring and colonizing *Klebsiella pneumonia*, which is resistant to carbapenems(Nseir et al., 2010). Nevertheless, there are other multivariable factors that influence Carbapenem-resistant *Klebsiella pneumonia* colonization and infection, including diabetes mellitus, prior antibiotic administration, people in nursing homes, using a urinary catheter, and having an aggressive tumor that has spread all over the body(Borer et al., 2012).

In this study, the colonization of *Klebsiella pneumonia* in the guts of intensive care unit patients at Near East University was investigated. And we found that 38(43.7%) of 87 patients were colonized, and 100% of those (43.7%) were found to be carbapenem-resistant strains. In addition, there was no relationship between gender (p-value = 0.339) or mean age (p-value = 0.235) of patients in the *Klebsiella pneumonia* infection. Previous research has shown that *K. pneumonia* is a major ICU pathogen with a wide range of antimicrobial susceptibilities, and our findings support this(Al Bshabshe et al., 2020). On the other hand, previous research in China found that patients aged ≥ 60 years are more likely to become infected with CRKP and colonized(Hu et al., 2020, pp. 2008–2018). Further, a previous study conducted in Greece showed that the male gender is more susceptible to colonizing carbapenemases–producing *K. pneumonia* (KPC-Kp) than the female(Papadimitriou-Olivgeris et al., 2013).

In accordance with the European Centre for Prevention and Control of Disease (ECDC), there has been an increase in antimicrobial resistance over the years. especially among Carbapenem-resistant *Klebsiella pneumonia*(Peñalva et al., 2019). In our study, we discovered that all isolates (38\38) were resistant to Imipenem, 71.1% (27\38) were resistant to Meropenem, and 86.8% (33\38) were resistant to Ertapenem. A previous study in Greece discovered that 100% of the isolates were resistant to Ertapenem, despite being susceptible or intermediate to Meropenem, and that all of the isolates were susceptible to Imipenem(Poulou et al., 2013). It turns out that other studies in Saudi Arabia found that the rate of Ertapenem and Meropenem resistance was high compared with Imipenem(Al Bshabshe et al., 2020). Other study participants included carbapenemase-producing Klebsiella pneumonia patients in the ICU of a public hospital in KwaZulu-Natal, South Africa. When tested for carbapenemase production, all of the isolated strains 100% were found to be resistant to ertapenem and meropenem, and 71.4% were resistant to imipenem(Madni et al., 2021).

There were some limitations to this study. The first is that the small sample size may have hampered the discovery of statistically significant associations between demographic variables (i.e., age and gender). The second limitation was that all of the data came from only one intensive care unit in a single hospital, which means Carbapenem-resistant *Klebsiella pneumonia* colonization may not be representative of the entire country. This study was also limited by the lack of data on molecular biology techniques, more research into the genes responsible for carbapenem resistance *Klebsiella pneumonia* may be beneficial.

CHAPTER VI
CONCLUSION AND RECOMMENDATIONS

The colonization of *Klebsiella pneumonia* with carbapenem resistance is concerning, and health authorities must pay close attention to reducing nosocomial infections, particularly in ICU settings, as well as an increase in the occurrence of severe and complicated infections, as well as an increase in mortality rates, particularly among critically ill patients. The experimental study's findings led to the conclusion that the carbapenem-resistant *Klebsiella pneumonia* (CRKP) strains are circulating in the intensive care unit (ICU) patients at Near East University at moderate prevalence, according to the results of an experimental study. An effective surveillance strategy must be developed for intensive care patients because they are the most vulnerable.

Future research should be conducted to support the main the findings of this study. As a result, researchers should try to find an answer to this question. And fully understanding the virulence patterns of *Klebsiella pneumonia* strains leads to colonizing the guts of patients, particularly intensive care patients, and using molecular biology techniques to determine the carbapenem-resistant pattern of *Klebsiella pneumonia* and the presence of carbapenem-resistant hypervirulent *K. pneumonia* (CR-hvKP) in the Turkish Republic of Northern Cyprus (TRNC).

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APPENDICES

Appendix A

YAKIN DOĞU ÜNİVERSİTESİ
BİLİMSEL ARAŞTIRMALAR ETİK KURULU

ARAŞTIRMA PROJESİ DEĞERLENDİRME RAPORU

Toplantı Tarihi :24.02.2022

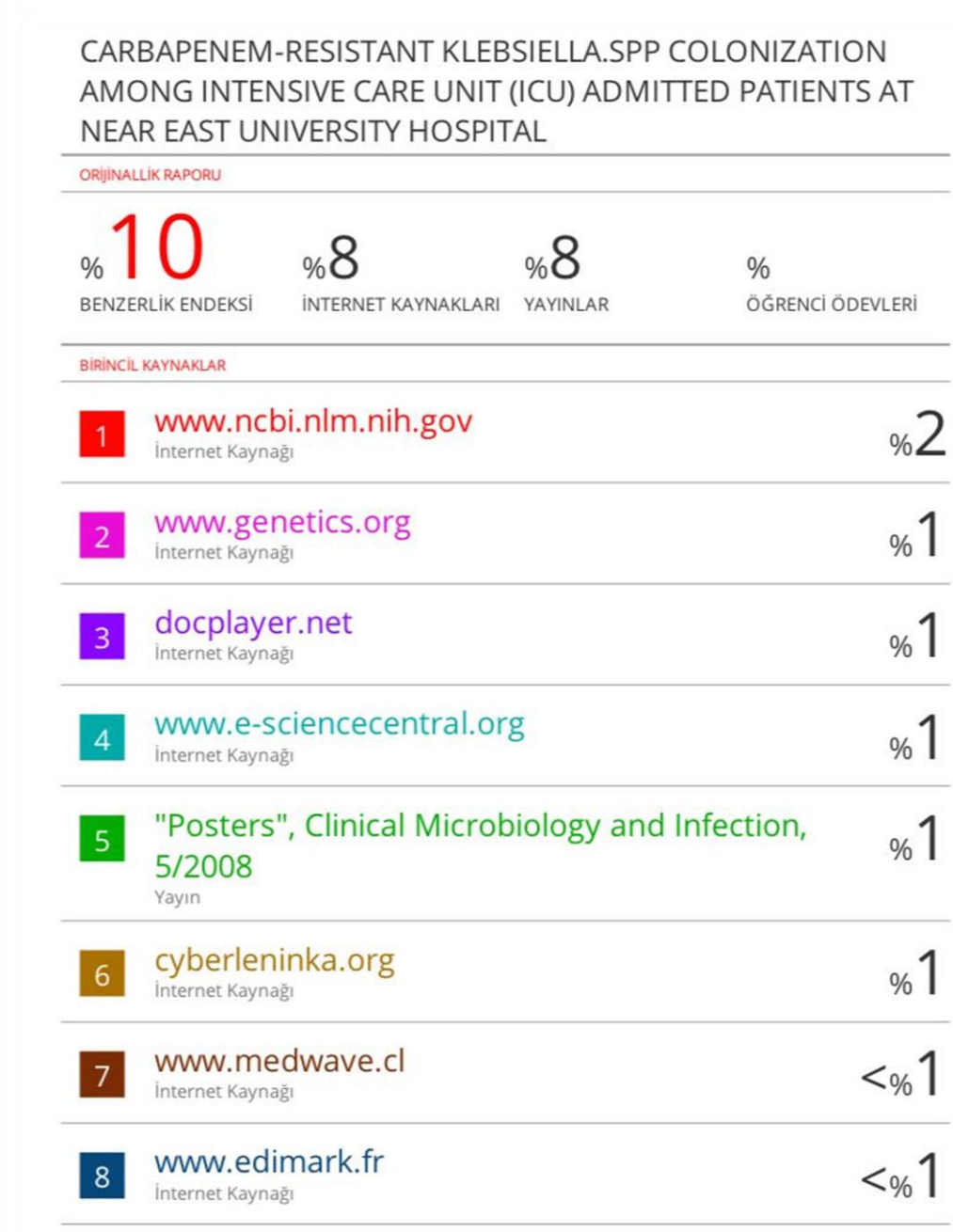
Toplantı No :2022/100

Proje No :1495

Yakın Doğu Üniversitesi SHMYO öğretim üyelerinden Doç. Dr. Meryem Güvenir'in sorumlu araştırmacısı olduğu, YDU/2022/100-1495 proje numaralı ve **"Yoğun Bakım Ünitelerinde İzlenen Hastaların Rektal Sürüntü Örneklerinde Karbapenem Dirençli *Klebsiella Pneumoniae* Kolonizasyonunun Araştırılması"** başlıklı proje önerisi kurulumuzca değerlendirilmiş olup, etik olarak uygun bulunmuştur.

Prof. Dr. Şanda Çalı
Yakın Doğu Üniversitesi
Bilimsel Araştırmalar Etik Kurulu Başkanı

Turnitin Similarity Report



CARBAPENEM-RESISTANT KLEBSIELLA.SPP COLONIZATION AMONG INTENSIVE CARE UNIT (ICU) ADMITTED PATIENTS AT NEAR EAST UNIVERSITY HOSPITAL

ORİJİNALLİK RAPORU

%10	%8	%8	%
BENZERLİK ENDEKSİ	İNTERNET KAYNAKLARI	YAYINLAR	ÖĞRENCİ ÖDEVLERİ

BİRİNCİL KAYNAKLAR

1	www.ncbi.nlm.nih.gov İnternet Kaynağı	%2
2	www.genetics.org İnternet Kaynağı	%1
3	docplayer.net İnternet Kaynağı	%1
4	www.e-sciencecentral.org İnternet Kaynağı	%1
5	"Posters", Clinical Microbiology and Infection, 5/2008 Yayın	%1
6	cyberleninka.org İnternet Kaynağı	%1
7	www.medwave.cl İnternet Kaynağı	<%1
8	www.edimark.fr İnternet Kaynağı	<%1

CURRICULUM VITAE

1. PERSONAL INFORMATION

NAME, SURNAME: **DATE of BIRTH and PLACE:**	HEDAYA OTHMAN MOHAMMED HASSAN 12\06\1986 Kuwait
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2. EDUCATION

YEAR	GRADE	UNIVERSITY	FIELD
2008	B.SC.	APPLIED UNIVERSITY	MEDICAL ANALYSIS

3. Job Experiences

Duration	DUTY	Institution
(01\03\2012)-(30\11\2019)	Senior Medical Laboratory Technologist	AL YADODAH Medical Lab
(01\03\2008)-(01\06\2011)	Lab Technician	AL-QADRI Medical Labs

4. FIELD OF INTERESTS

FIELDS OF INTERESTS	KEY WORDS
Medical Microbiology	Nosocomial infection ,Klebsiella pneumonia, colonization , ICU infection.