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Detection of Carbapenem Resistant Genes in Klebsiella pneumoniae from Various Clinical Samples.

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Detection of Carbapenem-Resistant Genes in *Klebsiella pneumoniae* from Various Clinical Samples.

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Thesis Approval

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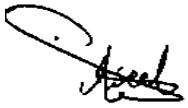
Declaration

I certify that this thesis, submitted for the Master's degree, is the result of my own research, unless otherwise acknowledged. I also confirm that this study, or any part of it, has not been submitted for a higher degree at any other university or institution.

Signed: 

Aseel Khaled Jamal ALSharif

Date: 09/08/2025

Dedication

I dedicate this work to all my family, especially to my father and mother for their patience to help me in my duties for so long as well as for supporting me continuously.

I also dedicate this work to my brothers and sisters whom always wanted to see me reaching here. To my teachers, supervisors and friends at al-Quds University who have been supporting me through the master thesis work. I will always appreciate all what they have done.

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Abstract

Background: *Klebsiella pneumoniae (K. pneumoniae*) is a significant cause of hospital-acquired infections. Penicillin, cephalosporins, and carbapenems are the β-lactam antibiotics that most frequently prescribed to treat this type of infection. However, this bacterium has become multidrug-resistant (MDR), and it is clear that rise resistance around the world lead to threaten public health on global scale. Carbapenem is often considered the last effective treatment option for multidrug-resistant gram-negative bacteria. Unfortunately, *K. pneumoniae* has developed resistance to the last effective beta-lactam antibiotic which is carbapenem. This resistance primarily results from the production of carbapenemases, such as *K. pneumoniae* Carbapenemase, KPC; Verona Integron Metallo Beta-lactamase, VIM; New Delhi Metallo-β-Lactamase-Mediated Carbapenem, NDM; and Oxacillin-Hydrolyzing Carbapenemases, OXA-48, which can be acquired in both hospital and community settings.

Aims: *K. pneumoniae* strains resistant to carbapenem (CR-Kp) are major health care-associated pathogens found globally. This study aimed to reveal the antibiotic resistance of clinical CR-Kp isolates and to find out whether carbapenemase genes, such as *bla* KPC, *bla* VIM, *bla* NDM, and *bla* OXA-48, are present, using multiplex PCR techniques.

Methodology: A total of 100 bacterial isolates were collected from Prince Alia Governmental Hospital in Hebron, Palestine. The isolates were identified as *K. pneumoniae* based on their colony morphology and subsequently confirmed using the VITEK 2 Compact system. Antibiotic susceptibility testing (AST) was determined using the same system. The ESBL was conducted by the double disk synergy test (DDTS). The screening for carbapenem resistance was carried out using meropenem (MEM) disks, followed by multiplex PCR to detect the presence of the carbapenemase genes *bla* KPC, *bla* VIM, *bla* NDM, and *bla* OXA-48

Result: Out of the 100 *K. pneumoniae* isolates included in this study, analysis indicated that 77 of the isolates (77%) showed extended-spectrum beta-lactamase (ESBL). Out of 100 isolates 35 representing (35%) were carbapenem resistant (CR). All of the 35 isolates were tested for the presence of carbapenemase genes. The results for the genes of carbapenemase were as follows: The *bla* OXA-48 gene was found in 32 of the 35 samples (91.4%); the *bla* NDM gene was in 29 of the 35 samples (82.8%); and the *bla* KPC gene was in 2 of the 35 samples (5.7%). However, the *bla* VIM gene was not found in any of the samples (0.0%). The co-occurrence of both *bla* NDM and *bla* OXA was observed in 26 out of 30 samples (74%). Conversely, the co-occurrence of *bla* KPC and *bla* OXA was found in only one isolate (28%).

Additionally, fifteen isolates that tested positive for extended-spectrum beta-lactamases (ESBL) but were sensitive to the meropenem (MEM) disk (carbapenem sensitive) were randomly chosen to be checked for carbapenemase genes. Out of the 15 isolates, blaVIM was found in 7 out of 15 samples (46.6%); while the bla OXA-48 gene was found in 4 out of 15 samples (26.6%). In contrast, the *bla* NDM gene was detected in only one sample (6.7%). Conversely, the co-occurrence of *bla* VIM and *bla* OXA was found in 4 out of 15 samples (26.6%).

Conclusion: The study findings clearly indicated that the rate of carbapenem-resistant *Enterobacteriaceae* (CRE) produced by *K. pneumoniae* isolates was high, making it a significant cause of healthcare-associated infections. Resistance to carbapenems is not limited to carbapenem-resistant isolates; some strains exhibiting this resistance can also be found among extended-spectrum beta-lactamase (ESBL) isolates. To ensure effective treatment, it is important to keep track of the local molecular epidemiology of CRE-resistant genes, and/or another reliable assay.

Keywords: **K.pneumoniae, Carbapenemases gene, multiplex PCR, Antibiotic susceptibility. blaKPC, blaVIM, blaNDM, and bla OXA-48.**

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List of Abbreviation:

Abbreviation	Terms
ATCC	American Type Culture Collection
AK	Amikacin
AMC	Amoxicillin /clavulanic acid
BA	Blood agar
Bp	Base pair
CAZ-30	Ceftazidime
CIP	Ciprofloxacin
C-Kp	Classical *K. pneumoniae*
CLSI	Clinical Laboratory Standard Institute
Cm	Centimeters
CN	Gentamicin
CPS	Capsular polysaccharides
CRE	Carbapenem resistance Enterobacteriaceae
CR-Kp	Carbapenem resistant *K. pneumoniae*
CTX-30	Cefotaxime
DNA	Deoxyribonucleic acid
ESPL	Extended-Spectrum Beta lactamases
ETP	Ertapenem
FOX-30	Cefoxitin
HvKp	Hypervirulent *K. pneumoniae*
I	Intermediate
KPC	*K. pneumoniae* Carbapenemase

KpSC	*K. pneumoniae* species complex
LPS	Lipopolysaccharides
MBLs	Metallo-Beta lactamases
MDR	Multi-Drug-Resistant
MEM	Meropenem
Min	Minutes
ML	Micro-liter
NDM	New Delhi Metallo-β-Lactamase-Mediated Carbapenem
OXA	Oxacillin-Hydrolyzing Carbapenemases
PBP	Penicillin-Binding proteins
PCR	Polymerase Chain Reaction
R	Resistance
S	Sensitive
TAE buffer	Tris-Acetate-EDTA buffer
TZP	Piperacillin/Tazobactam
UTI	Urinary Tract Infection
VIM	Verona Integron Metallo Beta-lactamase

Chapter One

Introduction and literature review

1.1 Characteristic of *K. pneumoniae*

The genus *Klebsiella* belongs to *the Enterobacteriaceae* family, the *Klebsiella* genus currently encompasses a broad range of species, which includes those within the *K. pneumoniae* species complex(KpSC) as well as other *Klebsiella* species such as *K. oxytoca, K. terrigena, K. pasteurii, K. huaxiensis, K. indica, K. michiganensis, K. spallanzanii K. pasteurii, andK. michiganensis*. These species exhibit an average nucleotide identity of only 90% with KpSC. The KpSC lacks a formal taxonomic classification and typically refers to closely related species that demonstrate an average nucleotide identity of 95%–96% with *K. pneumoniae*. According to phylogroups classified under KpSC have been identified, which include *K. pneumoniae,K. quasivariicola, K. africana K. variicola subsp. variicola, K. quasipneumoniae*subsp. *quasipneumoniae, K. quasipneumoniae* subsp. *similipneumoniae* , and *K. variicola* subsp. *tropica*(Dong et al.,2022).

It is gram-negative bacillus, capsulated, rod-shaped, mucoid, facultatively anaerobic, non-motile, non-spore-forming. The protective polysaccharide capsule is characteristic of *K. pneumoniae*. According to biochemical tests,*K. pneumoniae* is catalase and urease positive and tests negative for H_2S, indole, and cytochrome oxidase (Salauddin et al., 2019).

K. pneumoniae is prevalent in the environment and isconsider as a part of the normal flora in humans, particularly in the mouth, nose, and gastrointestinal tract (Rawy et al., 2020). However, it can become an opportunistic pathogen, particularly affecting individuals with compromised or weakened immune systems or even people that had pre-existing health issues such as liver disease, kidney failure, diabetes, malignancy, and chronic obstructive pulmonary disease, among others (Li et al., 2022).

Furthermore, *K. pneumoniae* is frequently responsible for hospital-acquired infections and poses significant risk to patients in hospitals; it is responsible for 3% to 8% of cases (Ashurst et al.,2024). Contamination of medical equipment and blood products, worsened by poor hygiene and sanitation practices in healthcare settings, are the primary routes of transmission (Ostria-Hernandez et al., 2018).

1.2 Pathogenic potential and infection caused by *K. pneumoniae*

The initial stage of a *K. pneumoniae* infection begins with the bacteria colonizing the host. This process involves the adhesion of the bacteria to the surfaces of mucosal and epithelial cells such as the upper respiratory tract. Fimbriae, the hairy structures on the bacterial surface, aid in this attachment by binding to specific sites and contributing to biofilm formation. The pathophysiology and colonization of *K. pneumoniae* are influenced by a number of virulence factors. These include the production of fimbriae, outer membrane proteins (OMPs), iron-binding siderophores, capsular polysaccharides (CPSs), and lipopolysaccharides (LPSs) (Abbas et al., 2024).

The factor that is associated with virulence CPS and LPS are crucial in *K. pneumoniae* infections. The Outer membrane is made up of lipid A, a core part, and O-polysaccharide antigens, an essential component of LPS, while CPS is the outer layer of *K. pneumoniae* that helps it resist phagocytosis. Siderophores, also referred to as iron carriers such as aerobactin, yersiniabactin, enterobactin, and salmochelin are found in the extracellular capsule. Iron is a vital competitive resource for pathogenic bacteria; thus, acquiring and utilizing host iron is a crucial strategy for *K. pneumoniae* to survive and colonize the host, even in the presence of immune cells (Karampatakis et al., 2023).

K. pneumoniae is capable of causing infections in various sites of the body, such as the urinary tract, lower biliary tract, upper respiratory tract, and gastrointestinal tract (Bengoechea et al.,2019). Recently, an identified variant of *K. pneumoniae*, referred to hypervirulent *K. pneumoniae* (hvKp) exhibits greater virulence than the classical strain (cKp), and can cause community-acquired infections, even in people immune competent who do not suffer from disease or infection(Asokan et al.,2025).

First reported and initially documented in Asia in cases of liver pyogenic abscesses, hvKp has since spread globally and is associated with infections in the eye, lung, and central nervous system (CNS). Additionally, hvKp has been connected to primary extrahepatic infections, such as osteomyelitis, bacteremia,meningitis, pneumonia, sepsis, and bronchitis (Asokan et al.,2025).

In comparison to classical *K. pneumoniae*, hypervirulent *K. pneumoniae* are considered hyper mucoid. Both classical and hypervirulent *K. pneumoniae* strains produce lipopolysaccharide (LPS), but hypervirulent *K. pneumoniae* has unique sticky parts called type 1 and type 3 fimbriae that enhance its attachment to surfaces of the host. Both strains release small molecules known as iron-scavenging siderophores, which specific receptors

on their membranes pick up. For instance, classical *K. pneumoniae* strains produce enterobactin, while hypervirulent *K. pneumoniae* strains typically release different kinds of siderophores, such as aerobactin that represent mobile elements such as plasmids and conjugation are involved in acquisition of virulence factors (Sun et al.,2025) as illustrated in Figure 1.1(Karampatakis et al., 2023).

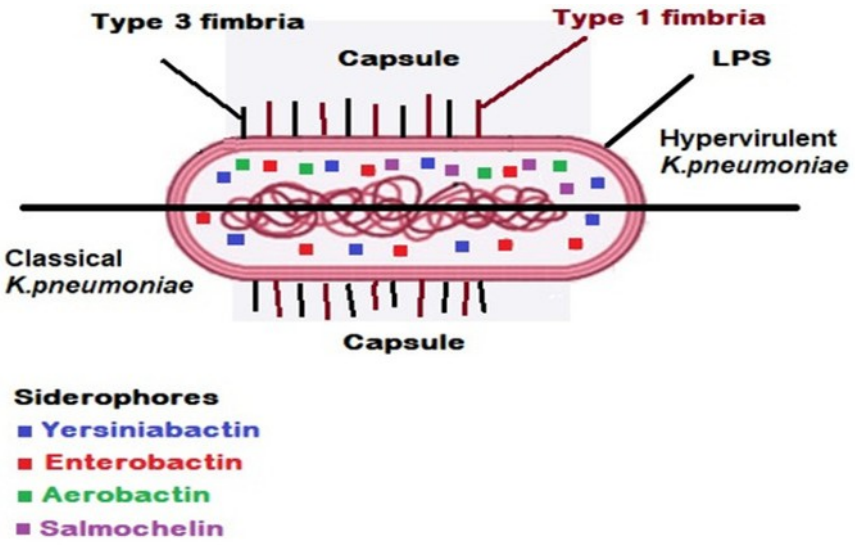

Figure 1.1: The difference between hypervirulence and classical *K. pneumoniae* (Karampatakis et al.,2023)

1.3Antibiotic and antibacterial resistance in *K. pneumoniae*

Treatment of *K. pneumoniae* involves a variety of antibiotics. Antibiotics with narrow spectrum and broad-spectrum such as cephalosporins, penicillins, carbapenems, aminoglycosides, and fluoroquinolones, have typically been used to treat these infections.The three beta-lactam antibiotics penicillins, carbapenems, and cephalosporins inhibit the formation of bacterial cell walls by focusing on penicillin-binding proteins (PBPs). The last stage of peptidoglycan synthesis, which is an important part of the bacterial cell wall, requires PBPs. Antibiotics that bind to inhibit PBPs stop peptidoglycan from properly cross-linking, weakening the cell wall and causing bacterial cell lysis (Sethuvel et al.,2023).

Nonetheless, bacterial resistance is currently on the rise; during the past 20 years, a notable increase in different multidrug-resistant (MDR) bacterial strains has been documented.bacterial isolate were classified as MDR when they showed resistance to at least one antibiotic in three distinct classes of antibiotics (Ristori et al.,2024).

Consequently, as the pathogen continues to evolve, *K. pneumoniae* is growing more and more multi-resistant. Conventional antibiotics are no longer effective against this pathogen due to the rise in drug resistance (Huy et al., 2024).

A significant factor contributing to this resistance such as the synthesis of extended-spectrum β-lactamases (ESBLs). Strains of *K. pneumoniae* that produce ESBLs show resistance to β-lactam antibiotics, which encompass penicillins, cephalosporins, and monobactams. Additionally, these strains frequently exhibit cross-resistance to other important classes of antibiotics, including aminoglycosides, fluoroquinolones, and chloramphenicol, thereby further restricting available treatment options (Riwu et al., 2020).

In Palestine, a study conducted by Rabaya et al, (2016) at "An-Najah National University in Nablus" analyzed the occurrence and molecular characteristics of extended-spectrum beta-lactamases (ESBLs) and metallo-beta-lactamases (MBLs) in clinical isolates of *K. pneumoniae* from various healthcare facilities between 2015 and 2016.The study found that a high number of these enzymes are present, ESBLs found in 92.2% and MBLs in 9.8% of the clinical samples "(Rabaya et al., 2016).

Carbapenems have traditionally been viewed as the final line of defense against multi-drug resistant (MDR) *K. pneumoniae*. However, carbapenem-resistance in *K. pneumoniae* bacteria has been detected globally in numerous healthcare facilities these CR-Kp strains exhibit resistance to any β-lactams and frequently to other critical treatment options (Annavajhala et al., 2019). A study carried out in Gaza from 2020 to 2022 found that the prevalence of carbapenem-resistant *K. pneumoniae* increased from 22.2% to 25.5% (Mansour et al., 2023).

1.4 Mechanism of resistance in *K. pneumoniae*

Different mechanisms enhance *K. pneumoniae* resistance to antibiotics. The most common ways that help *K. pneumoniae* resist antibiotics include the activity of efflux pumps, the formation of biofilms, point mutations that alter drug targets, reduced permeability of the membrane, and the production of certain enzymes like β-lactamases and aminoglycoside-modifying enzymes (Li et al., 2023).

1.4.1. Efflux pump

In *K. pneumoniae,* a number of MDR efflux pumps can be found comprising the major resistance-nodulation-division (RND), major facilitator superfamily (MFS), small multidrug resistance (SMR), and the antimicrobial extrusion protein family (MATE). Clinically, the efflux system of *K. pneumoniae* is dominated by the AcrAB-TolC and

OqxAB systems in the RND family, which is responsible for the energy-dependent expulsion of drugs (Li et al., 2024).

The AcrAB-TolC system is particularly influential in the resistance to β-lactam antibiotics. Regulatory genes associated with the AcrAB-TolC system, acrR and ramR, may undergo deletion mutations that result in the overexpression of the AcrAB-TolC efflux pump. This overexpression plays an important role in the resistance of *K. pneumoniae.* Additionally,the expression of a high level of its efflux pump is enhanced by the autosomal transfer of the OqxAB gene to plasmids. As a result, the heightened expression of OqxAB also exhibits a reduction in the susceptibility of *K. pneumoniae* to various antimicrobial drugs, which facilitates the development and emergence of drug resistance (Li et al., 2024).

1.4.2. Biofilm formation

This formation is a significant mechanism contributing to antibiotic resistance. Biofilms exhibit osmotic barrier properties that increase bacterial resistance to antimicrobial agents. Studies show that bacterial resistance to antimicrobial agents is ten times higher in the biofilm state than in the planktonic state (Li et al., 2024).

K. pneumoniae demonstrates a strong tendency to form biofilms through structures such as capsules and pili, which are essential for this process. These structures not only improve the bacterial adhesion to surfaces but also establish a protective environment that shields them from the host's immune system and antibiotic agents. Consequently, infections from *K. pneumoniae* that form biofilms are especially difficult to treat (Li et al., 2024).

1.4.3. Reduction of Membrane Permeability

K. pneumoniae acquires resistance through altering its outer membrane proteins (OMPs), especially porins. These outer membrane channels are mainly made-up from trimeric proteins that are primarily present in gram-negative bacteria, which enable antimicrobial drugs to enter the bacterium and bind to target proteins (Li et al., 2024). When mutations occur in *K. pneumoniae*, the outer membrane pore proteins may be absent or reduced. This leads to a change in membrane permeability and minimizes the amount of antimicrobial agents that enter into the bacterial cell, reducing drug inflow and preventing the medication from interacting with its intracellular target (Li et al., 2024).

The two nonspecific porins that *K. pneumoniae* mainly expresses, OmpK35 and OmpK36, are crucial for maintaining membrane permeability. Mutations of these porin genes can impact their functionality or expression, potentially resulting in their loss or altered performance. This diminished expression of the quantity of antimicrobial compounds that

can penetrate the bacterial cell significantly contributes to bacterial drug resistance (Li et al., 2024).

1.4.4. Enzymatic Modification

Various molecular mechanisms in *K. pneumoniae* utilized to counteract the effects of antibiotics, including mutations in DNA and changes in protein synthesis. A key resistance mechanism, especially against aminoglycoside antibiotics, is enzymatic modification (Li etal., 2024).

K. pneumoniae bacterium generates aminoglycoside-modifying enzymes (AMEs) that chemically modify aminoglycoside compounds, thereby neutralizing their antibacterial properties. These enzymes are categorized according to their modifying actions into acetyltransferases (AACs), nucleotidyltransferases (ANTs), and phosphotransferases (APHs). The genes responsible for these enzymes are generally located on plasmids and transposons, which promote their horizontal transfer among strains, leading to the swift dissemination of aminoglycoside resistance (Li et al., 2024).

Furthermore, *K. pneumoniae* demonstrates significant aminoglycoside resistance through the methylation of 16S rRNA by specific methyltransferases. The occurrence of 16S rRNA methylases has been increasingly documented with clinical isolates and is frequently linked to profiles of multidrug resistance (Li et al., 2024).

1.4.5. Production of β-Lactamases

One important way that *K. pneumoniae* becomes resistant to β-lactam antibiotics is through the synthesis of β-lactamases. These enzymes can be obtained through plasmids or naturally encoded within bacterial chromosomes. Gram-negative bacteria frequently produce β-lactamases, which give them resistance to penicillins, cephalosporins, and some carbapenems (Munita et al., 2016). The β-lactamases produce resistance by breaking down the β-lactam ring structure and altering penicillin-binding proteins (PBPs) that reduce drug-binding to the target (Huy et al., 2024).

β-lactamases are categorized into four primary classes—A, B, C, and D—based on their molecular structure and functional properties. A, C, and D compriseserine β-lactamases, whereas Class B enzymes, referred to as metallo-β-lactamases (MBLs), need divalent zinc ions for their hydrolytic activity (Bush et al., 2010).

Class A β-lactamases in this class, numerous gram-negative bacteria frequently contain class A β-lactamases, which are a broad group of proteins with different catalytic functions. They include carbapenemases like KPC (*K. pneumoniae* carbapenemase),

extended-spectrum β-lactamases (ESBLs) like CTX-M, and penicillinases like TEM-1 and SHV-1 (Bush et al., 2010).

Class B β-lactamases this classcan hydrolyzea wide variety of β-lactam antibiotics, including carbapenems, by using zinc ions. Prominent representatives of this class include New Delhi metallo-β-lactamase (NDM), Imipenemase (IMP), and Verona integron-encoded metallo-β-lactamase (VIM) (Bush et al., 2010).

Class C β-lactamases provide resistance against all penicillin's antibiotics and the majority of cephalosporins antibiotics, with AmpC being the commonly prominent enzyme. This enzyme is typically encoded within the chromosomes of several *Enterobacteriaceae* (Bush et al., 2010).

Class D β-lactamases these classes have the ability to hydrolyze oxacillin, which is the first way to distinguish them from class A penicillinases.(Also known as OXA-type β-lactamases). In fact, clavulanic acid does not effectively inhibit them. These are a large group of enzymes, and many different OXA types have been found that can break downcarbapenems, like OXA-23 from *Acinetobacter baumanii*, third-generation cephalosporins, like OXA-11 from *pseudomonas aeruginosa*, and OXA-48 which have different forms are often seen in samples from *K. pneumoniae* and other *Enterobacteriaceae*, and they have also been found in *Acinetobacterbaumanii* (Munita et al., 2016).

1.5 Carbapenems

Carbapenems represent a category of beta-lactam antibiotics that share structural similarities with penicillins, cephalosporins, and monobactams. Although they have a ring with five members, carbapenems have a carbon atom in place of the sulfur atom at position C-1, and a double bond is created between carbon atoms C-2 and C-3 (Jeon et al., 2015).

Carbapenems represent a category of beta-lactam antibiotics utilized to treat infections carried by bacteria that show ESBL. Introduced in the 1980s, these antibiotics possess the widest antibacterial spectrum among all beta-lactams and are effective against various types of ESBLs (Tarnberg et al., 2012).

Furthermore, carbapenems are thought to be the most potent treatment for multidrug-resistant *K. pneumoniae* and are often regarded as a final resort option, despite the appearance of carbapenem resistance in recent years (Fritzenwankeret al.,2018). The four carbapenems that are commonly utilized in clinical settings include imipenem, meropenem, doripenem, and ertapenem (Pyakurel et al., 2021).

Unfortunately, bacteria have the capability to improve, acquire, and obtain resistance to carbapenem antibiotics via several mechanisms. These mechanisms of resistance encompass the synthesis of carbapenemases (specific β-lactamases that break down carbapenems), the function of efflux pumps, reduced membrane permeability resulting from modified or absent porins, alterations in penicillin-binding proteins (PBPs), and structural mutations at antibiotic target sites (Aurilio et al., 2022).

1.6 Carbapenemase

In 1990, the term of "carbapenem-resistant *Enterobacteriaceae* "has appeared in Japan, since then escalated into a significant global public health concern. This development has diminished the availability of effective antibiotics and raised alarming issues due to its ability to result in infections that are difficult to treat, raising rates of morbidity and mortality (Iovleva et al., 2017).

Carbapenemases represent a specific category of β-lactamases distinguished by their extensive hydrolytic activity against a range of β-lactam antibiotics, such as penicillins, cephalosporins, monobactams, and carbapenems. The primary mechanism of resistance associated with carbapenemase production is the hydrolysis of the β-lactam ring, which effectively inactivates these antibiotics (Aurilio et al., 2022).

According to the Centers for Disease Control and Prevention (CDC) defines carbapenem-resistant *Enterobacteriaceae* (CRE) family members: (a bacterium that demonstrate resistance to at least one carbapenem antibiotic). Among these, (CR-Kp) is the most clinically significant species, representing a substantial threat in healthcare environments due to its correlation with elevated morbidity and mortality rates (Livorsi et al., 2018).

A study performed in October 2023 in Palestine "revealed that 83.33% of isolates were carbapenemase-producing Enterobacterales (CPE), and the majority of common species of CRE identified were *K. pneumoniae* (61.7%), *E. coli* (24.5%), and *E. cloacae* (12.8%)" (Ibaideya et al., 2024).

Another study by Thomsen et al. (2023) found that out of 14,593 samples, 7,023 (48.1%) were carbapenem-resistant *K. pneumoniae* (CR-Kp). Furthermore, documentation exists regarding the prevalence and dissemination of carbapenem resistance within *Enterobacteriaceae*; specifically, 3,668 (25.1%) of the samples were carbapenem-resistant *Escherichia coli*, while the last part, 3,902 (26.8%) isolates, represented 72 other species, as shown in Figure 1.2 (Thomsen et al., 2023).

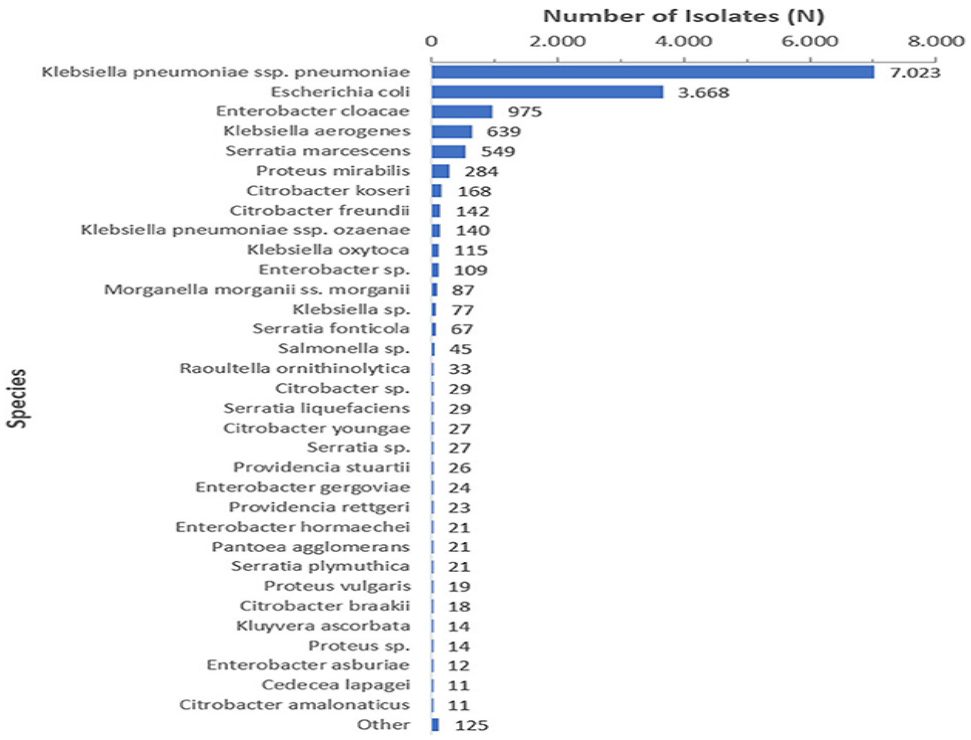

Figure 1.2: surveillance data from 14,593 clinical sample showed the distribution over an 11- years old (Thomsen et al., 2023).

1.6.1. Classification of carbapenemase

Carbapenemases encompass a wide variety of enzymes that were first discovered in *Enterobacteriaceae.*A, B, C, and D are four of them categorized according to β-lactamases. These share a common characteristic of having a serine residue in their active site, which facilitates the hydrolysis of the β-lactam ring in antibiotics, resulting in drug inactivation. In contrast, Class B β-lactamases, also call metallo-β-lactamases (MBLs), utilize divalent zinc ions to drive the hydrolysis process (Aurilio et al., 2022).

1.6.1.1. Class A Carbapenemases

Class A carbapenemases may be encoded either chromosomally, including BIC-1, SME, NmcA,SHV-38, PenA, FPH-114, and SFC-1, or plasmid mediated, including KPC, GES, and FRI-1. Some variants, like IMI, can be encoded by both chromosomal and plasmid sources; this class is represented in a group of bacteria, such as *P. aeruginosa* and *A. baumannii*. KPC (*K. pneumoniae* Carbapenemase) is the most prominent and has achieved global spread, being documented in the majority of clinical cases involving enterobacterial infections. Class A carbapenemases result in reduced susceptibility to imipenem and have the capability to dissolve and breakdown a broad spectrum of β-lactam antibiotics, including carbapenems (Aurilio et al., 2022).

1.6.1.2. Class B Carbapenemases

Class B carbapenemases, also referred to as metallo-β-lactamases (MBLs), are the most important and common type of carbapenemases found in hospitals, such as Imipenemase (IMP), Verona Integron-encoded Metallo-β-lactamase (VIM), and New Delhi. These are generally linked to mobile genetic elements such as integrons, transposons, andplasmids, which promote the horizontal movement of resistance genes between different bacteria (Aurilio et al., 2022). MBLs possess the ability to breakdown and dissolve all β-lactam antibiotics except aztreonam and are not susceptible to traditional β-lactamase inhibitors (Meletis et al., 2016).

1.6.1.3. Class C Carbapenemases

Class C carbapenemase enzymes have historically been categorized as cephalosporinases. Nevertheless, various studies have shown that these enzymes exhibit a restricted capacity to hydrolyze carbapenems. Resistance associated with Class C enzymes typically arises from their being coupled with reduced outer membrane permeability or increased activity of efflux pumps (Aurilio et al., 2022).

1.6.1.4. Class D Carbapenemases

Class D beta-lactamases represent a category of enzymes that were originally differentiated from class A penicillinases because of their capacity to hydrolyze oxacillin (OXA). Furthermore, in clinical settings, beta-lactamase combined with an inhibitor such as amoxicillin-clavulanic acid workwell against class A beta-lactamases but has no effect on class D carbapenemases. Various OXA variants have been identified, including enzymes capable of degrading third-generation cephalosporins, classified as extended-spectrum beta-lactamases (ESBLs) (Aurilio et al., 2022).

OXA enzymes are present in various types of bacteria. For example, OXA-11 is found in *Pseudomonas* spp., while OXA-23 is present in *Acinetobacter baumannii*. OXA-48, which is found in *K. pneumoniae*, has become more prevalent among clinical isolates of *K. pneumoniae*and other species of *Enterobacteriaceae* family (Evans et al., 2014).

1.6.2.2. Prevalence of carbapenemase in the Middle East

There has been a noticeable increase in carbapenem-resistant bacteria worldwide, particularly impacting the Middle East. Significant public health concerns have been raised in recent years by the appearance, emergence ,and spread of carbapenem-resistant bacteria in this area (Leylabadlo et al., 2015). The identification and management of bacteria that generate carbapenemase have become progressively challenging, mainly because of the horizontal transfer of plasmids that harbor resistance genes among various bacteria (Leylabadlo et al., 2015), reflecting poor hygiene practices.

KPC, VIM, NDM, and OXA are being the most commonly identified types of carbapenemase in the Middle East, as shown in Figure 1.3 (Leylabadlo et al., 2015). However, the occurrence of specific carbapenemases differs among Middle Easterncountries. A notable prevalence of KPC-producing strains has been documented in Jordan, Saudi Arabia, and Afghanistan. (Leylabadlo et al., 2015). Significant outbreaks of KPC-producing *K. pneumoniae* were reported; a study in Tel Aviv from 2004 to 2006 identified epidemic strains containing the *bla*KPC gene (Logan et al., 2017).

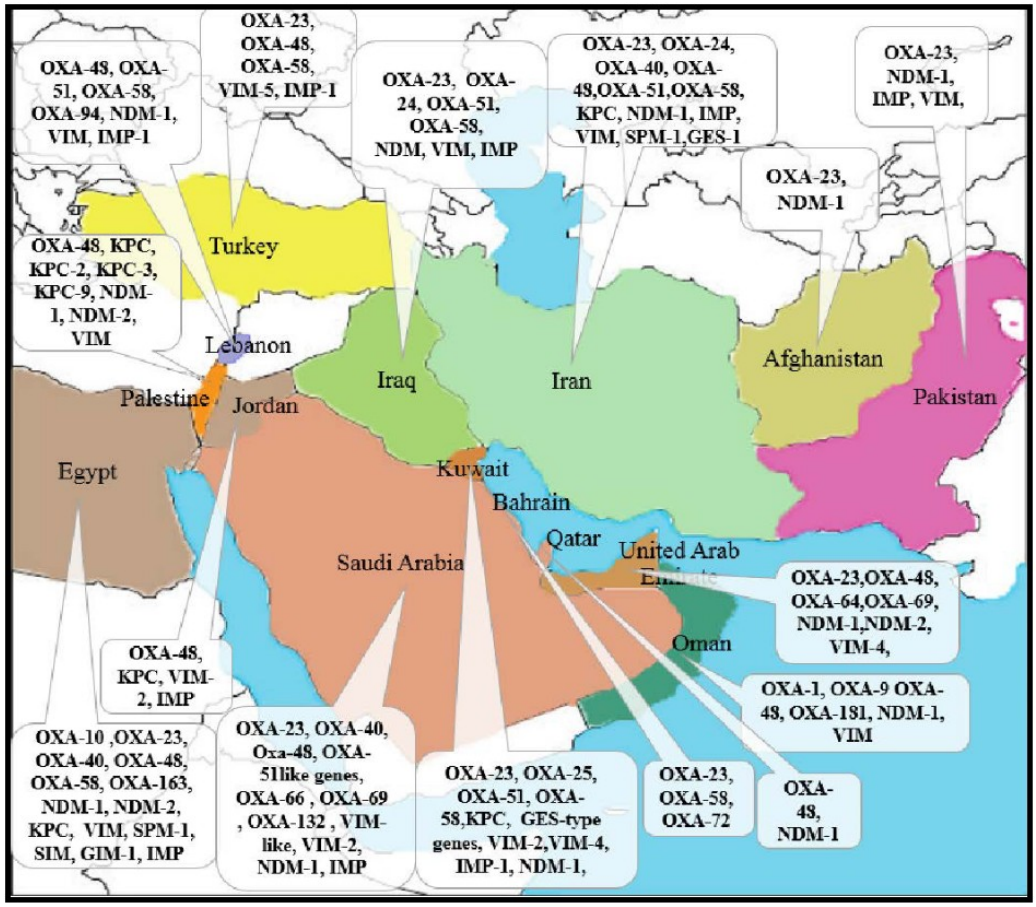

Figure 1.3: Geographical distribution of carbapenemases in the Middle East. (Leylabadlo et al., 2015).

Additionally, Pakistan has recorded some of the highest resistance levels linked to NDM-producing organisms, with sporadic cases noted in other Middle Eastern countries. However, resistance associated with OXA-type carbapenemases has also been frequently reported in Turkey and Pakistan, with occasional instances from other areas (Leylabadlo et al., 2015). But VIM enzymes have rapidly disseminated and spread throughout Europe, leading to numerous outbreaks, including Greece, Italy, and Turkey (Logan et al., 2017).

The variety and distribution of carbapenemase enzymes across Middle Eastern nations may be shaped by numerous factors, including historical events, cultural exchanges, armed conflicts, and troops, immigrants, and forced immigrants' movements. Furthermore, the cross-border transmission of these enzymes can promote the spread of antibiotic resistance, complicating public health initiatives (Leylabadlo et al., 2015).

1.7Carbapenemase genes in *K. pneumoniae*

The most clinically significant carbapenemase genes in *K. pneumoniae*, based on their enzymatic breakdown of carbapenems and prevalence in the Middle East, are KPC, VIM, NDM, and OXA-48 types (Poirel et al., 2012).

1.7.1. *K. pneumoniae* Carbapenemase (KPC) Gene (*bla* KPC)

K. pneumoniae carbapenemases (KPCs) are essential β-lactamase enzymes that compromise the efficacy of β-lactam antibiotics by hydrolyzing their β-lactam ring. KPC was initially discovered in the late 1990s in *K. pneumoniae* samples from New York City. Since that time, strains producing KPC have spread worldwide, with numerous reports emerging from the United States, especially along the eastern seaboard (Leylabadlo et al., 2015).

1.7.2. New Delhi -metallo-beta-lactamase (NDM) Gene (*bla* NDM)

NDM-1 is a metallo-β-lactamase that breaks down a broad spectrum of β-lactam antibiotics, such as penicillins, cephalosporins, and carbapenems. Its activity depends on two zinc ions present at its active site (Dortet et al., 2014).

The *bla* NDM gene, found on plasmids or transposons, facilitates horizontal gene transfer among gram-negative bacteria. In 2007, a Swedish man who contracted a carbapenem-resistant *K. pneumoniae* infection in New Delhi represented the initial discovery of NDM-1, which is the reason for the name 'NDM.' Since then, NDM-producing organisms have been reported worldwide, spreading through healthcare settings and travelers returning from Indian to other countries such as Europe, North America, East Asia, and Australia (Nordmann et al., 2011).

NDM-1 producers may have a high degree of carbapenem resistance. Plasmids that have the *bla* NDM-1 gene come in many different types and often include several other resistance genes that help bacteria resist different antibiotics, like oxacillinase-48 (OXA-48), cephalosporinase genes, extended-spectrum beta-lactamase (ESBL) genes, aminoglycoside resistance genes (16S RNA methylases), macrolide resistance genes (esterase), rifampin-modifying enzymes, and sulfamethoxazole resistance genes. Together these genes contribute to multidrug and pan-drug resistance (Nordmann et al., 2011).

1.7.3. Verona Integron metallo beta-lactamase (VIM) gene (*bla*VIM)

The Verona Integron-encoded metallo-β-lactamase (VIM) is a zinc-dependent enzyme that hydrolyzes the β-lactam ring, rendering β-lactam antibiotics ineffective. It is among the most prevalent metallo-β-lactamases (MBLs) associated with human infections.Over the last decade, this carbapenemase has emerged as a considerable threat. The widespread presence of the *bla*VIM gene is due to its position on gene cassettes of class 1 integrons, which are often linked to movable genetic elements like transposons and plasmids (Kohler et al., 2020).

In 1996 and 1997, VIM was first identified in *Pseudomonas aeruginosa* isolates from Verona, Italy (Logan et al., 2017); it is present in Enterobacteriaceae as well. The metallo-β-lactamase 1 (VIM-1) encoded by the Verona integron was initially identified in Greece in *K. pneumoniae* (Tijet et al., 2013).

1.7.4. Oxacillin-Hydrolyzing Carbapenemases (OXA) Gene (*bla* OXA-48)

The class D carbapenemase encoded by the *bla* OXA-48 gene, which is plasmid mediated, was first discovered in Turkey in a clinical isolate of *K. pneumoniae* in 2003. Although Turkey has a well-established prevalence, other Mediterranean nations have also reported isolated cases (Nordmann et al., 2011).

The OXA gene (*bla* OXA-48) encodes oxacillin-hydrolyzing carbapenemases, classified as β-lactamases primarily functioning as penicillinases. These enzymes can break down oxacillin and cloxacillin, as well as a range of cephalosporins like ceftazidime; their activity is not affected by EDTA or clavulanic acid, yet they are very effective at breaking down carbapenems. Therefore, it is difficult to identify OXA-48-type carbapenemase from ESBL OXA -48, which could result in an underestimation of their true prevalence (Nordmann et al., 2011).

1.8 Method to detect carbapenem-resistance in *K. pneumoniae* (CR-Kp)

Resistance to antibiotics, especially carbapenem, is an important issue. It is necessary to develop effective tools to detect and analyze this resistance in hospitalized patients. Laboratory detection of carbapenem-resistant *Enterobacteriaceae* (CRE) involves the use of both phenotypic and genotypic methods (Banerjee et al.,2017).

1.8.1. Phenotypic detection of CRE

The most widely used screening method for assessing susceptibility to carbapenem is the Kirby-Bauer disk diffusion method. In this procedure, samples of *K. pneumoniae* are usually spread on Mueller-Hinton agar plate, and disks that contain the antibiotics meropenem (10 μg), ertapenem (10 μg), and imipenem (10 μg) are placed on the plate. After the agar plate is incubated, the diameter of the zone of growth inhibition (clear areas around the antibiotic disks) are measured following the Clinical and Laboratory Standards Institute (CLSI) guidelines breakpoints; if the clear area is less than 22 mm for ertapenem or less than 19 mm for imipenem and meropenem, it shows resistance. If carbapenem resistance is detected, additional phenotypic testing is done to confirm carbapenemase production. These tests are the Carbapenem Inactivation Method (CIM), the Modified Hodge Test (MHT), and the Carba NP test (Lutgring et al., 2016).

1.8.1.1. Modified Hodge Test (MHT)

The Modified Hodge Test (MHT) identifies carbapenemase production by assessing the capability of a test organism to hydrolyze carbapenem antibiotics.In this test, a lawn of *E. coli* ATCC 25922 (a strain susceptible to carbapenem) is inoculated onto an agar plate. A carbapenem disk such as meropenem or ertapenem is positioned at the center of the plate, and the test isolate is streaked in a straight line from the disk's edge to the plate's edge. *E. coli* can grow toward the disk and create an indentation that resembles a cloverleaf, if the test organism generates carbapenemase, which will render the antibiotic inactive close to the disk.

The Modified Hodge test has shown adequate sensitivity in identifying class A carbapenemases, especially KPC enzymes, as well as class D carbapenemases. However, sensitivity for metallo-beta-lactamases (MBLs) like NDM was found to be low. Additionally, bacteria that make AmpC enzymes, especially if they also have changes in their porin proteins, can lead to incorrect positive or negative results, which affects the accuracy of the test, as a result of these limitations, the MHT was excluded from the CLSI guidelines in 2018 in favor of more precise testing methods (Rabaan et al., 2022).

1.8.1.2. Carba NP Test

The Carba NP test is a colorimetric assay intended to detect the production of carbapenemase in Enterobacterales. In comparison to other phenotypic methods, this approach is rapid, economical, and specific, demonstrating a sensitivity range of 73% to 100%; however, the Carba NP test exhibits low sensitivity in detecting OXA-48 and certain class A carbapenemases, and its effectiveness is reduced with mucoid isolates (Rabaan et al., 2022). Therefore, it is not a reliable test.

1.8.1.3. Carbapenem Inactivation Method (CIM)

The Carbapenem Inactivation Method (CIM), introduced in 2015, assesses the capacity of carbapenemases to deactivate carbapenem antibiotics. CIM is recognized for being very accurate, reliable, and affordable, showing better results in finding Carbapenem-resistant *Enterobacteriaceae* (CRE) than the Modified Hodge Test (MHT) and the Carba NP test. However, some studies show that OXA-48-like carbapenemases in Enterobacterales may not be easily detected using this method (Rabaan et al., 2022).

1.8.2. Genotypic detection for CRE

Genotypic methods serve as a complementary approach to phenotypic techniques, providingconfirmatory evidence of the resistance of bacterial isolates; therefore, this method is a choice for the detection of specific carbapenemase-producing genes in bacterial strains. This technique utilizes polymerase chain reaction (PCR), which relies on the amplification of particular genes. Despite the high costs, the need for specialized equipment and expertise, and the limitation to detecting only genes with known sequences, these molecular methods are both sensitive and efficient in terms of time (Rabaan et al., 2022).

1.9 Problem statement

Carbapenem-resistant *Klebsiella pneumoniae* (CR-Kp) has become a significant global public health issue, leading to community-acquired invasive infections. The rise and prevalence of antibiotic-resistant *Klebsiella pneumoniae* is primarily due to the production of enzymes that deactivate antibiotics, particularly various beta-lactamases. These resistant strains are now widespread in the community and are responsible for many treatment failures, posing a serious threat to the effectiveness of long-utilized antibiotics (Karampataki et al., 2023).

Consequently, carbapenem-resistant *Enterobacteriaceae (*CRE) are recognized as a global public health danger, ranking among the top causes of mortality in patients with hospital-acquired infections. The majority of carbapenem resistance is linked to the formation and production of carbapenemases, alongside other resistance mechanisms such as beta-lactamases extended-spectrum drugs, mutations in porins, and the existence of efflux pumps. Strains of carbapenem-resistant *Enterobacteriaceae* demonstrate resistance to a wide range of antimicrobial agents, including carbapenems, cephalosporins, beta-lactamase inhibitor combinations, ampicillin, ciprofloxacin, and trimethoprim-sulfamethoxazole (Wang et al., 2022).

The growing dissemination and prevalence of carbapenem-resistant *Enterobacteriaceae* (CRE), especially *Klebsiella pneumoniae*, in healthcare settings has resulted in a formidable treatment challenge due to the severely restricted availability of therapeutic alternatives (Karampataki et al., 2023). Therefore, strains of *K. pneumoniae* represent a considerable global health risk. It is essential to identify and comprehend the gene(s) responsible for carbapenem resistance to effectively manage the treatment of affected patients.

1.10 Aims and Objectives:

The main aim:

Screening of carbapenemase genes (*bla* KPC, *bla* OXA-48 *bla* NDM-1, *bla* VIM) in *Klebsiella pneumoniae* isolated from Hebron City, West Bank has not been carried out so far. Therefore, this study aims to investigate the CRE by using molecular techniques, including multiplex PCR for detection of the most prevalent carbapenemase genes.

The specific objectives:

- To identify and isolate *K. pneumoniae* from various clinical samples.
- To determine the phenotypic antibiotic resistance profile for *K. pneumoniae* isolates.
- To detect carbapenem resistance genes by multiplex PCR assay.

Chapter Two

Methodology

2.1 Material

Three different culture media from HIMEDIA Laboratories were used in this study for the culture of *K. pneumoniae* that include:

Blood Agar: A differential and enrichment medium that is composed of HM infusion (500 g/L), tryptose (10 g/L), sodium chloride (5.0 g/L), and agar (15 g/L) as a solidifying agent. The final pH at 25°C is approximately 6.8 ± 0.2. Blood agar supports the growth of a wide range of microorganisms.

MacConkey Agar: A selective and differential medium was used for the isolation of Gram-negative enteric bacteria and differentiation between lactose fermenters and non-fermenters. It contains peptic digest of animal tissue (1.5 g/L), casein enzymic hydrolysate (1.5 g/L), pancreatic digest of gelatin (17 g/L), lactose (10.0 g/L), bile salts (1.5 g/L), crystal violet (0.001 g/L), neutral red (0.03 g/L), sodium chloride (5.0 g/L), and agar (15.0 g/L). The final pH at 25°C is approximately 7.1 ± 0.2.

Mueller Hinton Agar (MHA): This medium was used for antimicrobial susceptibility testing using the disk diffusion method. It contains Acicase™ (17.5 g/L), HM infusion solids B (2 g/L), soluble starch (1.5 g/L), and agar (17.0 g/L). The final pH at 25°C is approximately 7.3 ± 0.1. MHA provides a standardized environment for accurate and reproducible antibiotic sensitivity testing.

For the antibiotic susceptibility testing, various antibiotics were used, including: Amikacin (AK, 10 µg), Ampicillin (AMP), Amoxicillin/clavulanate (AMC), Cefoxitin (FOX), Cefepime (CFPM), Ceftazidime (CAZ, 30 µg), Cefuroxime (CXM), Ciprofloxacin (CIP,

5 µg), Trimethoprim/sulfamethoxazole (SXT, 1.25/23.75 µg), Gentamicin (CN, 10 µg), Ertapenem, (ETP, 10 µg), Meropenem (MEM, 10 µg), and Piperacillin/tazobactam (TZP).

The materials used for the molecular detection of carbapenem-resistant *K. pneumoniae* (CR-Kp) were primarily obtained from Hylabs (Israel). A summary of these materials as summarized in Table 2.1.

(Table2.1) The material used for molecular detection of carbapenemase gene in *K. pneumoniae*

NO	Items	• Components	Supplier/company
1	Gel Electrophoresis	• Agarose powder • Tris EDTA buffer 1x • Microwave • Ethidium bromide solution • DNA ladder • Bio rad gel electrophoresis apparatus • UV-light documentation system	Hylabs
2.	PCR	• PCR tube • Thermos cycler device • Master mix • Heat block 95 C	
3.	Primers	• KPC Forward and Reverse • VIM Forward and Revers • NDM Forward and Reverse • OXA-48 Forward and Reverse	Hylabs

2.2 Methods

2.2.1. Specimens collections and storage

A total of 100 clinical specimens of *K. pneumoniae* were collected between November 2024 and February 2025 from Prince Alia Governmental Hospital in Hebron, Palestine. The isolates were stored in 25% glycerol made in Luria broth (LB) at -80°C until needed.

2.2.2. Sample Processing ofIsolates

The collected specimens were cultured on both blood agar and MacConkey agar plates and then incubated at 37 °C for 24 to 48 hours. After that, the characteristics of colony (colony color, size, and results from biochemical testing) were observed using standard microbiological methods.

2.2.3. Colony Morphology for *K. pneumoniae*

Cultural characteristics of *K. pneumoniae* isolates were evaluated based on the colonyappeared. This colony must to be pink on MacConkey agar due to lactose fermentation, mucoid, moderate or large according to size, circular or irregular in form, dome-shaped (convex or pulvinate) or spreader in elevation, and margins (curled, lobated, or entire).

2.2.4. Biochemical Confirmation Test

The biochemical tests, including catalase, urease, oxidase, motility, indole, and methyl red, were performed. Moreover, to eliminate variability in biochemical tests, the VITEK 2 automated system confirmed the result.

2.2.5. Antibiotic Susceptibility Test (AST)

AST was conducted for all collected samples at Prince Alia Governmental Hospital by using the VITEK 2 automated system (VITEK®2 ID & AST CARDS-BioMerieux, UK, Ltd.). Following recommendations from the Clinical and Laboratory Standards Institute (CLSI) guidelines.

2.2.6. Detection of ESBL in *K. pneumoniae*

To detect extended-spectrum beta-lactamase (ESBL) production, the DDST test was done. A suspension of *K. pneumoniae* bacterium was done using normal saline and adjusted to conform to the turbidity (0.5) McFarland standard. This adjusted suspension was subsequently inoculated by cotton swab onto the surface of a Mueller-Hinton agar. An amoxicillin-clavulanic acid (AMC) disk was positioned at the center of the plate, while 30 µg discs of cefotaxime and ceftazidime were placed 15 mm away from it. The plate was incubated overnight at 37°C.After that, for each disk the inhibition zone surrounding it was measured by ruler.A positive ESBL result was indicated if the inhibition zone resulting

from the combined action of ceftazidime or cefotaxime with clavulanic acid exceeded 5 mm compared to the inhibition zone of ceftazidime or cefotaxime alone (Khalifa et al.,2024).

2.2.7. Screening of CR in *K. pneumoniae*

The susceptibility to carbapenem was evaluated using the meropenem (MEM) disc for each isolate. A suspension of *K. pneumoniae* bacteria was done using normal saline and adjusted to conform to the turbidity (0.5) McFarland standard. This standardized suspension was then inoculated by cotton swab onto the surface of MacConkey agar to provide suitable susceptibly test plates. Disks containing 30 mg of MEM were placed on the plates, which were then incubated overnight at 37°C. Then, the diameter of the inhibition zone surrounding each disk was measured for each isolate. If there was an increase of ≥5 in the diameter of the inhibition zone around the MEM disk, isolates were categorized as producing carbapenem-resistant (CR) (Mohammadpour et al.,2025).

2.2.8. Molecular detection of carbapenem resistance genes in *K. pneumoniae* bymultiplex PCR

A total of fifty (50) isolates of *K. pneumoniae* were tested for carbapenemase genes: 35 from CR and 15 from ESBL.

2.2.8.1. DNA isolation

The boiling method was used to extract the isolates' deoxyribonucleic acid (DNA). Firstly, 100 µL of sterile, RNA-free distilled water was combined with a pure colony of *K. pneumoniae*. After that, the suspension was boiled for ten minutes at 95 °C in a water bath. Following boiling, the cell debris was precipitated using centrifugation for five minutes at 15,000 rpm. Finally, the extracted supernatant was utilized as a template for PCR amplification and stored at -20 C°for further use (Alshahrani et al., 2022).

2.2.8.2. PCR amplifications

Using the DNA template, multiplex PCR was used to amplify the target resistance genes.For the multiplex PCR, a total volume of 25 µL for each reaction was prepared that include DNA templet, primers (KPC, VIM, NDM, OXA), and nuclease free water as summarized in Table 2.2 (Alshahrani et al., 2022).

(Table 2.2)Volume of reagents in PCR reaction.

NO	Volume of reagents that used which included in each reaction mixture
1	12.5 µL of master mix
2	2 µL of DNA template (from extraction)
3	0.25 µL of each primer (KPC, VIM, NDM, OXA)
4	8.5 µL of nuclease-free water

Target genes were amplified on thermocycler instrument using the Eppendorf Master Cycler (*Eppendorf Mastercycler PCR Thermal Cycler. Eppendorf AG, Hamburg, Germany*) PCR amplification was performed for all carbapenem-resistant (CR) and ESBL-producing isolates under identical conditions within a single PCR run.The amplification consisted of 35 cycles, The following optimal cycling conditions summarized in Table 2.3(Ranjbar et al.,2020).

(Table 2.3)PCR cycling program.

NO	Steps	Temperate &Time
1	Initial heat activation	95°Cfor 3 minutes
2	Denaturation	95°C for 45 second
3	Annealing	57° C for 30second
4	Extension	72 °C for 1 minute
5	Final extension	72°C for 10 minutes

Certain oligonucleotide primers were used to detect the presence of carbapenemase genes in *K. pneumoniae* isolates. The target β-lactamase genes (*bla*KPC, *bla*NDM, *bla*VIM, and *bla*OXA-48) were amplified using these primers, and the expected amplicon sizes were determined according to Alshahrani et al. (2022). Details of the primer sequences, target

genes, and PCR product sizes are summarized in Table 2.4, which was applied to both carbapenem-resistant (CR) and ESBL-producing isolates.

(Table2.4) Target genes of β- lactamases used for PCR amplification along with corresponding primers sequences and expected amplicon sizes (Alshahrani et al.,2022).

NO	Gene	Primer	Sequence for CR gene	Band (bp)
1	*bla* KPC	KPC-F KPC-R	CATTCAAGGGCTTTCTTGCTGC ACGACGGCATAGTCATTTGC	538
2	*bla* NDM	NDM-F NDM-R	GGTTTGGCGATCTGGTTTTC CGGAATGGCTCATCACGATC	621
3	*bla* VIM	VIM-F VIM-R	GATGGTGTTTGGTCGCATA CGAATGCGCAGCACCAG	390
4	*bla* OXA-48	OXA-F OXA-R	GCTTGATCGCCCTCGATT GATTTGCTCCGTGGCCGAAA	281

2.2.8.3. Agarose gel electrophoresis

PCR products (6.00 µL) were analyzed by electrophoresis using 2% (w/v) agarose gel Electrophoresis. Agarose was prepared in TAE buffer 50X.The gel was run for 60 minutes at 90 volts.After electrophoresis, a gel documentation system was used to view and document the resultsunder ultraviolet light. To estimate the molecular sizes of the PCR amplicons, a 100-base pair (bp) DNA ladder (Gene DireX, Hy Labs) was incorporated into the gel (Alshahrani et al., 2022).

Statistical Analysis2.2.9.

IBM SPSS version 22.0 was used to analyze the study's outcome data about *K. pneumoniae* (SPSS Inc., Chicago, IL, USA).

2.2.10. Ethical consideration

The Health Professions College Al-Quds University's Research Ethics Subcommittee provided ethical guidance for this study

Chapter Three

Result

3.1 Identification of strains and demographic characteristics

In this study, 100 bacterial cultures of *K. pneumoniae* were collected from Princess Alia Governmental Hospital, Hebron, West Bank. The initial identification of *K. pneumoniae* isolates conducted based on the morphological traits of the colonies seen on MacConkey agar and blood agar. On blood agar, they were observed as large, white, mucoid colonies. While, the *K. pneumoniae* isolates manifested as large, mucoid, pink colonies resulting from lactose fermentation on MacConkey agar as illustrated in Figure 3.1.

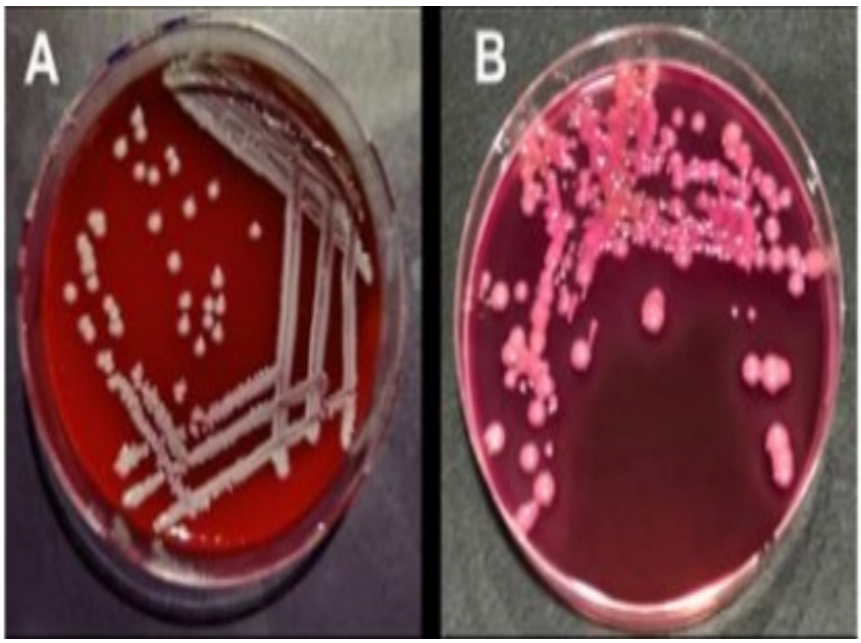

Figure 3.1 *K. pneumoniae* colony morphology (Alansary et al.,2022).

3.2 Gender and age distribution

The distribution of *K. pneumoniae* isolates revealed that 49% originated from females while 51% were from males patients, as shown in Figure 3.2 Regarding age distribution, *K. pneumoniae* was most commonly isolated from individuals aged 0 to 15 years, followed by those over 60 years. The prevalence then decreased across 46-60, 31-45, and 16-30 age groups respectively. The overall distribution of *K. pneumoniae* across these age groups are shown in Figure 3.3.

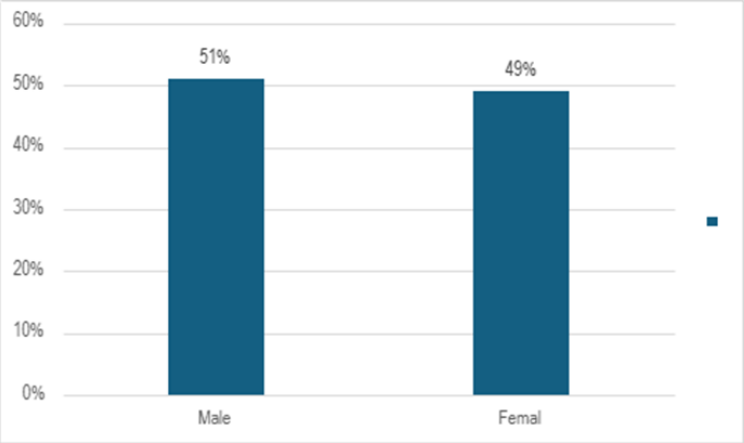

Figure 3.2 Distribution of *K. pneumoniae* according to gender

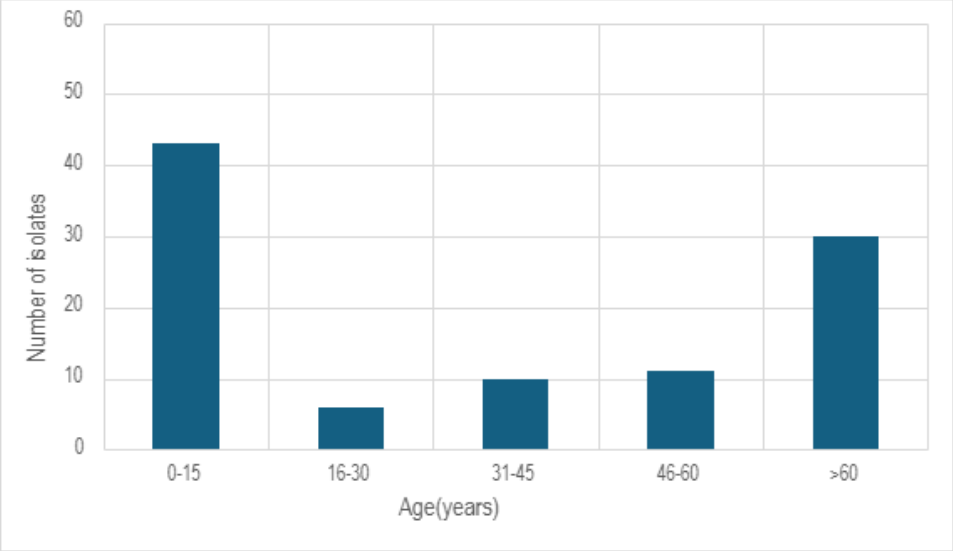

Figure 3.3 Distribution of *K. pneumoniae* according to age group.

3.3 Types of specimens

A total of one hundred isolates of *K. pneumoniae* were obtained from various clinical specimens. Of these, 51 isolates (51%) were obtained from urine, 21 isolates (21%) from wounds, 17 isolates (17%) from blood, 4 isolates (4%) from vaginal swabs, and 2 isolates (2%) from cerebrospinal fluid (CSF) and two isolates (2%) from throat swabs. Additionally, one isolate (1%) each from sputum and peritoneal fluid specimens, as illustrated in Figure 3.4.

These isolates were obtained from patients across various hospital wards. The pediatric ward had the highest percentage, 18 isolates (18%), followed by the medical ward, 17 isolates (17%); the neonatal ward, 16 isolates (16%); and the emergency ward, also 16 isolates (16%). Outpatient samples comprised 11 isolates (11%), while the surgical ward had 8 isolates (8%), the CCU ward had 6 isolates (6%), the obstetrics and gynaecology ward had 5 isolates (5%), the burn ward had 2 isolates (2%), and thehemodialysis ward had just 1 isolate (1%), as summarized in Table 3.1.

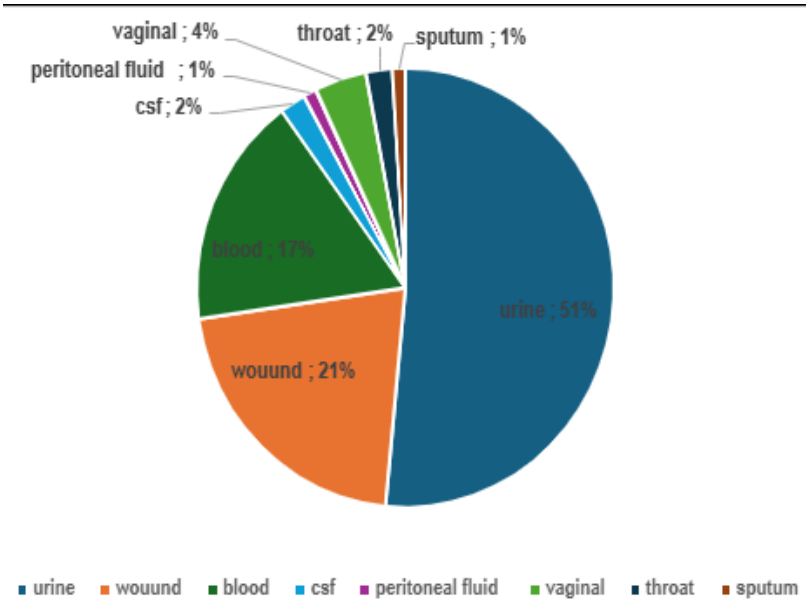

Figure 3.4 Distribution of *K. pneumoniae* isolates across various clinical specimens.

Table 3.1 Distribution of *K. pneumoniae* isolates across various departments.

Departments	%	Departments	%
Pediatric	18%	Surgical	8%
Medical	17%	CCU	6%
Emergency	16%	Obstetrics and Gynaecology	5%
Neonate	16%	Burns	2%
Outpatient	11%	Hemodialysis	1%

3.4 Antimicrobial susceptibility testing of *K. pneumoniae*

Based on the sensitivity data, amikacin (AK) showed the highest effectiveness, with 78% of isolates being sensitive to it. Both ertapenem (ETP) and meropenem (MEM) followed closely, each demonstrating a sensitivity rate of 65%. Gentamicin (CN) also performed well, with 62% sensitivity as shown in Figure 3.5.

Amoxicillin/clavulanic acid (AMC) and piperacillin/tazobactam (TZP) presented moderate sensitivity rates of 49% and 48%, respectively. Ciprofloxacin (CIP) had a nearly balanced profile, with 46% of isolates being sensitive. In contrast, lower sensitivity rates were observed with cefepime (CFPM) at 32%, ceftazidime (CAZ) at 21%, cefoxitin (FOX) and cefuroxime (CXM) at only 17%, and trimethoprim/sulfamethoxazole (SXT) at 34%, indicating reduced effectiveness as summarized in Table 3.2

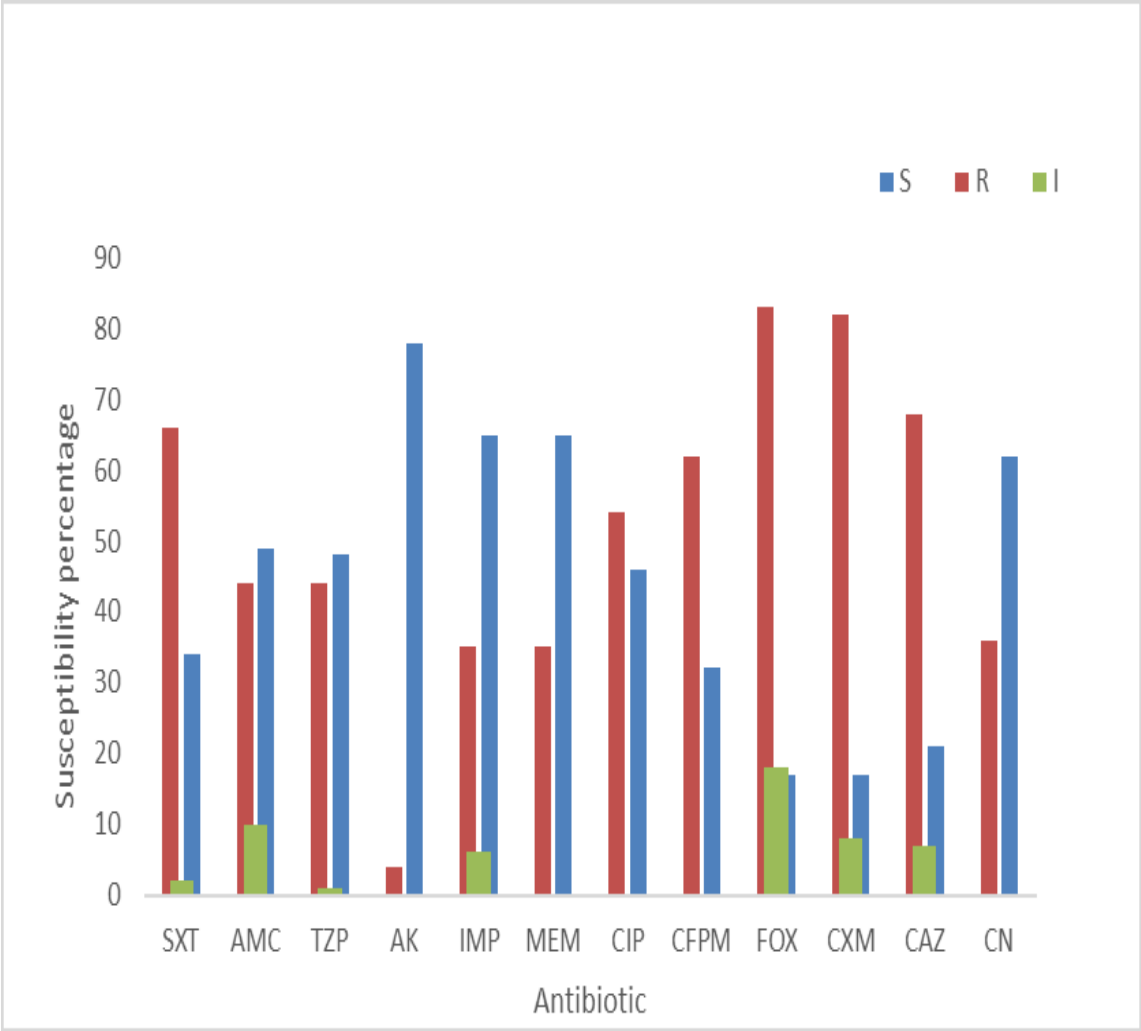

Figure 3.5 Antimicrobial susceptibility profile of *K. pneumoniae* isolates.

R: Resistance, S: Sensitive, I: intermediate

Table 3.2 Percentage of antibiotic susceptibility among 100*K. pneumoniae* isolates.

Antibiotics (Abbreviation)	Resistance	Sensitive	Intermediate
Amikacin (AK)	4%	78%	18%
Amoxicillin/clavulanic acid (AMC)	44%	49%	7%
Cefepime (CFPM)	62%	32%	6%
Cefoxitin (FOX)	83%	17%	0%
Ceftazidime (CAZ)	68%	21%	10%
Cefuroxime (CXM)	82%	17%	1%
Ciprofloxacin (CIP)	54%	46%	0%
Ertapenem (ETP)	35%	65%	0%
Gentamicin (CN)	36%	62%	2%
Meropenem (MEM)	35%	65%	0%
Piperacillin/tazobactam (TZP)	44%	48%	8%
Trimethoprim/sulfamethoxazole (SXT)	66%	34%	0%

3.5 Detection of ESBL in *K. pneumoniae*

All isolates of *K. pneumoniae* (100) were tested for ESBL production, the analysis revealed that 77 of isolates (77%) were recognized as ESBL producerswhereas the remaining 23 of isolates (23.0%) were classified as ESBL-sensitive, as illustrated in Figure 3.7.

A positive ESBL result was indicated if the inhibition zone resulting from the combined action of ceftazidime (CAZ) or cefotaxime (STX) in presence of clavulanic acid increased by ≥ 5 mm compared to the inhibition zone of ceftazidime or cefotaxime alone in the absence of clavulanic acidaccording to CLSI guidelines.

3.6 Screening for carbapenem-resistant *K. pneumoniae*

Among the 100 *K. pneumoniae* isolates tested, 35 isolates (35%) were identified as carbapenem-resistant (CR). Notably, all CR isolates were also confirmed as extended-spectrum β-lactamase (ESBL) producers. Additionally, 42 isolates (42%) were ESBL producers but not carbapenem-resistant. The remaining 23 isolates (23%) were sensitive to both carbapenems and ESBL production, as illustrated in Figure 3.7.

The diameter of the inhibition zone surrounding the MEM disk was measured for each isolate. Isolates were classified as carbapenem-resistant (CR) if the increase in the diameter of the inhibition zone was ≥ 5 mm, otherwise, they were considered sensitive, according to CLSI guidelines, as shown in Figure 3.6.

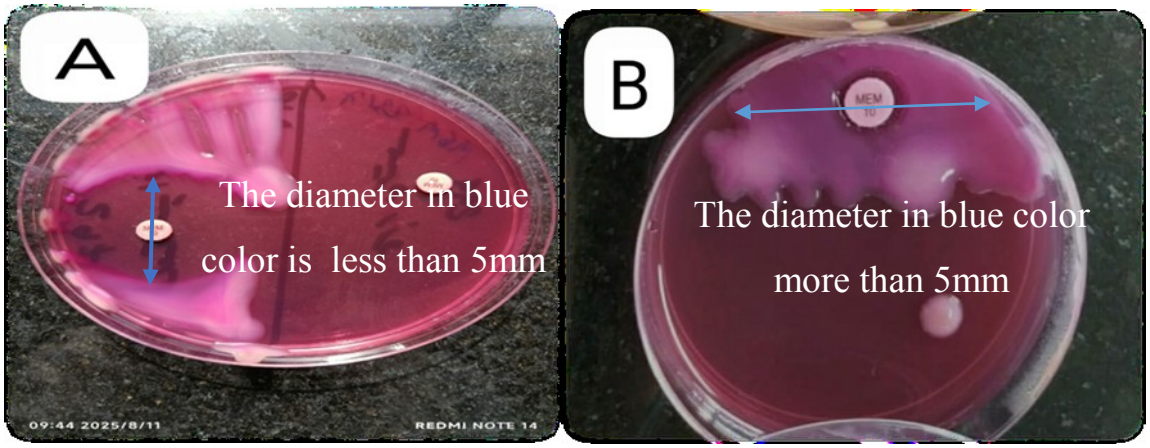

Figure 3.6 Screening for CR.A: sensitive for MEM disk, B: Resistance for MEM disk.

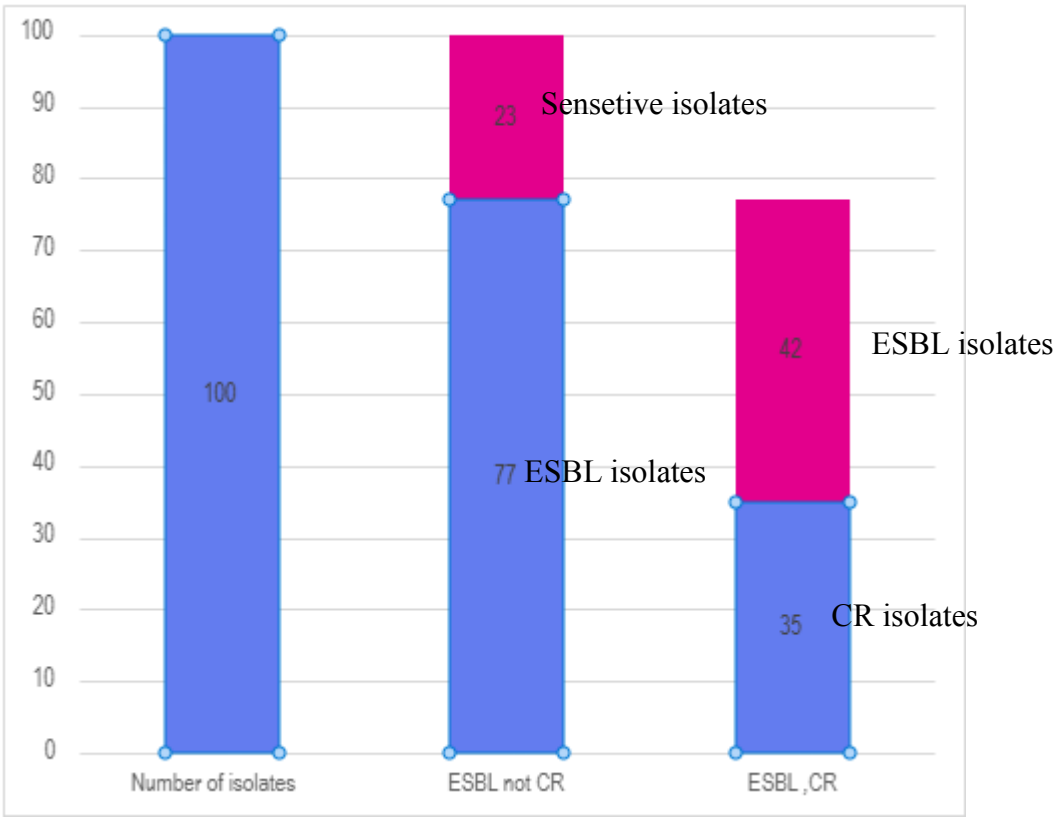

Figure 3.7 Percentage of CR and ESBL in *K. pneumoniae*

3.7 Genotypic detection of carbapenem resistance genes by multiplex PCR

A total of fifty (50) isolates were tested for carbapenemase genes: 35 from ESBL-producing and carbapenem-resistant (ESBL/CR) isolates, and 15 from ESBL-producing isolates that tested negative for carbapenem resistance. All thirty-five isolates 35/100(35%) of *K. pneumoniae* that were positive for carbapenem resistance using the MEM disk were included for carbapenemase gene detection. Additionally, fifteen isolates 15/42(34.8%) were randomly selected from the 42 ESBL-producing isolates that were carbapenem resistance-negative. These isolates showed sensitivity to MEM but were included to investigate the potential presence of carbapenemase genes.

3.7.1. Molecular detection of carbapenemase genes in CR *K. pneumoniae*

PCR analysis of the CR isolates showed amplification of a 281 bp fragment corresponding to the *bla* OXA-48 gene in 32 out of 35 samples, representing 91.4%. Furthermore, a 621 bp fragment associated with the *bla* NDM gene was detected in 29 out of 35 samples, representing 82.8%. In contrast, a 538 bp fragment corresponding to the *bla* KPC gene was identified in only 2 isolates, representing 5.7%. Notably, the target linked to *bla* VIM 390 was not detected in any of the 35 CR isolates that represent 0%. The co-occurrence of both *bla* NDM and bla OXA were observed in 26 out of 35 samples, accounting for 74.3%. Conversely, the co-occurrence of *bla* KPC and *bla* OXA 48 was found in only one sample, representing 2.86%, as shown in Figure 3.8. The percentage distribution for each gene is summarized in Figure 3.9.

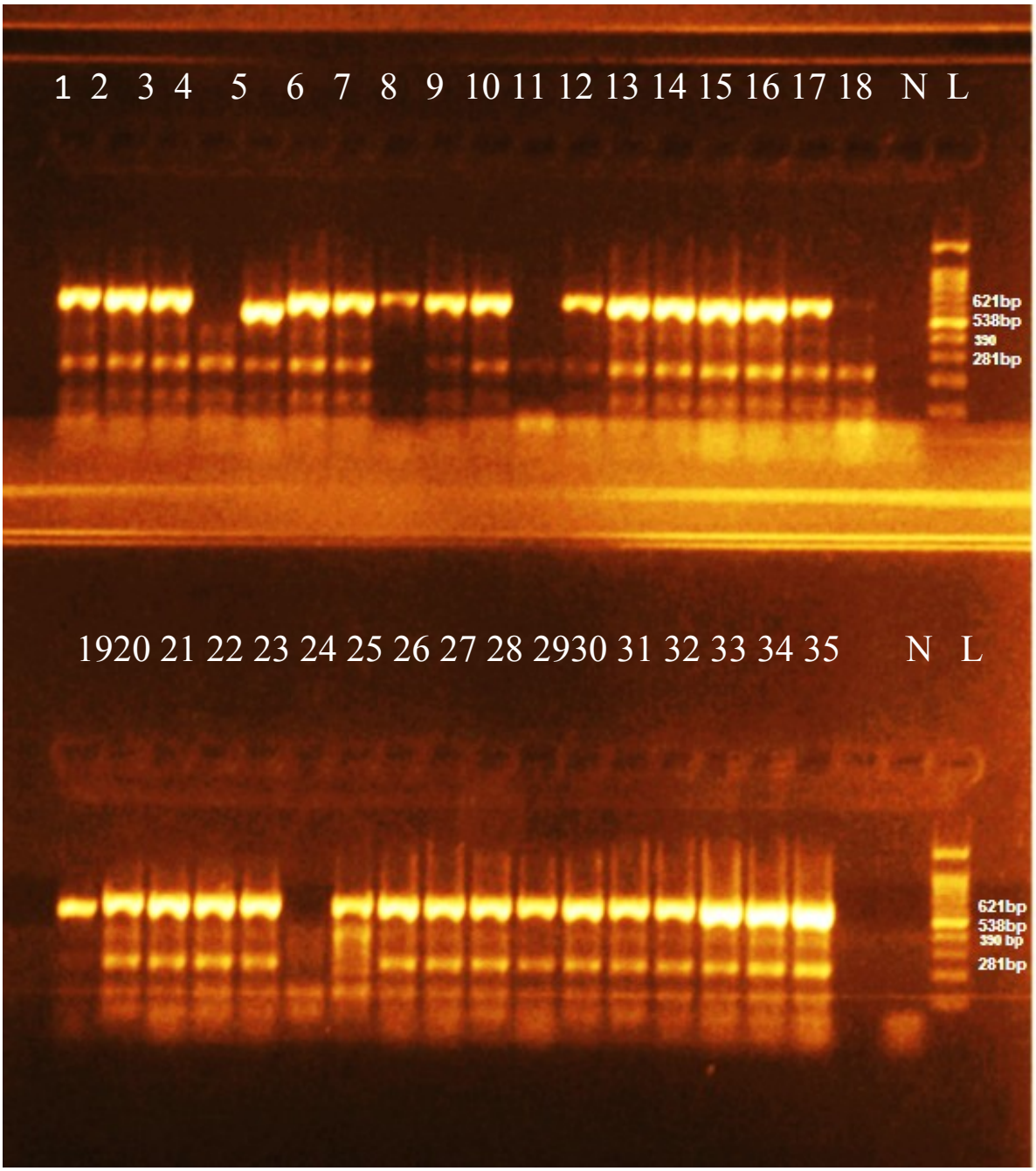

Figure 3.8 Agarose gel electrophoresis of multiplex PCR assay of CR-Kp. N: negative control, L: ladder of DNA (100bp).

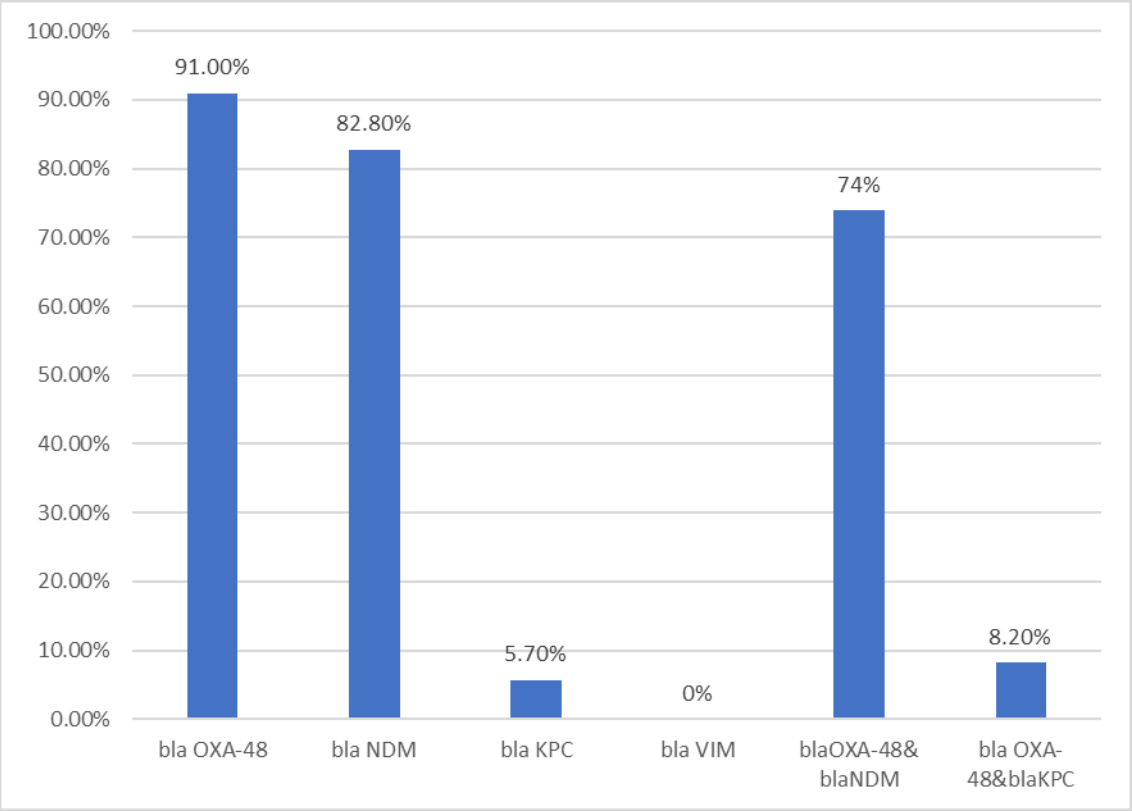

Figure 3.9 Distribution of carbapenemase genes in CR-Kp.

3.7.2 Detection of carbapenemase genes in ESBL in *K. pneumoniae*

The gel electrophoresis analysis of 15 ESBL isolates revealed that the carbapenemase gene**.** The *bla*VIM gene, PCR product of 390 bp, was detected in 7 out of 15 samples, representing for 46.6%. The *bla* OXA-48 gene, 281 bp, was found in 4 out of 15 samples, representing 26.6%. In comparison, the *bla* NDM gene, which is 621 bp long, was only found in one sample, representing 6.7%.Conversely, the co-occurrence of *bla* VIM and *bla* OXA was found in 4 out of 15 samples representing (26.6%) as illustrated in Figur3.10.The percentage distribution for each gene is summarized in Figure 3.11.

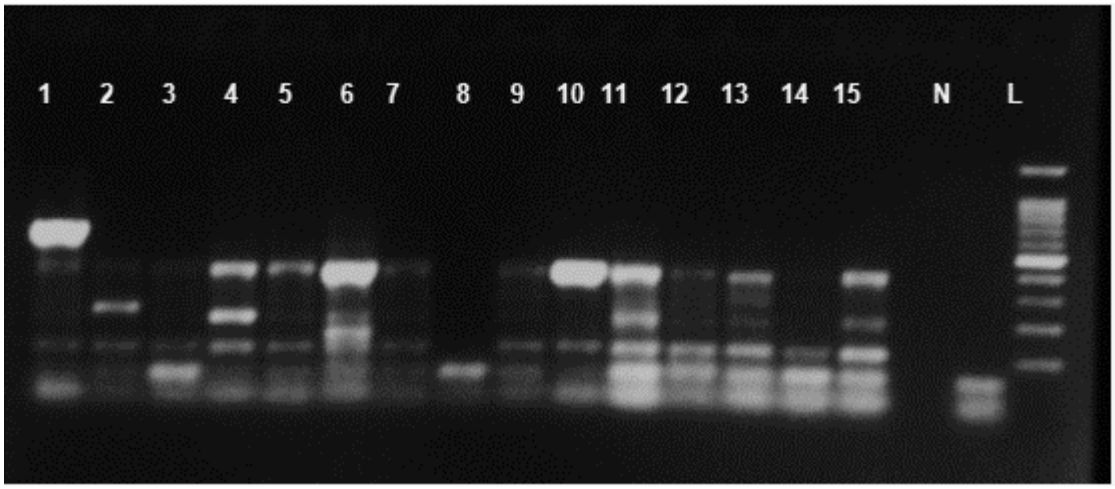

Figure 3.10 Agarose gel electrophoresis of multiplex PCR assay of ESBL *K. pneumoniae*. N: negative control, L: ladder of DNA (100bp)

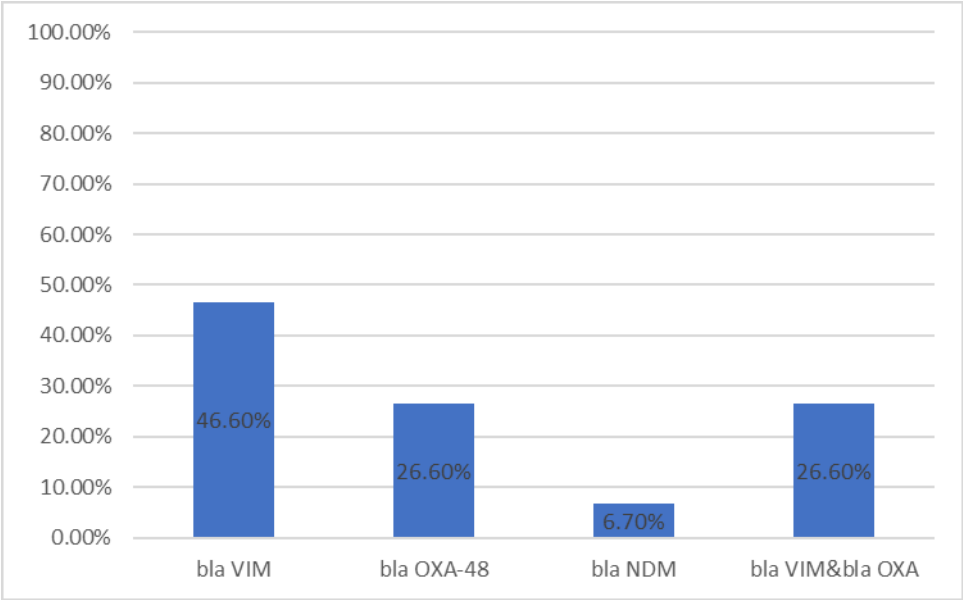

Figure 3.11 Distribution of carbapenemase genes in ESBL *K. pneumoniae*

Chapter Four

4.1 Discussion

K. pneumoniae is a well-documented cause of hospital-acquired infections. (Assoni et al., 2024). The primary antibiotic options have traditionally involved β-lactam antibiotics, particularly penicillin, cephalosporins, and carbapenems. However, the excessive and misuse of carbapenems has led to the emergence of carbapenem-resistant *K. pneumoniae* (CR-Kp) strains. These strains produce carbapenemase enzymes that lead to the promotion of many resistance genes. (Karampatakis et al., 2023).

There is a scarcity of published data on the prevalence of carbapenem resistance among *K. pneumoniae* isolates in Palestine. To close this disparity, the current study investigated the prevalence of CR-Kp 100 *K. pneumoniae* isolates collected from various clinical samples from various departments in Princess Alia Governmental Hospital in Hebron, Palestine, and tested the isolates for the distribution of carbapenemase genes.

The present study found that male patients account for 51% of *K. pneumoniae* cases, while female patients account for 49%. These outcomes are in agreement with a study reported by Saleem et al. (2022) in Saudi Arabia, in which 53% of *K. pneumoniae* cases were in male patients and 47% in female patients. (Saleem et al. 2022). In addition to similarly, Worku et al. (2024) in Ethiopia showed that male patients accounted for 55.7% of *K. pneumoniae* cases, but female patients accounted for 41.3%. The reason for the higher number of males involved could be due to the fact that there is a higher burden of comorbidities and lifestyle-related risk factors, as well as disparities in healthcare-seeking behaviors (Worku et al. 2024).

In the present study, *K. pneumoniae* isolates were prevalent in those aged 0-15 years. This was followed by prevalence among individuals over 60 years of age. This observation is supported by several related studies. For instance, the research conducted by Worku et al. (2022) highlights that the immunocompromised status of pediatric patients is a major reason for their increased susceptibility to *K. pneumoniae* infections. Furthermore, findings indicate that individuals over 60 years of age are even more vulnerable to these infections,

likely due to weakened immunity associated with aging and the prevalence of chronic diseases in elderly people (Worku et al. 2024).

In contrast, the age groups of 16 to 30 and 31 to 45 demonstrate a notable reduction in *K. pneumoniae* infections, which is often linked to efficient immune response and a lower number of risk factors among these individuals. Overall, the current findings support the conclusion that pediatric and geriatric populations are the most susceptible to *K. pneumoniae* infections (Worku et al., 2024).

Urine samples represented the highest proportion of *K. pneumoniae* isolates in this study, accounting for 51%, followed by wound swabs at 21% and blood samples at 17%. These findings are inconsistent with those reported by Elramli et al. (2024) in Libya, who also observed the highest percentages in urine and wound samples. However, a notable difference was observed in the distribution in the blood and sputum isolates, as their study reported a higher frequency of *K. pneumoniae* in sputum samples compared to blood (Elramli et al., 2024).

It is clear that the AST revealed that *K. pneumoniae* exhibited the highest sensitivityto the aminoglycoside antibiotics amikacin and gentamicin, showing sensitivities of 78/100(78%) and 62/100(62%), respectively. This contrasts with the finding from Sikarwar et al., (2011), who also reported high sensitivity to the aminoglycoside group but noted that sensitivity to ciprofloxacin was high,which was not observed in this study (Sikarwar et al., 2011).

In current study, the resistance rates to antibiotics that include β-lactamase inhibitors, specifically amoxicillin/clavulanate and piperacillin/tazobactam, were 44/100(44%). A study conducted in Saudi Arabia by Saleem et al. (2022) reported a resistance rate of 46.7% for amoxicillin/clavulanate, which is consistent with the findings of this study. However, for piperacillin/tazobactam, they observed a resistance rate of 33.4%, which is lower than this study (Saleem et al., 2022).

In contrast, another study in Saudi Arabia by Marzouk et al., in 2024 reported higher resistance rates for amoxicillin/clavulanate while noting a decrease in resistance to piperacillin/tazobactam compared to results of this study (Marzouk et al., 2024). These findings highlight the evolving nature of antibiotic resistance and emphasize the importance of continuous monitoring and adaptation of treatment strategies.

This study has shown an elevated rates of *K. pneumoniae* resistance to cephalosporins among bacteria. When bacteria exhibit resistance to this class of antibiotics in combination with extended-spectrum beta-lactamases (ESBL), the analysis revealed that 77/100 (77%) of the isolates were ESBL.

Regarding cephalosporin antibiotic, this study showed the following resistance rates for cephalosporins: cefoxitin 83/100 (83%), cefuroxime 82/100 (82%), ceftazidime 68/100

(68%), and cefepime 62/100 (62%). These rates are considerably greater than those reported in Palestine by Ibaideya et al. (2024), where the resistance rates for ceftazidime and cefepime were 53.2% and 43.6%, respectively (Ibaideya et al., 2024). In summary, the discrepancies in resistance rates could be attributed to differences in antibiotic usage practices, infection control strategies and hygiene, and regional epidemiological patterns.

In the present study, 66/100 (66%) of *K. pneumoniae* were shown to be resistant to the sulfamethoxazole (SXT). This resistance rate aligns with the high percentages (62.6%) reported by Marzouk et al. (2023) in Saudi Arabia (Marzouk et al., 2023). In contrast, a study conducted by Okafor et al. (2023) in South Africa revealed a much lower resistance rate of 41% for sulfamethoxazole (Okafor et al., 2023). Furthermore, resistance rates to trimethoprim-sulfamethoxazole in isolates of CR-Kp have been documented to vary between 50% and 98.2% (Lagadinou et al.,2024).

Antibiotic susceptibility testing revealed that *K. pneumoniae* is an MDR bacteria, exhibiting resistance to more than one antibiotic. The overall prevalence of multidrug-resistant *K. pneumoniae* (MDR-KP) among the clinical isolates was found to be 61.3%. This finding aligns with a study conducted by We classified bacterial isolates as, MDRE as in when they showed resistance to at least one antibiotic in three distinct classes of antibiotics. Ibaideya et al. (2024) in Palestine, which also reported a high rate 50.5% of MDR among 200 *K. pneumoniae* isolates (Ibaideya et al., 2024). Additionally, previous research from various regions has similarly documented elevated rates of MDR in *K. pneumoniae* isolates. For example, a study by Lagha et al. (2021) in Saudi Arabia found that *K. pneumoniae* strains exhibited high levels of resistance, recording a rate of 83.33% (Lagha et al., 2021).

The increase in MDR strains of *Klebsiella* has contributed to the rise of carbapenem-resistant *Enterobacteriaceae* (CRE). This work, revealed that35/100(35%) of *K. pneumoniae* isolates exhibited resistance to both meropenem and imipenem. This finding correlates to study conducted in northern Palestine by Aiesh et al. (2024), which reported a CRE prevalence of 39% in *K. pneumoniae* (Aiesh et al., 2024). However, another research study in Palestine by Ibaideya et al. (2024) found *K. pneumoniae* as the predominant species of carbapenem-resistant *Enterobacteriaceae* (CRE), representing (61.7%) of cases, that exceeds our observations (Ibaideya et al., 2024).

Multiple factors play a vital role in establishing and determining varying levels of resistance. The regional discrepancies can be linked to variations in antimicrobial stewardship practices, prescribing behaviors, and the local presences of multidrug-resistant organisms.

In the present study, a high prevalence of carbapenemase genes was noticed in *K. pneumoniae.* The *bla* OXA-48 gene was identified in 32/35(91.4%) of the samples, the *bla* NDM gene was found in 29/35 (82.8%) of the samples, and the *bla* KPC gene was found in 2/35(5.7%) of the samples; however, the *bla* VIM gene was not detected in any of the

samples 0/35 (0%). This finding is different from a study done in Palestine by Ibaideya et al. (2024), which reported the rates of carbapenemase genes in *K. pneumoniae* as 17% for the *bla* KPC gene and 16% for both *bla* OXA-48 and *bla* NDM but similar to the *bla*VIM gene, which was not found in their samples (Ibaideya et al., 2024).

Another study in Iran by Jafari-Sales et al. (2023) observed completely different results, showing that the most common carbapenemase genes in *K. pneumoniae* samples were *bla*KPC, found in 72.0% of the samples, followed by *bla*VIM in 12.0% and *bla* OXA-48 in 4.0%; however, the *bla* NDM gene was not found in any samples, whereas in this study, *bla* OXA-48 was the most prevalent, followed by *bla* NDM and *bla* VIM, which were not found in any sample (Jafari-Sales et al., 2023).

However, a study in Baghdad by Hamad et al. (2022) reported a high prevalence of carbapenemase genes among *K. pneumoniae* isolates, which is similar to this finding of this study. They identified the *bla* OXA48 gene as the most prevalent. However, Hamad et al. (2022) found the *bla*VIM gene in 28.75% of the isolates, while our study did not detect it in any sample. Moreover, the *bla*NDM gene had the lowest prevalence of the isolate, whereas prevalence in this study (Hamad et al., 2022).

In the present study found that many isolates harbored more than one carbapenemase gene. The study detected the co-occurrence of the genes *bla* NDM and *bla* OXA-48, as well as the co-occurrence of *bla* KPC and *bla* OXA-48. Notably, there was no co-occurrence of all three genes together. This finding supports the work of Alshahrani et al. (2022), which revealed the co-occurrence of *bla* OXA-48 and *bla* NDM only. On the other hand, Alshahrani et al. (2022) discovered the co-occurrence of three genes: *bla* NDM *bla* KPC, and *bla* OXA-48(Alshahrani et al., 2022).

The findings distinctly reveal discrepancies in the distribution of various carbapenemase genes across diverse geographical regions. Multiple factors contribute to these inconsistencies, such as differences in geographical locations, study demographics, sources of the isolations, and healthcare practices.

The carbapenemase gene was identified in ESBL, which carbapenem-sensitive strain, specifically *bla* VIM 390, which accounted for 7/15(46.6%) of the cases. Additionally, the *bla* OXA-48 gene was detected, representing 4/15 (26.6%). In contrast, the *bla* NDM gene was observed, constituting 1/15 (6.7%). These results indicate that the screening tests used to detect carbapenemase resistance are often unreliable. Specifically, in cases of ESBL *K. pneumoniae*, the MEM test for screening carbapenemase proved less effective compared to molecular techniques. Molecular tests successfully identified the presence of carbapenemase genes in *K. pneumoniae*. This finding demonstrates a reduction in susceptibility to cephalosporin antibiotics, likely due to the production and made of beta-lactamases.

Furthermore, a study conducted in Cairo in 2020 showed that all isolates, which were sensitive to carbapenem antibiotics and tested negative by the Modified Hodge Test (MHT) were found, by PCR, to carry one or more carbapenemase genes (Emira et al., 2020). Based on these findings, the Cairo results are considered questionable due to the poor performance of MHT, which may have underestimated carbapenemase production.

Additionally, a recent study conducted in Palestine in 2024 also detected carbapenemase genes in isolates that were sensitive to carbapenem antibiotics (Ibaideya et al., 2024). The main reasons to stand by the appearance of the *bla*VIM carbapenemase genes from ESBL *K. pneumoniae* may be due to both of them (ESBL and carbapenemase) conferring resistance to beta-lactam antibiotics or being found together on the same mobile genetic element, like a plasmid, so co-localization and transfer of different genes can spread these genes in bacteria both vertically and horizontally (Reyes et al., 2020).

A comparison between the secerning test by MEM disk diffusion and multiplex PCR can provide valuable insights, but it has inherent limitations. The phenotypic MEM disk diffusion test assesses bacterial resistance to specific antibiotics by reflecting the actual expression of resistance for this antibiotic. In contrast, molecular methods detect the presence of resistance genes (*bla* KPC, *bla* OXA, *bla* VIM and *bla* NDM) regardless of whether they are actively expressed. Consequently, discrepancies may arise: a resistance gene might be detected by PCR but not expressed in the disk diffusion test due to regulatory effects, gene silencing, or other physiological factors, as observed in some ESBL-producing isolates carrying carbapenemase genes. Conversely, if alternative or unidentified mechanisms are involved, phenotypic resistance may be observed without detection of a known gene. These findings emphasize the importance of interpreting phenotypic and molecular results as complementary rather than interchangeable.

During the multiplex PCR assay, certain technical limitations were observed that may have affected the resolution of some amplicons. Specifically, samples 33, 34, and 35 appeared to exhibit doublet bands of approximately 621 bp and 538 bp that could not be clearly resolved, suggesting a possible overlap in fragment size. In addition, a band of *bla* VIM approximately 390 bp may detected in sample 25 and several other samples. According to these findings, multiplex PCR can sometimes exhibit bias toward the amplification of specific targets due to primer–primer interactions, template competition, or variations in annealing efficiency.

4.2 Conclusion

Our study revealed a high prevalence of carbapenem-resistant *K. pneumoniae* isolates, predominantly carrying the *bla* OXA-48 then *bla* NDM gene, followed by *bla* KPC. The high prevalence of CRE in hospitals represent a critical public health concern.

The MEM test was found to be an unreliable method for detecting CRE-producing *K. pneumoniae* isolates, despite being not expensive and easy and simple to perform and use. Additionally, the recommended phenotypic methods for identifying enterobacteria are not always effective for *K. pneumoniae* isolates.On the other hand, despite being more costly and not frequently utilized in clinical microbiology labs, the multiplex PCR assay demonstrated exceptional results and was highly reliable

4.3 Recommendation

Future studies ensure strict standardization and calibration of antimicrobial susceptibility testing (AST) procedures, particularly in measuring the zone of inhibition and applying the ≥5 mm increase criterion for carbapenem resistance. Given the potential for minor measurement errors or variability in disk diffusion interpretation.To improve accuracy, we recommend repeating the AST with multiple observers, implementing automated zone measurement systems where possible, and confirming borderline or unexpected results using complementary methods such as MIC determination or molecular detection of carbapenemase genes.

In addition to, developing rapid detection methods and continuous monitoring for carbapenemase enzymes is crucial for ensuring early recognition and immediate application of hospitals control measures. This is particularly important for advancing future studies that highlight the significance of these bacteria and enhance our understanding of the possession of relevant genes and factors. Moreover, given the rise in bacterial resistance to antibiotics, it is advisable to explore alternative treatments derived from herbal extracts that can effectively inhibit the growth of these bacteria.

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الكشف عن الجينات المقاومة للكاربابينيم في بكتيريا الكلبسيلة الرئوية من عينات سريرية مختلفة.

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الملخص

خلفية الدراسة: تُعدّ الكلبسيلة الرئوية (*K. pneumoniae*) سببًا رئيسيًا للعدوى المكتسبة من المستشفيات. البنسلين والسيفالوسبورينات والكاربابينيمات هي المضادات الحيوية بيتا لاكتام التي توصف في أغلب الأحيان لعلاج هذا النوع من العدوى. ومع ذلك، أصبحت هذه البكتيريا مقاومة للأدوية المتعددة (MDR)، ومن الواضح أن تزايد المقاومة في جميع أنحاء العالم يؤدي إلى تهديد الصحة العامة على نطاق عالمي. غالبًا ما يُعتبر الكاربابينيم الخيار العلاجي الأخير الفعال للبكتيريا سالبة الجرام المقاومة للأدوية المتعددة. لسوء الحظ، طورت الكلبسيلة الرئوية مقاومة لآخر مضاد حيوي فعال من بيتا لاكتام وهو الكاربابينيم. تنشأ هذه المقاومة في المقام الأول من إنتاج الكاربابينيمات، مثل كاربابينيماز الكلبسيلة الرئوية، KPC؛ فيرونا إنتغرونميتالو بيتا لاكتاماز، VIM؛ نيودلهي ميتالو-β-لاكتاماز-ميدياتدكاربابينيم، NDM؛ والكاربابينيميز المحلل للأوكساسيلين، OXA-48، والذي يمكن الحصول عليه في كل من المستشفيات والمجتمعات المحلية.

هدف الدراسة :سلالات بكتيريا *K. pneumoniae* المقاومة للكاربابينيم (CR-Kp) هي مسببات أمراض رئيسية مرتبطة بالرعاية الصحية، وموجودة عالميًا. هدفت هذه الدراسة إلى الكشف عن مقاومة المضادات الحيوية لعزلات CR-Kp السريرية، ومعرفة ما إذا كانت جينات الكاربابينيماز، مثل *bla* KPC و *bla* VIM و *bla* NDM و *bla* OXA-48، موجودة، باستخدام تقنيات تفاعل البوليميراز المتسلسل

منهجية البحث :جُمعت 100 عينة بكتيرية معزولة من مستشفى الأميرة علياء الحكومي في الخليل. حُددت هذه العينات على أنها *K. pneumoniae* بناءً على شكل مستعمراتها، ثم تم تأكيدها باستخدام نظام Vitek2 Compact. حُددت حساسية المضادات الحيوية (AST) باستخدام النظام نفسه. أُجري اختبار ESBL باستخدام اختبار التآزر ثنائي القرص (DDTS). أُجري فحص مقاومة الكاربابينيم باستخدام أقراص الميروبينيم (MEM)، متبوعًا بتفاعل البوليميراز المتسلسل المتعدد (PCR) للكشف عن وجود جينات الكاربابينيماز *bla* KPC، *bla* VIM، *bla* NDM، و *bla* OXA-48.

النتائج: من بين 100 عزلة من بكتيريا *K. pneumoniae* المشمولة في هذه الدراسة، أشار التحليل إلى أن 77 من العزلات (77%) أظهرت بيتا لاكتاماز ممتد الطيف (ESBL). من بين 100 عزلة، كانت 35 (35%) مقاومة للكاربابينيم (CR). تم اختبار جميع العزلات الـ 35 بحثًا عن وجود جينات الكاربابينيماز. كانت نتائج جينات الكاربابينيماز كما يلي: تم العثور على جين *bla* OXA-48 في 32 من أصل 35 عينة (91.4%)؛ وكان جين *bla* NDM في 29 من أصل 35 عينة (82.8%)؛ وكان جين *bla* KPC في عينتين من أصل 35 عينة (5.7%). ومع ذلك، لم يتم العثور على جين bla VIM في أي من العينات (0.0%). لوحظ التواجد المشترك لكل من *bla* NDM و *bla* OXA في 26 عينة من أصل 30 عينة (74%). وعلى العكس من ذلك، وُجد التواجد المشترك لـ *bla* KPC و *bla* OXA في عينة واحدة فقط (28%). بالإضافة إلى ذلك، تم اختيار خمس عشرة عينة عشوائيًا، والتي كانت نتائج اختبارها إيجابية لإنزيمات بيتا لاكتاماز ممتدة الطيف (ESBL) ولكنها كانت حساسة لقرص الميروبينيم (MEM) (حساسة للكاربابينيم)، لفحص جينات الكاربابينيماز. من بين العينات الخمس عشرة، وُجد *bla*VIM في 7 عينات من أصل 15 عينة (46.6%)؛ بينما وُجد جين *bla* OXA-48 في 4 عينات من أصل 15 عينة (26.6%). في المقابل، وُجد جين *bla* NDM في عينة واحدة فقط (6.7%). وعلى العكس من ذلك، تم العثور على حدوث *bla* VIM و *bla* OXA في 4 من أصل 15 عينة (26.6%).

الخلاصة: أشارت نتائج الدراسة بوضوح إلى ارتفاع معدل إنتاج البكتيريا المعوية المقاومة للكاربابينيم (CRE) من عزلات بكتيريا *K. pneumoniae*، مما يجعلها سببًا رئيسيًا للعدوى المرتبطة بالرعاية الصحية. ولا تقتصر مقاومة الكاربابينيمات على العزلات المقاومة للكاربابينيم؛ إذ يمكن أيضًا العثور على بعض السلالات التي تُظهر هذه المقاومة بين عزلات بيتا لاكتاماز واسعة الطيف (ESBL). ولضمان فعالية العلاج، من المهم متابعة الوبائيات الجزيئية المحلية للجينات المقاومة للكاربابينيم، و/أو إجراء تحليل موثوق آخر.

الكلمات الدالة : جين الكاربابينيمات، تفاعل البوليميراز المتسلسل المتعدد، حساسية المضادات الحيوية. *bla*KPC، *bla*VIM، *bla*NDM، و *bla* OXA-48