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Occurrence and Molecular Characterization of Carbapenems Resistant *Escherichia coli* in Thi-Qar Province

A Dissertation

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

﴿ قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا

إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ ﴾

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Dedication

To

The Soul of My Father

To

The Kindest Heart My Mother

To

My Brothers and Sisters

To

My Dear Wife and Soul of Her Father

To

My Lovely Son Afadal

Mostim

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Summary

Carbapenems are powerful β -lactam antibiotics that are currently the drugs of choice for treating the most serious infections caused by multidrug-resistant (MDR) bacteria. Antibiotic resistance has become a critical issue in managing bacterial infections today. Carbapenems resistant *Escherichia coli* poses an emerging threat to the healthcare system due to the limited therapeutic options available. This study was conducted to determine the occurrence and characteristics of carbapenems resistant *E. coli* isolates recovered from different clinical specimens in Thi-Qar Hospitals.

This cross-sectional study was performed on a total of 2203 clinical specimens randomly collected from patients hospitalized or attending at two major Hospitals in Thi-Qar province over a ten-month period in 2024. All *E. coli* isolates were identified using traditional biochemical tests and confirmed by the VITEK-2 system. Antibiotic susceptibility was evaluated using the discs diffusion method against twenty-three antibiotics from eleven classes. The phenotypic characterization of extended-spectrum β -lactamases (ESBLs), AmpC β -lactamases, and carbapenemases production was conducted using discs diffusion methods. The presence of carbapenem resistance determinants and integrons (class 1 and class 2) was investigated through polymerase chain reaction (PCR) methods. The PCR products of *bla*_{NDM-1} and *bla*_{OXA} positive isolates were sequenced.

Escherichia coli was isolated from 186 specimens, accounting for 15.35% of the total positive bacterial culture. The *E. coli* isolates exhibited a high frequency of resistance to most antibiotics tested, with resistance rates ranging from 11.29% against imipenem to 100% against ampicillin. Carbapenems resistance was identified in 40 isolates (21.51%), with the highest resistance rates to doripenem (80.00%), followed by meropenem (55.00%) and imipenem (52.50%). Of the carbapenems resistant isolates, 27 (67.50%) exhibited extensive drug resistance (XDR), while 13 (32.50%) were recognized as possibly pan-drug resistant (PDR). All isolates showed a multiple antibiotic

resistance (MAR) index greater than 0.2, with 12 (30.00%) isolates exhibiting a maximum MAR index of 1, demonstrating resistance to all tested antibiotics. Confirmatory test for ESBLs production revealed that 10 isolates (25.00%) were positive. However, 28 (70%) of isolates were positive to plasmidic AmpC but none of the isolates tested positive for chromosomal AmpC production. Carbapenemases production was suggested by Double Discs Synergy Test (DDST) and Combined Discs Test (CDT), indicating that 11 isolates (27.50%) were metallo- β -lactamases (MBLs) producers. Additionally, 2.5% of the isolates were identified as class A carbapenemase or OXA-48 producers using the Modified Hodge Test (MHT). A Lateral Flow Immunoassay (LFIA) showed that 11 isolates (27.50%) were positive for NDM enzyme and 1 isolate (2.50%) was positive for OXA-48 enzyme.

Among the carbapenemase genes studied, 34 isolates (85.00%) harbored at least one carbapenemase gene. Analysis revealed that *bla*_{NDM-1} was the most prevalent carbapenemase gene found in 24 isolates (60.00%), followed by *bla*_{OXA} in 16 isolates (40.00%), *bla*_{OXA-48} in 6 isolates (15.00%), *bla*_{OXA-51} in 2 isolates (5.00%), and *bla*_{VIM} in 2 isolates (5.00%). Co-occurrence of carbapenemase resistance genes was commonly detected among isolates, with six different combinations observed. The presence of class 1 integrons was confirmed in all isolates, and class 2 integrons were detected in 32.50% of the tested isolates. Sequencing results for twenty-four isolates harboring *bla*_{NDM-1} confirmed 22 of them as *bla*_{NDM-1} variants. Sequencing of the 16 isolates containing *bla*_{OXA} confirmed 11 as *bla*_{OXA-1} variants.

In conclusion, carbapenems resistant *E. coli* isolates harboring carbapenemase genes are currently widely spread in Thi-Qar Hospitals. The co-production of carbapenemase genes associated with integrons may increase the risk of spreading MDR isolates in these Hospitals. Rapid identification of carbapenems resistant isolates and appropriate infection control measures are essential to prevent the further spread of infections caused by these bacteria in Iraqi Hospitals.

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List of Abbreviations

Abbreviation	Key
AMR	Antimicrobial Resistance
bp	Base Pair
CLSI	Clinical and Laboratory Standards Institute
CFU	Colony Forming Unit
COVID-19	Corona Virus Disease of 2019
°C	Degree Celsius
DAEC	Diffusely Adherent <i>E. coli</i>
EAEC	Enteraggregative <i>E. coli</i>
EHEC	Enterohemorrhagic <i>E. coli</i>
EIEC	Enteroinvasive <i>E. coli</i>
EPEC	Enteropathogenic <i>E. coli</i>
ETEC	Enterotoxigenic <i>E. coli</i>
EDTA	Ethylene Diamine Tetra Acetic Acid
ESBLs	Extended Spectrum β -Lactamases
XDR	Extensively Drug Resistant
ExPEC	Extraintestinal Pathogenic <i>E. coli</i>
ExPEC	Extraintestinal Pathogenic <i>E. coli</i>
FDA	Food and Drug Administration
GIM	German Imipenemase
g	Gram
IMP	Imipenemase
IMI	Imipenem-Hydrolyzing β -Lactamase
<i>intI1</i>	Integron 1 Gene
<i>intI2</i>	Integron 2 Gene
IPEC	Intestinal Pathogenic <i>E. coli</i>
IPEC	Intestinal Pathogenic <i>E. coli</i>
KPC	<i>Klebsiella pneumoniae</i> -Carbapenemase
LPS	Lipopolysaccharide
MBLs	Metallo- β -Lactamases
μ M	Micro Molar

µg	Microgram
µL	Microliter
Mg	Milligram
mL	Milliliter
Mm	Millimeter
mM	Millmolar
AmpC	Molecular Class C β-Lactamase
MHA	Mueller-Hinton Agar
MDR	Multidrug Resistant
NMEC	Neonatal Meningitis <i>E. coli</i>
NDM	New Delhi Metallo-β-Lactamase
NMC	Not Metalloenzyme Carbapenemase
OXA	Oxacillinases
PDR	Pan Drug Resistant
PBPs	Penicillin Binding Proteins
pH	Potential of Hydrogen
Rpm	Round Per Minute
SPM	Sao Paulo Metallo-β-Lactamase
SIM	Seoul Imipenemase
SME	<i>Serratia marcescens</i> Enzyme
STEC	Shiga Toxin-producing <i>E. coli</i>
U	Units
UTIs	Urinary Tract Infections
UPEC	Uropathogenic <i>E. coli</i>
OXA-48	Variant of Oxacillinases
OXA-51	Variant of Oxacillinases
VIM	Verona Integron-Encoded Metallo-β-Lactamase
WHO	World Health Organization
<i>bla</i> gene	β-Lactamase Gene

Chapter One

INTRODUCTION

1.1. Introduction

Gram-negative *E. coli* is a common inhabitant of the normal intestinal flora (Pokharel *et al.*, 2023). While this organism is typically harmless, it has the ability to acquire a diverse range of mobile genetic elements that contain genes responsible for producing various virulence factors (Pakbin *et al.*, 2021). These factors enable *E. coli* to evade the body's immune system and develop resistance to commonly prescribed antibiotics (Kibret and Abera, 2011; Bessaiah *et al.*, 2021). As a result, it can emerge as a human pathogen capable of causing a wide variety of intestinal and extra-intestinal diseases (Mueller and Tainter, 2023).

However, managing these infections is becoming increasingly complicated (Hooton *et al.*, 2004). This complexity arises from the excessive, inappropriate, irregular, and indiscriminate use of antimicrobial agents, which reduces the effectiveness of drugs and leads to the emergence of strains resistant to many classes of antimicrobial agents (WHO, 2014; Baym *et al.*, 2016). Antimicrobial resistance (AMR) is a significant medical issue and is regarded by the WHO as perhaps the most urgent challenge facing medical science today (WHO, 2021).

Escherichia coli infections are typically treated with β -lactam antibiotics, such as penicillins, cephalosporins, monobactams, and carbapenems (Pitout, 2012). Among the various β -lactams, carbapenems are the most potent and have the broadest spectrum of activity against both Gram-positive and Gram-negative bacteria, making them commonly referred to as "antibiotics of last resort" for patients suspected of harboring MDR bacteria (Paterson and Bonomo, 2005; Torres *et al.*, 2007). However, the dramatic increase in carbapenems usage, particularly after the Corona Virus Disease of 2019 (COVID-19) pandemic, which heightened the risk of bacterial co-infections and inappropriate antibiotics use, has led to higher rates of antibiotic resistance, presenting a growing public health challenge worldwide (Ghosh *et al.*, 2021; Jomehzadeh *et al.*, 2022).

Carbapenemases are significant contributors to carbapenems resistance (Pitout *et al.*, 2015). These enzymes primarily occur in Gram-negative bacteria and can be intrinsic or acquired through transferable genes encoding carbapenemases (Codjoe and Donkor, 2017). Numerous types of carbapenemases have been discovered and characterized, belonging to either molecular classes A and D (serine carbapenemases) or class B (metallo- β -lactamases, MBLs). The genes that encode these enzymes are associated with mobile genetic elements, facilitating their rapid spread in clinical settings (Castanheira *et al.*, 2019). Therefore, understanding the mechanisms of carbapenem is crucial for selecting appropriate therapeutic strategies and implementing effective infection control measures (Miriagou *et al.*, 2010). Although molecular characterization of carbapenem-resistant *E. coli* has been conducted in several countries, there is limited data available from Iraq, particularly in Thi-Qar province.

1.2. Aims of the Study

The aim of the present study to determine the occurrence, molecular characterization of the carbapenems resistant *E. coli* isolates collected from Hospitals in Thi-Qar.

This study is carried out to achieve the following objectives:

1. To evaluate the occurrence and antibiotic susceptibility profiles of *E. coli* isolates from clinical specimens in Thi-Qar hospitals.
2. To identify the spectrum of *E. coli* isolates that are resistant to carbapenems.
3. To investigate the dissemination of MDR, XDR, and PDR among carbapenems resistant isolates.
4. To detect β -lactamases production phenotypically among carbapenem resistant isolates.
5. To assess the distribution and diversity of the main class A, class B, and class D carbapenemases determinants, as well as integron genes among isolates exhibiting carbapenems resistant phenotypes.

Chapter Two

LITERATURE REVIEW

2. Literature Review

2.1. Historical Overview of *Escherichia coli*

Escherichia coli was first described in 1885 by German pediatrician Theodor Escherich, who was studying the gut flora of newborns. He initially named the bacterium '*Bacterium coli commune*,' describing it as a short, plump rod (Escherich, 1885). In 1919, the bacterium was officially renamed *Escherichia coli* in honor of its discoverer (Castellani and Chalmers, 1919). Initially, Escherich considered *E. coli* to be nonpathogenic; however, it gradually became recognized as a cause of infantile diarrhea (Robins-Browne, 1987; Jaybhaye and Deb, 2021). Later, Bray identified that certain isolates of *E. coli* were the primary cause of diarrhea among children (Bray, 1945). Further investigations have also linked *E. coli* to urinary tract infections (UTIs), sepsis, and newborn meningitis (Hacker, 1990).

2.2. General Properties of *E. coli*

Escherichia coli is a relatively small, Gram-negative, straight, non-spore-forming bacterium that is facultatively anaerobic (Campbell and Reece, 2002; Gaitaev *et al.*, 2023). This bacterium has a rod shape, measuring approximately 0.4 μm in diameter and 2-3 μm in length (Poli *et al.*, 2005). Due to the presence of peritrichous flagella, *E. coli* is capable of motility, though very few strains are non-motile (Riley *et al.*, 2018). Fimbriated strains can be either motile or non-motile (Leimbach *et al.*, 2013). They possess only one or two layers of peptidoglycan in their thin cell wall (Köhler and Dobrindt, 2011). Some *E. coli* strains, particularly those isolated from extra-intestinal infections, have an outer membrane covered by a polysaccharide capsule (Whitfield, 2006).

2.3. Growth Conditions and Biochemical Properties

These bacteria can be grown and maintained on standard culture media with minimal nutrient requirements (Welch, 2006). *E. coli* is widely

recognized as a lactose-fermenting, Gram-negative rod that can thrive in both aerobic and anaerobic environments (Croxen and Finlay, 2010). It can grow at a broad range of temperatures, from 15°C to 45°C (Poli *et al.*, 2005). *E. coli* can tolerate a pH range of 4.5 to 9.5, with optimal growth occurring at pH 7.0. Under ideal conditions, it can divide in as little as 20 minutes (Erjavec, 2023). Morphologically, *E. coli* colonies exhibit a smooth surface and a convex shape. When cultured on MacConkey medium, colonies appear flat, dry, and pink, while on Eosin Methylene Blue medium, they display a green metallic sheen (Al-Temeamy, 2014). Biochemical testing typically shows that *E. coli* gives positive results for catalase, indole, and methyl red, generates acid and gas, and can reduce nitrate to nitrite. However, it produces negative results for Voges-Proskauer, urease, oxidase, and hydrogen sulfide (H₂S) production (AL-Ganimi, 2014).

2.4. Taxonomy

Currently, the genus *Escherichia* comprises six species: *E. coli* (Castellani and Chalmers, 1919), *E. hermannii* (Brenner *et al.*, 1982), *E. fergusonii* (Farmer *et al.*, 1985), *E. albertii* (Huys *et al.*, 2003), *E. marmotae* (Liu *et al.*, 2015), and the recently described *E. ruysiae* (van der Putten *et al.*, 2021). The majority of species within this genus are classified as *E. coli* (Parks *et al.*, 2021). Biochemically, *E. coli* is distinct from the other species and is classified in the following manner (Faner *et al.*, 2017):

Domain: Bacteria

Kingdom: Eubacteria

Phylum: Proteobacteria

Class: Gammaproteobacteria

Order: Enterobacterales

Family: Enterobacteriaceae

Genus: *Escherichia*

Species: *E. coli*

2.5. *Escherichia coli* Serotypes

In 1947, Kauffmann proposed a serological typing scheme to classify *E. coli* based on the bacteria's somatic (O antigen), capsular (K antigen), and flagellar (H antigen) proteins. The heat-stable O antigens, which may be shared among different members of the Enterobacteriaceae family, are found in the polysaccharide portion of lipopolysaccharides (LPS) (Megrian *et al.*, 2020). The H antigens are associated with flagella, meaning only flagellated bacteria possess these antigens. On the other hand, K antigens are usually associated with the capsule and, occasionally, with the fimbriae (Lillington *et al.*, 2014). Consequently, a strain's serogroup is defined by its O antigen, while its serotype is determined by the combination of O and H antigens (Liu *et al.*, 2020). Currently, there are approximately 186 different *E. coli* O-groups, 53 H-types, and 67 K antigen types (Denamur *et al.*, 2021). The most notable *E. coli* serotype is O157:H7, which is one of the primary serotypes linked to Enterohemorrhagic *E. coli* (EHEC) infections. K1-encapsulated strains account for about 80% of *E. coli* neonatal meningitis (Kim, 2012).

Although serotyping provides useful information regarding specific pathotypes (such as *E. coli* O157:H7, which is exclusively identified as an EHEC serotype), it is not always beneficial for other *E. coli* strains. Some strains with the same O type may belong to different pathogenic groups (DebRoy *et al.*, 2011). For instance, strains in the O26 serogroup are found in both Enteropathogenic *E. coli* (EPEC) and Enterohemorrhagic *E. coli* (EHEC) groups (Meng *et al.*, 2001).

2.6. *Escherichia coli* Pathotyping

Escherichia coli encompasses both commensal and pathogenic strains. While commensal *E. coli* rarely causes illness, except in immunocompromised individuals or when normal gastrointestinal barriers are compromised, some strains have adapted to pathogenic and can induce a

wide range of diseases through the acquisition of specific virulence factors (Kaper *et al.*, 2004). Generally, *E. coli* can be categorized based on its ability to cause infections within the gastrointestinal system (Intestinal Pathogenic *E. coli*, or IPEC) or outside of it (Extraintestinal Pathogenic *E. coli*, or ExPEC) in both healthy and immunocompromised individuals (Sora *et al.*, 2021). Phylogenetic analyses have identified five main phylogenetic groups of *E. coli*: A, B1, B2, D, and E. Intestinal and commensal isolates primarily belong to groups A and B1 (Da Silva and Mendonca, 2012). ExPEC, while phylogenetically and epidemiologically distinct from IPEC, mainly belong to group B2 and, to a lesser extent, group D. They can colonize various anatomical sites and cause a range of infections outside the intestinal tract, including newborn meningitis and UTIs, which are the most prevalent (Allocati *et al.*, 2013; Jensen *et al.*, 2014).

Although pathotyping classification schemes are useful for categorizing *E. coli* strains, it is important to note that absolute categorization cannot always be achieved based solely on antigenic differences and virulence factors (Kaper *et al.*, 2004). Furthermore, many virulence genes are located on plasmids, phages, or chromosomal pathogenicity islands, allowing for the transfer of virulence genes between *E. coli* strains (Hedberg *et al.*, 1997). A brief description of the key features of different *E. coli* infections is provided below:

2.6.1. Intestinal Pathogenic *E. coli* (IPEC)

Intestinal infections, particularly diarrhea, pose a significant public health issue and are a leading cause of morbidity and mortality among infants and young children, especially in developing countries (Kaper *et al.*, 2004). Diarrhea in humans primarily occurs due to the consumption of contaminated food and water, often influenced by animal or human waste (Some *et al.*, 2021). Intestinal Pathogenic *E. coli* encompasses several pathotypes according to their virulence factors, which include patterns of

bacterial attachment to host cells, the effects of this attachment, the production of various toxins, and invasiveness (Kaper *et al.*, 2004). This classification leads to six pathotypes: Enteropathogenic *E. coli* (EPEC), Enterohemorrhagic *E. coli* (EHEC)/Shiga toxin-producing *E. coli* (STEC), Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), Enteroaggregative *E. coli* (EAEC), and Diffusely Adherent *E. coli* (DAEC) (Servin, 2014).

2.6.2. Extraintestinal Pathogenic *E. coli* (ExPEC)

Extraintestinal Pathogenic *E. coli* typically reside in the intestinal tract of humans and other animals. From this reservoir, they can emerge to cause infections at sites outside the intestines (Manges *et al.*, 2019). In addition to characteristics specific to these bacteria, host-specific factors are also necessary for the development of disease. ExPEC strains are responsible for a range of human infections in both healthcare-associated and community settings (Foxman, 2003; Russo and Johnson, 2003). There are two main ExPEC pathotypes. The first is uropathogenic *E. coli* (UPEC), which is a significant cause of lower UTIs, such as cystitis, as well as systemic infections in human (Pitout, 2012). These systemic infections can include upper UTIs (pyelonephritis) (Isla *et al.*, 2017), bacteremia, nosocomial pneumonia (Park *et al.*, 2019), cholecystitis, peritonitis, and osteomyelitis (George and Manges, 2010). The second pathotype is neonatal meningitis *E. coli* (NMEC), which accounts for approximately 30% of meningitis in neonates (Foxman, 2003; Nielubowicz and Mobley, 2010).

Extraintestinal Pathogenic *E. coli* isolates possess a broad range of virulence factors, including toxins, adhesions (particularly type I and P fimbriae), LPS, polysaccharide capsules, proteases, and invasins. These factors are often encoded by genes located in mobile genetic elements (Dobrindt and Hacker, 2008). Rather than serving solely as typical virulence factors directly linked to infection, these factors appear to enhance ExPEC's

fitness, including capabilities like iron-uptake systems, bacteriocins, proteases, and adhesins. This increased adaptability and competitiveness allow ExPEC to better colonize the human body (Pitout, 2012). Specific virulence factors, such as type I fimbriae, the K1 capsule, and various cytotoxic elements, are crucial for NMEC's ability to cause meningitis (Kim, 2012).

Uropathogenic *E. coli* is the primary cause of community-acquired UTIs in individuals of all ages and genders, though they are most common in women due to their shorter urethra (Raeispour and Ranjbar, 2018). It is estimated that approximately 20% of women over the age of 18 will experience an UTI at some point in their lives (Foxman, 2010). Common symptoms include frequent urination, pain during urination, and a persistent urge to urinate even when the bladder is empty. Symptoms indicating a kidney infection include fever and flank pain, accompanied by the typical symptoms of a lower UTIs (Lane and Takhar, 2011).

Neonatal meningitis *E. coli* is associated with significant morbidity and mortality. Despite advancements in intensive care, survivors often experience neurological complications such as hearing loss and delays in development or cognitive impairment (Chandran *et al.*, 2011; Theodoridou *et al.*, 2013). Infections caused by NMEC in newborns typically occur following hematogenous spread (Zhao *et al.*, 2018). A critical factor for the progression of meningitis is the ability of *E. coli* to spread into the central nervous system (Kalliopi, 2013).

2.7. Antimicrobials Therapy

2.7.1. Antimicrobials Used to Treat *E. coli* Infections

Antimicrobial drugs are among the most significant discoveries of the 20th century, having saved millions of lives from infectious diseases (Salam *et al.*, 2023). They are defined as natural compounds produced by

microorganisms, as well as semi-synthetic and synthetic agents (Lima *et al.*, 2020). These drugs specifically target distinct mechanisms in bacteria, leading either to the cessation of cell growth (bacteriostatic) or to bacterial death (bactericidal). There are various antibiotics available, each differing in terms of their mechanism of action (Sebastian *et al.*, 2021).

The majority of these antimicrobials cause alterations at the metabolic and physiological level, including modifications in cell wall synthesis (such as β -lactams), inhibition of protein synthesis (such as aminoglycosides, macrolides, and tetracyclines), blockage of metabolic pathways (such as sulfonamides and trimethoprim), and interference with DNA replication (such as fluoroquinolones) (Pitout, 2015; Kapoor *et al.*, 2017).

The choice of antimicrobials depends on several factors, including the site and severity of the infection, antibiotic susceptibility testing, and patient-specific considerations such as allergies and pregnancy (Obaidat *et al.*, 2018). Generally, *E. coli* is known to be one of the more commonly susceptible bacteria within the Enterobacteriaceae family. Various antimicrobial agents are recommended for treating community and hospital infections caused by *E. coli*, including β -lactams, fluoroquinolones, aminoglycosides, nitrofurantoin, and trimethoprim-sulfamethoxazole (Pitout, 2012).

2.7.2. Evolution of Antimicrobials Resistance

Antimicrobial resistance (AMR) is a natural phenomenon associated with the emergence and spread of resistance genes. WHO defines AMR as the ability of microorganisms to resist the effects of antimicrobial agents after being previously susceptible to them (WHO, 2020). This resistance arises because these microorganisms develop new resistance capabilities (Adhikari, 2020; Nadeem *et al.*, 2020). As a consequence of the overuse and misuse of antimicrobials, particularly the inappropriate use of antimicrobials (Khan *et al.*, 2019; Tang *et al.*, 2023). AMR leads to decreased efficacy of

antimicrobials, making patient treatment more expensive and complicated or even impossible (Tzouveleakis *et al.*, 2014). In some cases, resistance can extend to an entire class of antimicrobial agents, presenting a significant challenge and effectively returning us to the pre-antibiotic era (Nordmann *et al.*, 2011).

Over the last decade, the WHO has identified the evolution and dissemination of AMR as a critical global health threat since 2015 (WHO, 2015; Galindo-Méndez, 2020). As a result of developing resistance in microorganisms, antimicrobials resistance lead to significant rates of mortality, morbidity, and economic costs annually (Boolchandani *et al.*, 2019). Antimicrobial-resistant microorganisms are responsible for more than 2.8 million infections and over 35000 deaths each year in the United States, according to the Centers for Disease Control and Prevention (CDC) (CDC, 2019). If the current rate of AMR continues to rise, it predicts that infections caused by resistant bacteria will become the leading cause of death globally, surpassing cancer, diabetes, and cardiovascular diseases (O'Neill, 2016). It is estimated that more than 3 million people may die each year, resulting in an economic loss exceeding 100 trillion US dollars by 2050 due to MDR strains, particularly carbapenem resistant strains, which are already spreading across the globe (O'Neill, 2016; Vilalta, 2018).

Among the pathogenic bacteria that pose the greatest threat to human health are members of the Enterobacteriaceae family, particularly *E. coli*, which are prevalent in the community (Galindo-Méndez, 2020). Due to its specific ecology, *E. coli* can serve as a sensor for AMR. This organism has developed resistance traits over many years (Rodríguez-Martínez *et al.*, 2011). *E. coli* is listed as a critical priority pathogen by the WHO for its role in developing drug-resistant infections, contributing to millions of deaths, and it is part of a group of 12 bacterial families that present the most significant danger to human health (Tagliabue and Rappuoli, 2018).

According to the CDC, carbapenem-resistant Enterobacterales, including *Klebsiella* species, *E. coli*, and *Enterobacter* species, are among the most critical emerging threats of AMR globally (CDC, 2019). Overall, *E. coli* strains have evolved resistance to major classes of antibiotics such as β -lactams, quinolones, aminoglycosides, and sulfonamides, resulting in increased morbidity and mortality (El Bouamri *et al.*, 2015). Since the first reported cases, *E. coli*'s resistance to antibiotics has expanded substantially in recent years and is now endemic in certain regions of North America, Europe, the Mediterranean, and South Asia (Bonomo *et al.*, 2018).

2.8. Carbapenems

Carbapenem antibiotics are crucial antimicrobials frequently recommended as a last line of antibiotics for critically ill patients or those suspected of harboring antibiotic-resistant bacteria (Aurilio *et al.*, 2022). As a key subclass of β -lactam antibiotics, carbapenems have shown sustained efficacy even in the presence of resistance to other β -lactam antibiotics. This is due to their unique structure making them resistant to hydrolysis by many β -lactamase enzymes (Hansen, 2021).

Carbapenems provide a broad spectrum of activity against both Gram-positive and Gram-negative bacteria, offering more effective treatment options compared to penicillins, cephalosporins, or β -lactam/ β -lactamase inhibitors (Bedenic *et al.*, 2014). The introduction of these agents has significantly reduced morbidity and mortality rates associated with various life-threatening diseases, making antibiotics a cornerstone of therapy for bacterial infections (Rice, 2008). Additionally, carbapenems are the third most widely used class of antibiotics worldwide for community-acquired infections in intensive care units and the first choice for hospital-acquired infections (Versporten *et al.*, 2018).

2.8.1. Chemistry of Carbapenems

Carbapenems are semi-synthetic derivatives of thienamycin, a β -lactamase inhibitor that was isolated from the culture broths of the soil-dwelling organism *Streptomyces cattleya* (Henry, 2019). Although thienamycin was first described in 1976 (Kropp *et al.*, 1976), its instability in aqueous solution hindered its use for therapeutic purposes (Kahan *et al.*, 1983). As a result, it served as a model for the development of more stable derivatives. A carbapenem features a fused 4:5 β -lactam ring and shares a similar molecular structure with other β -lactams (Papp-Wallace *et al.*, 2011). However, in carbapenems, the sulfur atom at position 1 in the structure is replaced by a carbon atom (Lo *et al.*, 2008; Henry, 2019). The presence of additional side chains and their specific arrangement significantly enhances their affinity for penicillin-binding proteins (PBPs) and also provides resistance to many β -lactamases (Figure 2-1) (Queenan and Bush, 2007; Lo *et al.*, 2008). Furthermore, the unsaturation between the carbon atoms C2 and C3 contributes to the increased chemical reactivity of carbapenems compared to penicillins and cephalosporins (Dalhoff and Thomson, 2003).

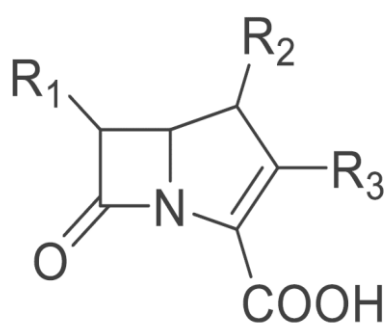


Figure (2-1): Chemical Structure of Carbapenems (Lima *et al.*, 2020).

2.8.2. Mechanism of Action of Carbapenems

Carbapenems, like other β -lactam antibiotics, are inhibitors of cell wall synthesis. Specifically, they target important enzymes, Penicillin Binding Proteins (PBPs), necessary for the synthesis of the peptidoglycan layer of bacterial cell walls (Breilh *et al.*, 2013). PBPs are cytoplasmic

membrane proteins that consist of two types of enzymes: carboxypeptidases and transpeptidases. These enzymes are responsible for forming peptidoglycan layers in the cell wall and maintaining its structural integrity (Lister, 2007). Among these, transpeptidase is considered the primary target enzyme for carbapenems (Harbottle *et al.*, 2016; Sheppard *et al.*, 2016). When a β -lactam molecule binds to PBPs, it inhibits the complete transpeptidation of peptidoglycan, thereby disrupting cell wall formation (Figure 2-2) (Elshamy and Aboshanab, 2020). This action subsequently inactivates intracellular autolytic inhibitor enzymes, leading to cell lysis and bacterial death. Consequently, carbapenems are classified as bactericidal agents (Sumita and Fukasawa, 1995; Bonfiglio *et al.*, 2002).

For carbapenems to exert their effects on PBPs, they must penetrate the bacterial cell wall through porins in the outer membrane (Bedenic *et al.*, 2014). The affinity of carbapenems for specific PBPs varies among different bacteria, allowing for the categorization of carbapenems based on their relationships with these proteins (Lo *et al.*, 2008). The distinction between carbapenems and other β -lactams often comes from their affinity for PBP1a or PBP1b, while the differences among carbapenems are generally determined by their binding to PBP-2 or PBP-3 in Gram-negative bacteria (Lister, 2007). For instance, imipenem shows the strongest binding to PBP-2, followed by PBP-1a and PBP-1b, and lastly PBP-3. In contrast, ertapenem and meropenem have the highest affinity for PBP-2, followed by PBP-3, and also interact with PBP-1a and PBP-1b. Doripenem binds to PBP-1, PBP-2, and PBP-4 in *Staphylococcus aureus*, PBP-3 in *Pseudomonas aeruginosa*, and PBP-2 in *E. coli* (Zhanel *et al.*, 2007). Thus, the varying binding affinities of carbapenems for different PBPs may help explain the differences in their antimicrobial activity (Lo *et al.*, 2008).

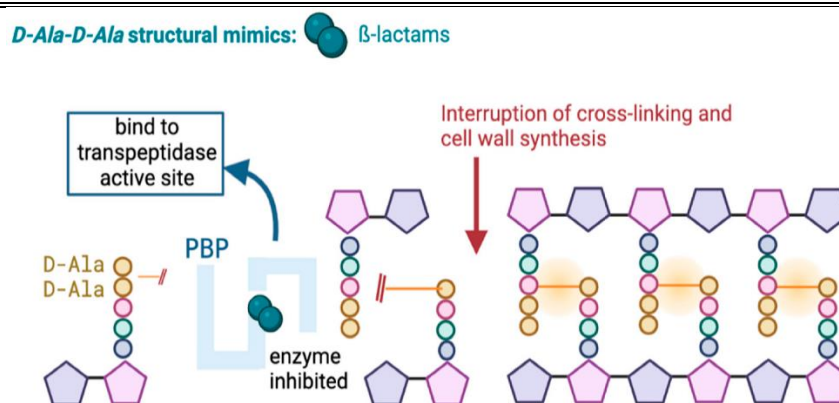


Figure (2-2): Mode of Action of β -Lactams Antibiotics (Mó *et al.*, 2024).

2.8.3. Types of Carbapenems

There are currently four types of carbapenems that are approved and used clinically:

2.8.3.1. Imipenem

The first carbapenem was imipenem, a synthetic derivative of thienamycin approved for medical use in 1982 (Figure 2-3) (Neu, 1982). Imipenem exhibits greater chemical stability than thienamycin (Eliopoulos *et al.*, 1982). However, like thienamycin, imipenem is rapidly metabolized by renal dehydropeptidase-1 (DHP-1), leading to low recovery levels of imipenem in urine. This limitation poses a significant challenge for clinical application (Huo *et al.*, 2019). To address this issue, imipenem is administered in combination with cilastatin in a 1:1 ratio. Cilastatin is a reversible inhibitor of DHP-1, which prevents the inactivation of imipenem and mitigates the nephrotoxicity associated with its hydrolyzed metabolites (Papp-Wallace *et al.*, 2011). Overall, this carbapenem is slightly more potent against Gram-positive bacteria compared to other agents. Imipenem shows strong binding affinity for PBPs, demonstrates good stability against β -lactamases, and is an effective treatment for *P. aeruginosa* and *Acinetobacter* species, which are often associated with hospital-acquired critical infections. Over the past two decades, imipenem has been used by more than 26 million patients (Tahri *et al.*, 2017). It is important to note that the United States

Food and Drug Administration (FDA) does not approve imipenem for the treatment of meningitis or infections related to the central nervous system, as it can induce seizures in individuals with significant risk factors, including renal failure or structural brain disease (Kattan *et al.*, 2008).

2.8.3.2. Meropenem

Meropenem is the first carbapenem that is resistant to hydrolysis by human DHP-1. It was discovered in 1995 and approved for use in 1996 (Blumer *et al.*, 1997). Unlike imipenem, meropenem does not require concurrent administration with cilastatin because the 1-B-methyl group makes it resistant to hydrolysis by DHP-I (Figure 2-3) (Shah and Isaacs, 2003). This antibacterial agent is well-tolerated and, when used alone, is as effective as the combination of imipenem and cilastatin (Dougherty and Pucci, 2011).

As one of the smallest β -lactam antibiotics, meropenem has a very broad spectrum of activity. Its compact size allows it to easily penetrate the cell wall of various Gram-negative bacilli (Hellinger and Brewer, 1999). Over the years, the evidence of meropenem's clinical efficacy, demonstrated through its widespread use, has led physicians to regard this carbapenem as one of the most reliable and accessible options for treating critically ill patients with hospital-acquired infections (Shah and Isaacs, 2003). Meropenem is approved by the FDA for the treatment of bacterial meningitis in adults and children older than three months. Its range of activity is similar to that of imipenem, including effectiveness against *P. aeruginosa* and *Acinetobacter* species, but it tends to be slightly more potent against Gram-negative aerobic bacteria (Kattan *et al.*, 2008).

2.8.3.3. Ertapenem

Ertapenem, discovered in 2001 (Figure 2-3), is known for its potent action against a wide range of bacterial pathogens, particularly those that produce ESBL and/or AmpC type β -lactamases, especially among Gram-

negative enteric bacteria (Shah and Isaacs, 2003; Kattan *et al.*, 2008). Compared to imipenem and meropenem, ertapenem is more resistant to degradation by DPH-I, but it is less effective against *P. aeruginosa*, *Acinetobacter* species, and enterococci. This limited efficacy is the reason it is not recommended for use in clinical situations where nosocomial infections are suspected (Kattan *et al.*, 2008; Shah, 2008).

The presence of a meta-substituted group at position 2 of ertapenem is believed to enhance its pharmacokinetic profile (Chattopadhyay *et al.*, 1983). With a longer elimination half-life and a once-daily dosing regimen, ertapenem is a crucial treatment option for community-acquired bacterial infections, particularly those involving a diverse flora of anaerobic and aerobic bacteria. Examples include community-acquired pneumonia and complicated intra-abdominal, skin, or UTIs (Kattan *et al.*, 2008; Shah, 2008).

2.8.3.4. Doripenem

Doripenem is a structural analogue of meropenem, with a longer half-life. It was approved by the FDA in 2007 (Figure 2-3) (Hilas *et al.*, 2008). Doripenem is notable for its ability to stabilize renal enzyme inactivation and to resist β -lactamases, including extended-spectrum β -lactamases (ESBLs). When compared to other carbapenems, doripenem has enhanced activity against both Gram-positive and Gram-negative bacteria. This antibiotic demonstrates bactericidal activity against a variety of bacterial species. It is used to treat nosocomial pneumonia, complicated intra-abdominal infections, and complicated UTIs (Alvarez-Lerma *et al.*, 2009). However, due to its high cost, doripenem is not used as frequently as imipenem and meropenem (Otter *et al.*, 2015).

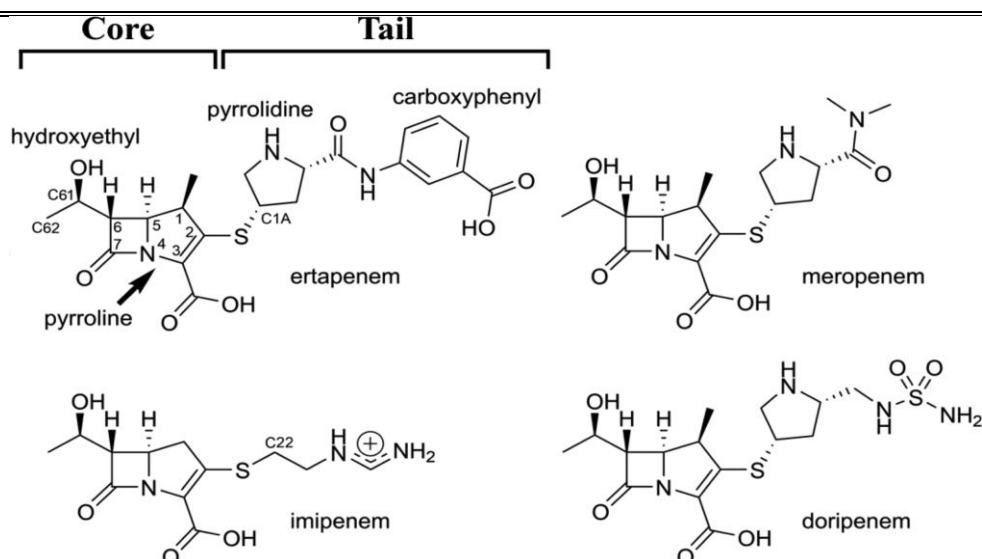


Figure 2-3: Chemical Structures of Ertapenem, Meropenem, Imipenem, and Doripenem (Stewart *et al.*, 2015).

2.8.4. Mechanisms of Carbapenems Resistance

Carbapenems resistance have unfortunately become a significant public health concern worldwide (Jomehzadeh *et al.*, 2022). Resistance to carbapenems primarily occurs through the production of carbapenemases, which is the main mechanism driving this resistance (Bush and Rasmussen, 1997; Livermore and Neil, 2000). Additionally, there are mechanisms of resistance that do not involve carbapenemase production (Codjoe and Donkor, 2017; Elshamy and Aboshanab, 2020).

2.8.4.1. Carbapenemases Production

One key mechanism of carbapenem resistance is the hydrolysis of carbapenems by carbapenemase enzymes (Figure 2-4) (Nordmann *et al.*, 2012). These enzymes belong to a diverse family of β -lactamases that can hydrolyze a variety of antibiotics, including penicillins, cephalosporins, monobactams, and carbapenems, rendering these drugs ineffective (Queenan and Bush, 2007). Carbapenemases work by binding to the drug and breaking the amide bond of a four-membered β -lactam ring, which prevents the antibiotic from binding to the PBPs in the bacterial cell wall (Bush and Bradford, 2020). The presence of carbapenemase gene combinations, alongside other antimicrobial resistance genes housed on mobile genetic

elements, poses a significant challenge, especially when found in Enterobacteriaceae. This situation leads to an increased acquisition of resistance to new classes of antibiotics (Zimmerman *et al.*, 2013). The uncontrolled transfer of mobile genetic elements within and between species has contributed to the evolution of bacteria that are resistant to nearly all antimicrobials (Gazin *et al.*, 2017).

There are several classifications for carbapenemases, but the two most commonly used are functional classification, according to Bush-Jacoby-Meideros (Table 2-1) (Bush *et al.*, 1995), and structural classification, according to Ambler (Ambler, 1980). The functional classification categorizes known β -lactamases into four major functional groups (groups 1-4), with multiple subgroups under group 2 differentiated by specific substrate or inhibitor profiles. In this classification, carbapenemases are mainly found in groups 2f and 3, while OXA is categorized under group 2d (Bush *et al.*, 1995).

Table 2-1: Substrate and Inhibition Profiles of Carbapenemases (Queenan and Bush, 2007).

Molecular Class	Functional Group	Hydrolysis Profile ^a					Inhibition Profile ^b	
		Penicillins	Early Cephalosporins	Extended Spectrum Cephalosporins	Aztreonam	Carbapenems	Clavulanic Acid	EDTA
A	2f	+	+	+	+	+	+	-
B	3	+	+	+	-	+	-	+
D	2d	+	+	±	-	±	±	±

a Symbols: +, strong hydrolysis; ±, weak hydrolysis; -, no measurable hydrolysis. **b** Symbols: +, reported inhibition; ±, variable inhibition among β -lactamase family members; -, no inhibition reported.

The Ambler classification, based on amino acid homology, has resulted in four major classes of β -lactamases (Ambler, 1980). While these classes correlate well with functional schemes, they lack detailed information about the enzymatic activity of the enzymes. The majority of

carbapenemases fall into three of the four known classes: A, B and D (Halat, and Moubareck, 2020).

2.8.4.1.1. Class A Carbapenemases

Ambler class A carbapenemases utilize a serine residue at their active site and can hydrolyze all β -lactams, including aztreonam. They are all inhibited by clavulanate and tazobactam, categorizing them within the group 2f functional subgroup of β -lactamases (Simon *et al.*, 2019; Albichr *et al.*, 2020). These β -lactamases have appeared sporadically in clinical isolates since their initial discovery over 20 years ago and have been detected either as single isolates or in small outbreaks (Yang *et al.*, 1990; Nordmann *et al.*, 1993). Class A carbapenemases include *Klebsiella pneumoniae* Carbapenemases (KPC), Non-Metalloenzyme Carbapenemase (NMC), *Serratia fonticola* Carbapenemase (SFC), *Serratia marcescens* Enzyme (SME), Imipenem-Hydrolyzing β -Lactamase (IMI), and Guiana Extended-Spectrum β -Lactamase (GES) (Philippon *et al.*, 2016; Sawa *et al.*, 2020).

SME was first detected in England from two *S. marcescens* isolates collected in 1982 (Yang *et al.*, 1990). The SME β -lactamase has been identified sporadically throughout the United States (Queenan *et al.*, 2000; Gales *et al.*, 2001). Infections caused by SME-producing *S. marcescens* have occurred as isolated incidents or in small clusters, with a maximum of 19 isolates. Notably, SME-producing *S. marcescens* isolates from different geographical locations are not identical, as defined by Pulsed-Field Gel Electrophoresis. However, some degree of clonal spread may exist when several isolates are collected from one location (Troillet *et al.*, 1999; Queenan *et al.*, 2006).

The IMI and NMC enzymes have been identified in rare clinical isolates of *E. cloacae* in the United States, France, and Argentina (Pottumarthy *et al.*, 2003; Radice *et al.*, 2004). NMC and IMI share 97% amino acid identity and are related to SME, with approximately 70% amino

acid identity. The genes for SME, NMC, SFC, and IMI β -lactamases are chromosomally located, with no evidence of association with mobile elements, which may contribute to their rarity (Rasmussen *et al.*, 1996).

In contrast, the genes for KPC and GES enzymes are plasmid-encoded (Queenan and Bush, 2007). KPCs are the most clinically significant and differ from other functional group 2f enzymes as they are found on transferable plasmids, and their substrate hydrolysis spectrum includes third-generation cephalosporins, such as cefotaxime (Miriagou *et al.*, 2003). Additionally, the KPC family has the highest potential for spread, being most frequently found in *K. pneumoniae* (initially discovered in the United States in 1996). Most concerning, treatment of infections caused by these organisms is extremely challenging due to their MDR, resulting in high mortality rates (Bratu *et al.*, 2005). Numerous reports have documented KPC in other Enterobacterales, including *E. coli*, *K. oxytoca*, *Salmonella enterica*, *Citrobacter freundii*, *Enterobacter aerogenes*, *E. cloacae*, *Proteus mirabilis*, and *S. marcescens* (Boutal *et al.*, 2018; Fernández *et al.*, 2018).

Another less frequently encountered carbapenemase genes related to this class is the GES family, first described in 2000 from an *E. cloacae* isolate in Greece (Giakkoupi *et al.*, 2000) and in a *K. pneumoniae* isolate from French Guiana (Poirel *et al.*, 2000). The genes encoding GES were found in integrons on transferable plasmids. At least nine GES variants have been described, with GES-9 recently identified in a *P. aeruginosa* isolate from France (Poirel *et al.*, 2005). Although rare, GES enzymes have been identified globally (Castanheira *et al.*, 2004; Jeong *et al.*, 2005).

2.8.4.1.2. Class B Carbapenemases

One of the major carbapenemase families that holds significant clinical importance globally is the metallo- β -lactamases (MBLs), which belong to Class B (Walsh *et al.*, 2005). MBLs function through a mechanism of hydrolysis that involves the interaction of the β -lactam ring with a divalent

cation (Zn^{+2}) at the active site. As a result, MBLs can be inhibited by EDTA, a chelator of Zn^{+2} and other divalent cations (Nucleo *et al.*, 2013). These enzymes are characterized by their ability to hydrolyze most β -lactam antibiotics, except aztreonam. Additionally, they show resistance to β -lactamase inhibitors (Neyestanaki *et al.*, 2014; Wu *et al.*, 2019). Functional analyses have classified these β -lactamases as distinct (belonging to functional group 3) from those that employ a serine-based hydrolytic mechanism (Bush, 1988).

The first MBLs identified were chromosomal enzymes present in environmental and opportunistic pathogenic bacteria, such as *Bacillus cereus* (Lim *et al.*, 1988), *Aeromonas* spp. (Iaconis and Sanders, 1990), and *Stenotrophomonas maltophilia* (Saino *et al.*, 1982). These bacteria rarely cause serious nosocomial infections, as they are typically opportunistic pathogens, and their chromosomal MBL genes are not easily transferable (Queenan and Bush, 2007). In contrast, there has been a notable increase in the detection and spread of acquired or transferable families of these metalloenzymes. The genes for these enzymes are often located within various integron structures, where they have been incorporated as gene cassettes (Van Duin and Doi, 2017).

The most common MBL enzymes include New Delhi Metallo- β -Lactamase (NDM), German-Imipenemase (GIM), Seoul-Imipenemase (SIM), Verona Integron-Encoded Metallo- β -Lactamase (VIM), São Paulo Metallo- β -Lactamase (SPM), and Imipenemase (IMP) (Simon *et al.*, 2019; Albichr *et al.*, 2020).

The IMP enzyme was the first to be detected in this class, isolated from an imipenem-resistant *P. aeruginosa* isolate in Japan in 1991 (Watanabe *et al.*, 1991). Since then, the IMP family has been identified worldwide in *A. baumannii* and Enterobacteriaceae (Cornaglia *et al.*, 2011; Shanthi and Balagurunathan, 2014). IMP is commonly transferred between bacteria,

particularly Gram-negative species, via class 1 or class 3 integrons (Pongchaikul and Mongkolsuk, 2022). Up to 33 varieties of the IMP type have been detected globally (Braun *et al.*, 2018).

Similarly, VIM was first recognized in Verona, Italy, in a *P. aeruginosa* clinical isolate recovered in 1997 (Gupta, 2008). This enzyme has also been discovered in *E. coli* and *K. pneumoniae* (Giakkoupi *et al.*, 2003) and has been reported in many countries worldwide (Cornaglia *et al.*, 2011). VIM and IMP share some similarities, conferring resistance to all β -lactam antibiotics except monobactams and being susceptible to all β -lactam inhibitors (Marsik and Nambiar, 2011). These genes are typically found within class 1 integrons (Scoulica *et al.*, 2004).

As for GIM, it was first discovered in a *P. aeruginosa* isolate from patients in Germany in 2002 (Rieber *et al.*, 2012). Since then, it has not been detected elsewhere (Wendel *et al.*, 2013). GIM shows approximately 30% homology to VIM, 43% homology to IMPs, and 29% homology to SPM. Like most MBL genes, GIM has been found within class 1 integrons on plasmids (Walsh, 2005; Queenan and Bush, 2007).

SPM is an MBL first identified in *P. aeruginosa* in São Paulo, Brazil, in 2002 (Wozniak *et al.*, 2021). It shares a similar genetic structure with IMP, exhibiting 35.5% amino acid identity, and phenotypically does not hydrolyze clavulanic acid or aztreonam (Poirel *et al.*, 2004). However, SPM has only been detected in single clones that have caused multiple hospital outbreaks with high mortality rates in Brazil. The reason for SPM's restricted geographic distribution, in contrast to other MBLs that spread worldwide, remains unknown (Marra *et al.*, 2006; do Nascimento *et al.*, 2020). Genetic analysis of the regions surrounding the SPM gene revealed that it is not part of an integron but instead is associated with common regions containing a novel type of transposable structure with potential recombinase and promoter sequences (Toleman *et al.*, 2002; Poirel *et al.*, 2004).

The SIM enzyme was first isolated from *A. baumannii* and *P. aeruginosa* through a large-scale screening of imipenem non-susceptible strains in Seoul, Korea (Lee *et al.*, 2004). SIM shows the closest amino acid identity to the IMP family, ranging from 64% to 69%. All bacterial isolates harboring the SIM characteristics exhibit a MDR phenotype with low minimum inhibitory concentration values for meropenem and imipenem (Perez *et al.*, 2007; Gupta, 2008). The gene encode SIM is likely to transfer horizontally among bacteria since it is located on a gene cassette inserted into integron class 1, indicating the potential for further spread of resistance (Gupta, 2008). Since their initial discoveries, SPM, GIM, and SIM have not spread beyond their countries of origin. However, VIM and IMP continue to be detected worldwide, with an overall trend showing these MBLs moving from *P. aeruginosa* into the Enterobacteriaceae family (Queenan and Bush, 2007).

More recently, NDM enzymes were discovered for the first time in a *K. pneumoniae* isolate from a Swedish patient who had been hospitalized in New Delhi, India, in 2009 (Yong *et al.*, 2009). NDM-producing bacteria have garnered worldwide attention because this gene can be disseminated from India to other countries through travel (Walsh *et al.*, 2011). Unlike several other genes, NDM is easily spread within Enterobacteriaceae and among unrelated species, as the organisms producing this gene can colonize the human gastrointestinal tract for an extended period and also spread through contaminated water and ecological surfaces (Coetzee and Brink, 2011; Khan *et al.*, 2019). Currently, NDM is one of the most prevalent carbapenemases in all Enterobacteriaceae, as well as in *P. aeruginosa* (Cornaglia *et al.*, 2011; Cantón *et al.*, 2012). At least 21 variants of NDM have been described and identified (Rahman *et al.*, 2018).

2.8.4.1.3. Class D Carbapenemases

Class D carbapenemases, identified as a functional group (2d), are a subset of oxacillinase-type enzymes. These enzymes hydrolyze the oxacillin at a much faster rate than classical penicillins like benzylpenicillin. They represent one of the most prevalent families of β -lactamases discovered in the late 1970s and early 1980s (Matthew, 1979; Simpson *et al.*, 1980). Class D carbapenemases require serine for their catalytic activity, and their genes can be found on both plasmids and chromosomes (Nowak and Paluchowska, 2016).

These enzymes are classified into narrow- and broad-spectrum types based on their hydrolysis profiles against β -lactam antibiotics (Queenan and Bush, 2007). The OXA-2 and OXA-10 β -lactamases are examples of narrow-spectrum enzymes that confer resistance to penicillins and some early cephalosporins (Poirel *et al.*, 2010). Conversely, broad-spectrum OXA enzymes have expanded substrate profiles that include carbapenem antibiotics. These enzymes exhibit high activity against penicillins, intermediate activity against broad-spectrum cephalosporins, and weak hydrolytic activity against carbapenems (Queenan and Bush, 2007). Despite this, OXA-producing bacteria typically show resistance to carbapenems due to additional resistance mechanisms such as permeability defects or the production of other β -lactamases (Tzouvelekis *et al.*, 2012; Poirel *et al.*, 2012).

Class D carbapenemases were classified separately from other serine β -lactamases (Huovinen *et al.*, 1988). They had mainly been identified in *A. baumannii*, Enterobacteriaceae and *P. aeruginosa* (Naas and Nordmann, 1999). They were functionally described as penicillinases capable of hydrolyzing oxacillin (Bush, 1988), and were generally variably inhibited by clavulanic acid while being not fully inhibited by EDTA (Table 2-1) (Bush *et al.*, 1995; Livermore *et al.*, 2013). The OXA class D carbapenemases

represent a heterogeneous group comprising over 100 genetically distinct enzymes, subdivided into various subgroups based on amino acid sequences (Queenan and Bush, 2007).

The first OXA β -lactamase with carbapenemase activity was purified from a MDR *A. baumannii* strain isolated from a patient in Scotland (Paton *et al.*, 1993). Sequencing revealed that this enzyme belonged to the OXA class D family and was subsequently renamed OXA-23 (Donald *et al.*, 2000). Six groups have been identified that hold significant clinical importance due to their widespread, including OXA-23-like, OXA-24/40-like, OXA-48-like, OXA-51-like, and OXA-58-like. The majority of OXA carbapenemases, except for OXA-48-like, have been primarily identified in *A. baumannii* (Poirel *et al.*, 2010; Evans *et al.*, 2013).

OXA-48-like enzymes, especially OXA-48, are particularly notable among class D carbapenemases (Poirel *et al.*, 2012). OXA-48 displays tenfold greater activity than other OXA enzymes and has been identified in *K. pneumoniae*, from which it has spread to other Enterobacteriaceae. Variants related to OXA-48, such as those denoted as OXA-181, OXA-162, and OXA-232, showcase distinct geographic distributions and associated resistance genes (Giani *et al.*, 2012). All these variants are classified within the OXA-48-like category, differing only by one to five amino acid substitutions or deletions (Poirel *et al.*, 2012).

OXA-51-like enzymes are the most abundant and are present in all *A. baumannii* strains, potentially as a natural component of their chromosome (Heritier *et al.*, 2005). Recently, the gene encoding this enzyme has also been identified on plasmids, which facilitates its spread among various *Acinetobacter* species (Lee *et al.*, 2009). The major concern regarding these carbapenemases is their ability to confer resistance to carbapenems. Their capacity for rapid mutation and the expansion of their activity range significantly limit therapeutic options (Stewart *et al.*, 2018).

2.8.4.2. Other Mechanisms of Carbapenems Resistance

In addition to producing carbapenemase enzymes, *E. coli* and other members of the Enterobacteriaceae family can exhibit resistance through non-carbapenemase mediated mechanisms (Figure 2-4). While carbapenemases specifically target carbapenems, these alternative mechanisms are unspecific and often associated with MDR (Tzouveleakis *et al.*, 2012). Examples include the production of other β -lactamases, loss of porins, and overexpression of efflux pumps. All of these mechanisms aim to prevent the antibiotic from penetrating the bacterial cell (Codjoe and Donkor, 2017).

Firstly, Enterobacteriaceae can produce various types of β -lactamases, such as AmpC. These enzymes are not classified as carbapenemases because their ability to hydrolyze carbapenems is very weak or absent (Queenan *et al.*, 2010). However, they can contribute to carbapenems resistance when there are permeability defects, as they can bind to the carbapenem molecule and prevent it from reaching its target (Goessens *et al.*, 2013; Meletis, 2016). Specifically, the plasmid-encoded AmpC (CMY-2) is commonly found in *E. coli* and other Enterobacteriaceae worldwide, can leading to resistance to carbapenems (Philippon *et al.*, 2002).

Secondly, the overexpression of genes that encode efflux pumps is another significant mechanism associated with carbapenems resistance in Enterobacteriaceae (Moyá *et al.*, 2012). Efflux systems actively pump out drugs after they enter the cell. The action of efflux pumps contributes to MDR because they tend to be non-specific, capable of extruding many different antibiotics (Manchanda *et al.*, 2010; Wong *et al.*, 2019). Five families of efflux pumps are responsible for increased antibiotic resistance, with three primarily found in pathogenic bacteria: the Multidrug and Toxic Compound Extrusion (MATE) family, the Major Facilitator Super (MFS) family, and the Resistance Nodulation-cell Division (RND) family (Nowak

and Paluchowska, 2016). Among these, the RND family pumps, such as AcrAB or AcrAB-like pumps, are recognized as significant mechanisms of antibiotic resistance (Weston *et al.*, 2018). Both the AcrAB efflux pump and the CusABC efflux complex are present in *E. coli*. The genes responsible for these mechanisms of resistance can easily transfer between bacteria via plasmids (Courvalin, 1994).

Lastly, reduced expression or mutations in porins also contribute to the limited penetration of carbapenems into the bacterial cell (Bialek-Davenet *et al.*, 2017). Porin channels are nonspecific pathways in the outer membrane of Gram-negative bacteria, essential for antibiotic uptake (D'Souza *et al.*, 2019). A decrease in the expression of outer membrane porins leads to reduced susceptibility to imipenem and meropenem (Nowak and Paluchowska, 2016). Furthermore, previous studies indicate that the outer membrane porins of *E. coli* are involved in the multi-drug resistance phenotype (Mach *et al.*, 2008). Choi and lee (2019) constructed mutants of porins (specifically OmpC and OmpF mutations) in *E. coli* to show that porins play a distinct role in antibiotic resistance and membrane integrity.

Rarely, *E. coli* can develop carbapenems resistance through mutations or other modifications that decrease the production levels or binding affinity of PBPs (Yamachika *et al.*, 2013).

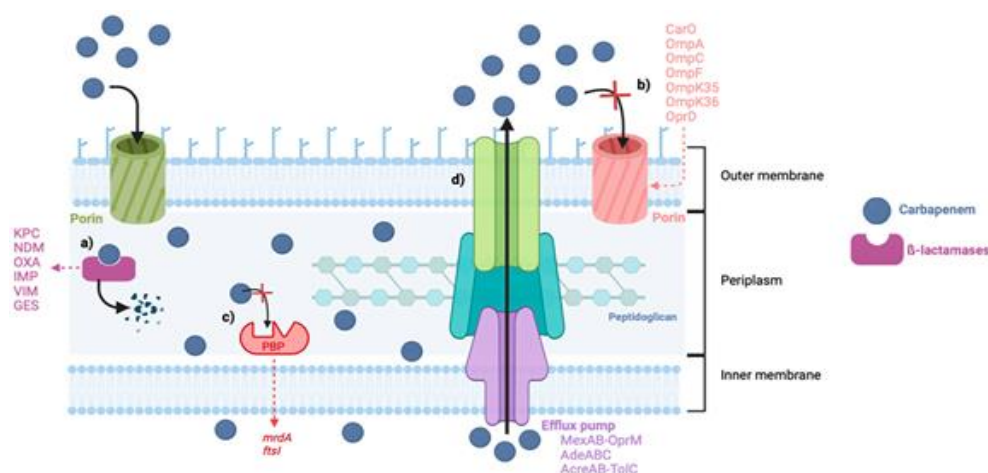


Figure (2-4): Carbapenems Resistance Mechanisms in Gram-Negative Bacteria. (a) Enzymatic hydrolysis of the carbapenem. (b) Porin downregulation decrease permeability. (c) Production of low-affinity PBPs. (d) Upregulation of efflux pumps (Mó *et al.*, 2024).

2.9. Integrons

The emergence of antibiotic resistance in pathogenic bacteria is primarily attributed to horizontal genetic transfer. This process is facilitated by certain mobile genetic elements that promote the movement of genes both within and between cells. These include insertion sequences, transposons, plasmids, and integrons (Wang *et al.*, 2008). While insertion sequences and transposons are mobile and can insert into random locations along with their carried resistance genes (Kaushik *et al.*, 2018). Integrons are not self-movable; it can become mobile when associated with other genetic elements like plasmids or transposons (Xia *et al.*, 2013). Integrons are considered particularly significant because their insertion relies on site-specific recombination, which enables the mobilization of resistance genes (Stalder *et al.*, 2012). Integrons are predominantly found in Gram-negative bacteria. However, recent research has also detected integrons in Gram-positive species (Xu *et al.*, 2010). Approximately 10% of bacterial genomes that have been partially or completely sequenced contain this genetic element (Boucher *et al.*, 2007).

All integrons share three essential core features (Figure 2-5), whose combined activities capture, integrate, and subsequently express exogenous genes as part of gene cassettes (Boucher *et al.*, 2007; Cambray *et al.*, 2010). The first feature is the *IntI* gene, which encodes the integron integrase (ntI) (Messier and Roy, 2001). This IntI catalyzes recombination between incoming gene cassettes and the second core feature, an integron-associated recombination site known as attI (Partridge *et al.*, 2000). Once a gene cassette is integrated, it is expressed through the third core feature, an integron-associated promoter called Pc (Collis and Hall, 1995; Cambray *et al.*, 2010).

Integrations acquire new genes in the form of gene cassettes (Hall *et al.*, 1991). These cassettes can exist in either a circular form when not integrated into a cassette array or as a linear form when inserted into an integron (Partridge *et al.*, 2009; Boucher *et al.*, 2007). Gene cassettes are mobile, non-replicating elements that typically consist of an Open Reading Frame and a recombination site known as attC (Hall *et al.*, 1991; Stokes *et al.*, 1997). Despite containing coding sequences, gene cassettes generally lack promoters (Xu *et al.*, 2009). Gene cassettes are captured through recombination between the attI site of the integron and the attC site from the cassette, a process mediated by the IntI enzyme (Moquet *et al.*, 2011; Lima *et al.*, 2014). As a result, the genes carried on these elements can be expressed, relying on external promoters for expression (Cury *et al.*, 2016). This process is reversible; cassettes can be excised as free circular DNA elements (Hocquet *et al.*, 2012). Therefore, they facilitate the dissemination of antimicrobial resistance by transferring plasmids, transposons, or integrons (Yang *et al.*, 2017; Huang *et al.*, 2020).

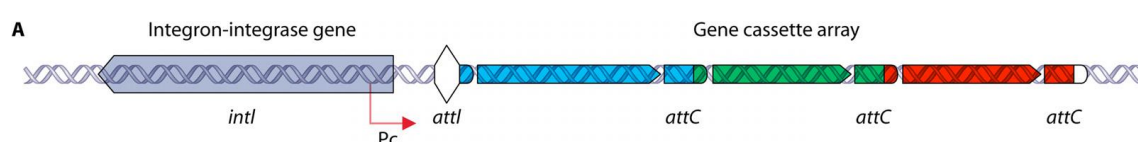


Figure 2-5: The Basic Structure of Integrons (Gillings, 2014).

Different classes of integrons can be distinguished based on the amino acid sequence of IntI (Miao *et al.*, 2019), with IntI1 defining ‘class 1’, IntI2 as ‘class 2’, IntI3 as ‘class 3’, and so on. Class 1, class 2, and class 3 were initially identified in association with mobile genetic elements, while class 4 is associated with chromosomal integrons (Malek *et al.*, 2015). To date, more than 194 gene cassettes associated with class 1-3 integrons have been identified, often conferring resistance to most classes of antibiotics, including all known β -lactams, aminoglycosides, chloramphenicol,

trimethoprim, rifampin, erythromycin, quinolones, fosfomycin, and lincomycin (Moura *et al.*, 2009; Partridge *et al.*, 2009). They may also carry other adaptive genes associated with environmental stresses or Open Reading Frames that code hypothetical proteins of unknown function (Elsaied *et al.*, 2007).

Class 1 and Class 2 integrons are frequently detected and well-characterized, primarily among bacteria belonging to the Enterobacteriaceae family, including *E. coli* (Han *et al.*, 2012). However, other classes of integrons that confer antibiotic resistance have also been described in clinical contexts (Gillings, 2014). Class 1 integrons are widely disseminated among Gram-negative bacteria in humans and animals (Gillings, 2017). Although not exclusively located on plasmids and transposons, their presence in these mobile genetic elements accounts for their widespread distribution and significant association with MDR strains in Enterobacteriaceae (Leverstein *et al.*, 2003; Fluit and Schmitz, 2004). There are three types of recombination sites recognized in these integrons: attI1, attC, and secondary sites by IntI1 (Xu *et al.*, 2011). Similarly, class 2 integrons are commonly associated with the Tn7 transposon, which carries both its recombination site attI2 and the promoter Pc (Cambray *et al.*, 2010). However, the amino acid sequence homology of the *IntI2* gene is found to be less than 50% compared to *IntI1*, and it is non-functional due to an internal stop codon (TAA). This leads to the production of a shorter, inactive polypeptide that is unable to catalyze the recombination reaction (Barlow and Gobius, 2006).

Chapter Three

MATERIALS AND

METHODS

3. Materials and Methods

3.1. Materials

3.1.1. Laboratory Equipment

Table (3-1): Equipment

No.	Equipment	Company	Origin
1.	Autoclave	Marubeni	Japan
2.	Bench Centrifuge	Hettich	Germany
3.	Deep Freezer	GFL	Germany
4.	Electric Oven	Memmert	Germany
5.	Electrophoresis Unit	FSF-SPBT	UK
6.	Gel Documentation System	E-Graph	Japan
7.	Hot Plate	Labtech	Korea
8.	Incubator	Memmert	Germany
9.	Laminar Flow Cabinet	Marubeni	Japan
10.	Magnetic Stirrer	Scilogex	Korea
11.	Microscope	Olympus	Japan
12.	Mini Spin Centrifuge	Eppendorf	Germany
13.	Nanodrop	Avans	China
14.	PCR Cabinet	Aura PCR	Italy
15.	PCR Vortex	Exispin	European
16.	pH Meter	Radiometer	Denemark
17.	Refrigerator	Gree	China
18.	Sensitive Balance	Kern	Germany
19.	Thermocycler Apparatus	Bioneer	Korea
20.	VITEK-2 System	BioMerieux	France
21.	Vortex	Vortex mixer	Taiwan
22.	Water Bath	Memmert	Germany
23.	Water Distiller	GFL	Germany

3.1.2. Laboratory Instruments

Table (3-2): Instruments

No.	Instruments	Company	Origin
1.	Dry Cotton Swabs	Difco	UK
2.	Ependroff Tubes	Sterellin Ltd	UK
3.	Forceps	China	China
4.	Glass Flasks (100-1000) ml	Jlassco	India
5.	Micropipette Set (1-1000) ml	Eppendorf	Germany
6.	Millipore Filters (0.22) mm	Difco	UK
7.	PCR Tubes	Eppendorf	Germany
8.	Petri Dishes	Himedia	India
9.	Slides with Covers	Sliprand	China
10.	Standard Loop (0.01) ml	Himedia	India
11.	Swabs with Media	Difco	UK
12.	Test Tubes	Pyrex	UK
13.	Tips (10, 100, 1000) ml	Difco	UK

14.	Wooden Sticks	Superem	China
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3.1.3. Chemicals

Table (3-3): The Chemicals that Have Been Used in the Study.

No.	Chemicals	Company	Origin
1.	Agar-agar	Himedia	India
2.	Agarose	Promega	USA
3.	Bromophenol Blue	Promega	USA
4.	EDTA	AppliChem	Germany
5.	Ethanol (96%)	BDH	UK
6.	Ethidium Bromide	Sigma	USA
7.	Glycerol (C ₃ H ₈ O ₃)	Fluke	Germany
8.	Gram Stain Kit	Himedia	India
9.	Hydrochloric Acid (HCl)	BDH	UK
10.	Hydrogen Peroxide (H ₂ O ₂)	Teba	Iraq
11.	Isoamyl Alcohol	BDH	UK
12.	Kovac's Reagent	Himedia	India
13.	Mcfarland (0.5) Standard	BioMerieux	France
14.	Methyl Red	BDH	UK
15.	Normal Saline	Alhikma	Iraq
16.	Nuclease Free Water	Ambion	USA
17.	Peptone	Himedia	India
18.	Potassium Hydroxide (KOH)	BDH	UK
19.	Sodium Chloride (NaCl)	BDH	UK
20.	Soduim Hydroxide (NaOH)	Fisher	Germany
21.	Sucrose	Difco	UK
22.	Sulphuric Acid	BDH	UK
23.	Tetramethyl-P-Phenylene Diamine Dihydrochloride	BDH	UK
24.	Tris-Borate-EDTA Buffer	Promega	USA
25.	Tris-EDTA Buffer	Promega	USA
26.	Trypton	Difco	USA
27.	Urea Solution	Mast Diagnostic	UK
28.	Yeast Extract	Bioworld	USA
29.	α-Naphthol (C ₁₀ H ₈ O)	BDH	UK

3.1.4. Culture Media

Table (3-4): The Culture Media that Have Been Used in the Study.

No.	Medium	Company	Origin
1.	Blood Agar Base	Himedia	India
2.	Brain Heart Infusion Broth	Himedia	India
3.	Eosin Methylene Blue	Neogen	USA
4.	MacConkey Agar	Oxoid	UK
5.	MR-VP Broth	Oxoid	UK
6.	Muller-Hinton Agar	Oxoid	UK
7.	Nutrient Broth and Agar	Himedia	India
8.	Pepton Water	BDH	UK
9.	Simmon-Citrate Agar	Himedia	India

10.	Triple Sugar Iron Agar	Biolife	Italy
11.	Tryptic Soy Broth	Biolife	Italy
12.	Urea Base Agar	Difco	USA

3.1.5. Standard *E. coli* Strain

Table (3-5): The Standard *E. coli* Strain.

Strain	Identifier	Key Characteristics	Source
<i>E. coli</i>	American Type Culture Collection, ATCC 25922	Susceptible to ampicillin, cephalosporins and gentamicin	University of Kufa, Faculty of Medicine, Department of Microbiology

3.1.6. Antibiotic Discs

Twenty-three types of antibiotic discs belonging to eleven classes applied in this study are listed in the Table (3-6).

Table (3-6): The Antibiotic Discs that Have Been Used in the Study (Bioanalyse, Turkey).

Antibiotics Class	Antibiotic Subclass	Antibiotic Name	Symbol	Concn. (µg)
Penicillins	Aminopenicillins	Ampicillin	AM	25
β-Lactam/β-Lactamase Inhibitors	Amoxicillin-Clavulanic Acid		AMC	20/10
	Ampicillin/Sulbactam		AMS	10/10
Cephams	Cephalosporin III	Ceftazidime	CAZ	30
		Cefotaxime	CTX	30
		Ceftriaxone	CRO	30
	Cephalosporin IV	Cefepime	FEP	10
	Cephamicin	Cefoxitin	FOX	30
Monobactams	Aztreonam		ATM	30
Carbapenems	Imipenem		IMI	10
	Meropenem		MRP	10
	Doripenem		DOR	10
Aminoglycosides	Amikacin		AK	10
	Gentamicin		CN	10
	Tobramycin		TOB	10
Quinolones	Fluroquinolones	Ciprofloxacin	CIP	5
		Levofloxacin	LEV	5
	Quinolones	Nalidixic Acid	NA	30
Tetracyclines	Doxycycline		DO	10
	Tetracycline		TE	30
Folate Pathway Inhibitors	Trimethoprim		TMP	10
Phenicols	Chloramphenicol		C	10
Nitrofurans	Nitrofurantoin		F	100

3.1.7. Kits

Table 3-7: The Kits that Have Been Used in the Study.

Kit	Components	Company (Country)
DNA Ladder	Ladder consists of 13 dsDNA fragment (100, 200, 300, 400, 500, 600, 700, 800, 900, 1000).	Bioneer (Korea)
Go Taq Green Master mix	2X Green Taq Reaction buffer pH 8.5, 400 μ M dATP, 400 μ M dGTP, 400 μ M dCTP, 400 μ M dTTP, and 3mM MgCl ₂ .	Promega (USA)
KPC/IMP/NDM/VIM/OXA, Combo Test Kit (LFIA)	Test cassette, Dilution buffer	Jiangsu Medomics Medical Technology (China)
Presto™ Mini gDNA Bacteria Kit	GT Buffer 30 ml, Proteinase K, GB Buffer 40 ml, Wash (W1) Buffer 30 ml, Wash (W2) Buffer 45 ml, Elution Buffer 30 ml, 2ml collection tubes, GD Colum	Geneaid (USA)
VITEK-2 System Cards	VITEK glass test tubes, Densichek meter, VITEK GV- ID plus card.	BioMérieux (France)

3.1.8. Primers

Table (3-8): The genes and primer's sequences that used (Macrogen, Korea)

Class	Gene Name	Primer Name	Sequence (5' → 3')	Product Size (bp)	Reference
A	<i>bla</i> _{KPC}	KPC-F	ATGTCACTGTATCGCCGTCT	540	Shanmugakani <i>et al.</i> , 2019
		KPC-R	TTTTCAGAGCCTTACTGCCC		
	<i>bla</i> _{NMC}	NMC-F	GCATTGATATACCTTTAGCAGAGA	371	Radice <i>et al.</i> , 2004
		NMC-R	CGGTGATAAAATCACACTGAGCATA		
	<i>bla</i> _{IMI}	IMI-F	CCATTACCCATCACAAAC	440	Woodford <i>et al.</i> , 2007
		IMI-R	CTACCGCATAATCATTTGC		
<i>bla</i> _{GES}	GES-F	ATGCGCTTCATTCACGCAC	846	Wang <i>et al.</i> , 2006	
	GES-R	CTATTTGTCCGTGCTCAGG			
B	<i>bla</i> _{NDM-1}	NDM-1-F	GGTTTGGCGATCTGGTTTTTC	621	Farzana <i>et al.</i> , 2013
		NDM-1-R	CGGAATGGCTCATCACGATC		
	<i>bla</i> _{IMP}	IMP-F	CATGGTTTGGTGGTTCTTGT	448	Farzana <i>et al.</i> , 2013
		IMP-R	ATAATTTGGCGGACTTTGGC		
	<i>bla</i> _{VIM}	VIM-F	GATGGTGTTTGGTCGCATA	390	Farzana <i>et al.</i> , 2013
		VIM-R	CGAATGCGCAGCACCAG		
	<i>bla</i> _{GIM}	GIM-F	TCGACACACCTTGGTCTGAA	477	Poirel <i>et al.</i> , 2011
		GIM-R	AACTTCCAACCTTTGCCATGC		
	<i>bla</i> _{SPM}	SPM-F	GGGTGGCTAAGACTATGAAGCC	447	Poirel <i>et al.</i> , 2004
		SPMR	GCCGCCGAGCTGAATCGG		
	<i>bla</i> _{SIM}	SIM-F	TACAAGGGATTCGGCATCG	250	Poirel <i>et al.</i> , 2011
		SIM-R	TAATGGCCTGTTCCCATTGTG		
D	<i>bla</i> _{OXA (ESBL)}	OXA-F	GGCACCAGATTCAACTTTCAAG	564	Dallenne <i>et al.</i> , 2010
		OXA-R	GACCCCAAGTTTCCTGTAAGTG		
	<i>bla</i> _{OXA-48}	OXA-48-F	TTGGTGGCATCGATTATCGG	744	Poirel <i>et al.</i> , 2004
		OXA-48-R	GAGCACTTCTTTTGTGATGGC		
	<i>bla</i> _{OXA-51}	OXA-51-F	TAATGCTTTATCGGCCTTG	353	Sharma <i>et al.</i> , 2023
OXA-51-R		TGGATTGCACTTCATCTTGG			
Integrons	<i>IntI1</i>	<i>IntI1</i> -F	CAGTGGACATAAGCCTGTTC	160	Dillon <i>et al.</i> , 2005
		<i>IntI1</i> -R	CCCGAGGCATAGACTGTA		
	<i>IntI2</i>	<i>IntI2</i> -F	CACGGATATGCGACAAAAAGGT	789	
		<i>IntI2</i> -R	GTAGCAAACGAGTGACGAAATG		

3.2. Methods

3.2.1. Study Design

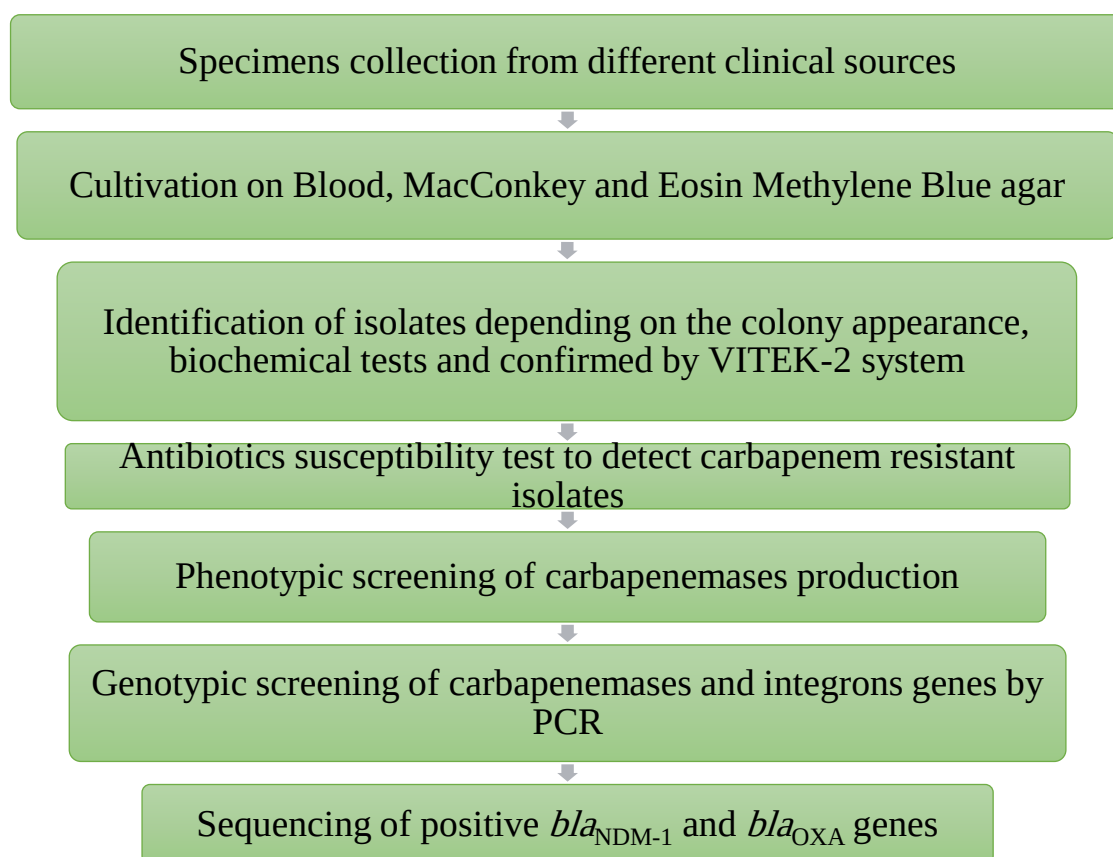


Figure (3-1): Design of the Study.

3.2.2. Specimens Collection

This cross-sectional prevalence survey was conducted at two major Teaching Hospitals, Al-Hussein Teaching Hospital and Al-Nasiriyah Teaching Hospital, in Thi-Qar province over a period of ten months, from January to October 2024. Before the investigation began, ethics approval was obtained from the College of Science at Al-Qadisiyah University and Thi-Qar Health Directorate. Various clinical specimens were collected randomly from patients hospitalized or attending at these Hospitals, who clinically suspected by physician to infections. These specimens included blood, cerebrospinal fluid (CSF), sputum, burn and wound exudates, body fluids, and urine. For each patient, medical records were reviewed, which included information on gender, age, and hospitalization. All specimens were labeled

and transported immediately to the Microbiology Laboratory at the College of Dentistry, Thi-Qar University, for processing, following the guidelines described by Collee *et al.* (1996).

For the collection of burn and wound exudates, a sterile swab was used to collect specimens from the infected site under appropriate precautions. The swab was transported immediately in a transport media test tube to the laboratory for culture. For sputum specimens, patients were instructed to cough deeply at the beginning of the collection to minimize saliva contamination. Sputum was collected early in the morning after the patient rinsed their mouth with water. A sterile wide-mouthed container was used for sputum collection via expectoration. Clean-Catch midstream urine was collected in a sterile, wide-mouthed, screw-capped container from patients suspected of having a UTI, and the container was transported to the laboratory immediately.

Blood specimens were collected after cleaning the skin with 70% ethanol followed by 2% iodine. Five milliliters of blood were collected from adults and 1 to 2 milliliters from pediatric patients. This blood was immediately inoculated into 50 milliliters of Brain-Heart Infusion broth. Blood culture bottles were subsequently incubated at 37°C for 24 hours to 7 days, and after the incubation period, the grown bacterial colonies were sub-cultured on solid media. Body fluids were aspirated using needles by physician, collected into sterile screw-top tubes, and transported to the Microbiology Laboratory within one hour for culture and analysis.

3.2.3. Cultivation of Specimens

All specimens were cultured aseptically on blood agar, MacConkey agar, and Eosin Methylene Blue (EMB) agar plates, then incubated aerobically for 24 hours at 37°C. Using the standard loop method, urine specimens were streaked onto the aforementioned media. A urine culture was considered positive if it yielded a bacterial count of 10^5 colony-forming

units (CFU)/ml. For blood cultures, any broth that showed turbidity was sub-cultured onto media using sterile techniques. Broth that remained clear was kept for further incubation and regarded as negative for growth if it remained clear after 7 days of incubation. After overnight incubation, the culture plates were examined for growth.

Bacterial isolates were identified based on their morphology. On blood agar, *E. coli* colonies are typically non-hemolytic, although some strains isolated from infections may be β -hemolytic. On MacConkey agar, most *E. coli* are lactose fermenters, and the colonies usually exhibit flat, dry pink surfaces with darker pink areas where precipitated bile salts have formed around them (Manishimwe *et al.*, 2021). The colony morphology of *E. coli* on EMB agar exhibits a distinctive greenish metallic sheen. Further identification of *E. coli* was typically performed based on Gram reactions and traditional biochemical tests following the schemes provided by MacFaddin (2000). In addition, *E. coli* isolates underwent confirmatory identification using the VITEK-2 system.

3.2.4. Culture Media Preparation

3.2.4.1. Ready-Prepared Culture Media

The media used in the present study are listed in Table (3-4). Each medium was prepared according to the manufacturer's instructions. Sterilization was performed using an autoclave set to 121 °C and a pressure of 15 lbs/in² for 15 minutes. The sterilized media were then poured into sterile petri dishes or tubes and incubated at 37°C for 24 hours to ensure there was no contamination. After incubation, the media were stored at 4 °C until needed.

For blood agar, following sterilization and cooling to 45°C, 5% human blood was added. Urea agar base was supplemented with a 20% sterile urea solution, which was prepared by dissolving 20 g of urea in 100 ml of distilled

water (DW) and then filtering it through a 0.22-micron millipore syringe filter. Each prepared medium was labeled and stored at 4°C in a refrigerator.

3.2.4.2. Maintenance Medium

The maintenance medium consists of brain heart infusion broth as a basal medium, supplemented with 20% glycerol. After autoclaving at 121°C for 15 minutes and allowing it to cool to 56°C, 5 ml of the medium is dispensed into sterile tubes and stored at 4°C until needed. This medium is used to maintain bacterial isolates for long-term storage at -70°C for several months (Harley and Prescott, 2002). For short-term preservation, a pure bacterial isolate colony is streaked onto nutrient agar slants, incubated at 37°C for 18 to 24 hours, and then stored at 4°C in the refrigerator. Frozen stock cultures can be sub-cultured on fresh blood agar plates and incubated in aerobic conditions at 37°C for 24 hours (Mahon *et al.*, 2007).

3.2.4.3. Motility Medium

The motility medium was prepared by melting 0.5 g of agar-agar in 100 milliliters of brain heart infusion broth. This mixture was then distributed into test tubes and autoclaved at 121°C for 15 minutes (MacFaddin, 2000).

3.2.5. Biochemical Tests

The present study employed several conventional biochemical tests to identify *E. coli*, following the guidelines set by MacFaddin (2000). The results of all tests are presented in Table (3-9).

3.2.5.1. Indole Test

For the indole test, the conventional tube method was utilized. Fresh colonies of the bacteria to be tested were inoculated into a tube of peptone water and incubated for 18 to 48 hours at 37°C. After incubation, a few drops of Kovac's reagent (composed of 5 g of para-dimethyl aminobenzaldehyde in 57 milliliters of isoamyl alcohol, with 25 milliliters of HCl added

gradually) were added to the broth culture, which was then shaken gently. The formation of a pink ring indicated a positive result.

3.2.5.2. Citrate Utilization Test

Fresh isolate colonies from the culture plate were taken using a straight wire loop and inoculated into Simmon's citrate slant. The slants were then incubated at 37°C for 48 to 72 hours. A positive test was indicated by a color change from green to blue and the growth of colonies along the streak line.

3.2.5.3. Methyl Red Test

Colonies from an overnight culture were inoculated into a Methyl Red/Voges-Proskauer (MR-PV) broth tube. This broth was incubated at 37°C for 24 hours. The methyl red reagent was prepared by dissolving 0.1 g of methyl red in 300 ml of 96% ethanol and then diluting it to 500 ml with DW. The reagent was stored at 4°C and allowed to warm to room temperature before use. Five drops of the reagent were added to the tube, and the result was read immediately. A positive test was indicated by the development of a red color.

3.2.5.4. Voges-Proskauer Test

MR-PV broth was inoculated with overnight bacterial culture and was incubated at 37°C for 48 hours. Two drops of 40% KOH solution (40 gm of KOH in 100 ml of DW) followed by 6 drops of 5% solution of α -naphthol (5 gm of α -naphthol in 100 of absolute ethanol) were added to tube. A positive test was represented by the development of a pink color 15 minutes after the addition of the reagents.

3.2.5.5. Urease Test

Colonies of isolated bacteria from an overnight culture were streaked onto the surface of a urea agar slant. The tube was incubated at 37°C and was read after 24 hours, and every day for 6 days. The test was positive if a purple pink color appeared.

3.2.5.6. Oxidase Test

The oxidase reagent was prepared by dissolving 0.1 g of tetramethyl-p-phenylenediamine dihydrochloride in 10 milliliters of DW. This solution was stored in a dark bottle. To perform the test, a strip of filter paper was soaked with a few drops of the freshly made 1% oxidase reagent. An overnight growth colony was picked using a sterile wooden stick and applied to the filter paper. A deep purple color appearing within 10 seconds indicates a positive result.

3.2.5.7. Catalase Test

To conduct the catalase test, a sterile inoculating loop or wooden applicator stick was used to pick the center of a colony from an overnight culture plate. The specimen was placed on a clean glass slide, and a drop of 3% (V/V) hydrogen peroxide (H_2O_2) was added to the smear. The rapid production of bubbles, indicating the release of oxygen, signifies a positive result.

3.2.5.8. Triple Sugar Iron Test (TSI)

Using a straight inoculation needle, a colony isolated from an overnight growth culture plate was picked and stabbed into the center of the medium all the way to the bottom of the tube. The needle was then used to streak the surface of the agar slant in a zigzag pattern. The slant was then incubated at 37°C for 24 hours. The expected results are as follows:

- An acid-acid reaction (yellow slant and yellow butt) indicates fermentation of dextrose, lactose, and/or sucrose.
- An alkaline-acid reaction (red slant and yellow butt) indicates fermentation of dextrose only.
- An alkaline-alkaline reaction (red slant and red butt) indicates the absence of carbohydrate fermentation.

Presence of blackening in the medium indicates the presence of hydrogen sulfide (H_2S), while bubbles or cracks in the agar signify gas production.

3.2.5.9. Motility Test

An overnight growth colony of isolated bacteria was inoculated into the motility medium by stabbing the center of the medium to a depth of 2 cm with a straight inoculation needle. The inoculated medium was then incubated at 37°C for 24 to 48 hours. The formation of a diffuse zone of growth spreading from the line of inoculation indicates a positive motility test.

Table (3-9): The Biochemical Tests for Identification of *E. coli* Isolates.

Test	Result
Methyl Red	Positive
Motility	
Catalase	
Indole	
Voges-Proskauer	Negative
Simmons Citrate	
Oxidase	
Urease	
TSI	Acid/acid with gas production

3.2.5.10. Confirmatory Diagnosis by VITEK-2 System

The VITEK-2 system is one of the fastest and most accurate automated techniques for identifying bacteria. It utilizes a biochemical profile produced by plastic reagent cards, which contain microliter amounts of various biochemical test media in 30 wells to identify organisms. The system measures color changes in the card caused by the metabolic activity of the microbes using a photometer. An inoculum from cultivated specimens is automatically deposited into the card, while color changes due to microbial activity are measured periodically. Data is processed, stored, and printed using a computerized database, and the system offers a variety of cards for identifying both Gram-negative and Gram-positive bacteria (Maina and Kagotho, 2014). The procedure is outlined below:

1. In a sterile manufacturer's tube, suspended a single isolated pure colony of bacteria in 3 ml of physiological saline.

2. Compared the bacterial suspension to a standard turbidity solution using a turbidity measuring device to adjust the concentration. The final turbidity within the tube should be between 0.5 and 0.63.
3. Followed the diagnostic Gram stain, placed the tubes into the VITEK-2 cassette, which contains a specific strip for Gram-negative bacteria.
4. A rack holding the tubes and cassette is then introduced into the system. Initially, it is positioned in the filler area, where the machine automatically filled the cassette with the bacterial suspension. Once this process is completed, the device signals the end of the filling.
5. The system cuts the cassettes and send data as a digital signal to the second field reader. Each sample's data is provided by a tube rack that moves away from the instrument and connects to the VITEK-2 system.
6. Leave the cassettes at 37°C for 6-8 hours, after which the results for bacterial identification can be read.

3.2.6. Antibiotics Susceptibility Test

The antibiotic susceptibility testing for different *E. coli* isolates was conducted using the Kirby-Bauer discs diffusion method (Bauer *et al.*, 1966) with Mueller-Hinton agar (MHA), following the guidelines recommended by the Clinical and Laboratory Standards Institute (CLSI, 2025). A total of twenty-three antibiotic discs (Table 3-6) were tested. To begin, *E. coli* isolates were grown on fresh nutrient agar plates for 18 to 24 hours. Three to five identical colonies were then transferred into 2 ml of tryptic soy broth and incubated for 8 hours at 37°C. The turbidity of the broth was adjusted to the 0.5 McFarland standard, which corresponds to approximately 1.5×10^8 CFU/ml. Next, a sterile cotton swab was dipped into the bacterial suspension, and excess suspension was removed by squeezing the swab against the wall of the test tube. The inoculum was then evenly distributed across the entire surface of the MHA plate by swabbing three times while rotating the plate approximately 60 degrees each time. After inoculation, the plates were

allowed to stand at room temperature for 5 minutes to let the inoculum dry. A maximum of six antibiotic discs were then placed in a circular pattern on a 100 mm inoculated plate. Following a 15-minute incubation period at room temperature, the plates were placed in an incubator set at 37°C for 18 hours. Finally, the diameter of each inhibition zone, including the diameter of the discs, was measured with a ruler and recorded in millimeters.

The Multiple Antibiotic Resistance (MAR) index was calculated for each isolate by dividing the number of antibiotics to which the isolate showed resistance by the total number of antibiotics tested (Krumperman, 1983).

$$\text{MAR index} = \frac{\text{Number of antibiotics against which isolate showed resistance}}{\text{Total number of antibiotics tested}}$$

MDR was defined as a non-susceptibility to at least one agent in three or more antibiotic classes (11 classes). XDR was defined as non-susceptibility to at least one agent in all but one or two antibiotic classes which sensitive. PDR was defined as non-susceptible to all antibiotic classes (Magiorakos *et al.*, 2012).

3.2.7. Phenotypic Screening of β -Lactamases

All carbapenem resistant *E. coli* isolates were further tested for β -lactamases production as follows:

3.2.7.1. Screening of Extended-Spectrum β -Lactamases (ESBLs)

3.2.7.1.1. Initial Screening of ESBLs

The production of ESBLs were initially screened using the discs diffusion method with ceftazidime, ceftriaxone, cefotaxime, and aztreonam (30 μ g/disc each), according to the CLSI (2016) recommendations. Isolates that exhibited an inhibition zone of ≤ 22 mm for ceftazidime, ≤ 25 mm for ceftriaxone, and ≤ 27 mm for cefotaxime and aztreonam were suspected to produce the ESBL enzymes.

3.2.7.1.2. Confirmatory Screening of ESBLs

Potential ESBL-producing isolates were further analyzed using the discs approximation test, which assesses the inhibitory effect of clavulanic acid, following the criteria of Kiratisin *et al.* (2008) and CLSI (2016). Four antibiotic discs (cefotaxime, ceftazidime, ceftriaxone, and aztreonam, 30 µg each) were placed around a central disc of amoxicillin-clavulanic acid (20/10 µg) at a distance of 20 mm from center to center on MHA plates inoculated with the bacteria being tested for ESBLs production. The plates were incubated aerobically at 37°C for 18 hours, and the diameter of any inhibition zones around the antibiotic discs was measured in mm using a ruler. An increase in the inhibition zone diameter between the central amoxicillin-clavulanic acid disc and any of the four antibiotic discs indicating resistance or intermediate susceptibility was recorded, leading to the conclusion that the bacteria were ESBLs producer (CLSI, 2016).

3.2.7.2. Screening of AmpC β-Lactamases

3.2.7.2.1. Initial Screening of AmpC

All carbapenem resistant isolates were tested for susceptibility to cefoxitin using the standard discs diffusion method. Isolates with an inhibition zone diameter of 18 mm or less were considered to be potential producers of AmpC β-lactamases (Plasmidic) (Coudron *et al.*, 2003).

3.2.7.2.2. Confirmatory Screening of AmpC

The Ceftazidime-Imipenem Antagonism Test (CIAT) was performed to detect the production of inducible AmpC β-lactamase (Chromosomal), following the method described by Cantarelli *et al.* (2007). The test involved placing a 10 µg imipenem disc 20 mm from a 30 µg ceftazidime disc on a MHA plate that had been inoculated with a 0.5 McFarland bacterial suspension. The plate was then incubated for 24 hours at 35°C. For comparison, a cefoxitin disc was also placed 20 mm from the ceftazidime disc. A visible reduction in the inhibition zone around the ceftazidime disc,

due to antagonism from either the imipenem or ceftioxin discs, was interpreted as a positive result for the production of inducible AmpC β -lactamases.

3.2.7.3. Screening of Carbapenemases

3.2.7.3.1. Combined Discs Test (CDT)

The presence of class B carbapenemases (MBLs) was evaluated in all isolates that were non-susceptible to carbapenems. There is no specific CLSI guideline for the detection of MBLs, so the procedure follows the method described by Yong *et al.* (2002). A bacterial suspension of the test isolate was prepared in peptone water and incubated at 37°C for 2 hours. The turbidity of the suspension was matched with the 0.5 McFarland turbidity standard before inoculation on MHA. Discs of imipenem (10 μ g) and imipenem-EDTA (10 μ g + 750 μ g), as well as meropenem (10 μ g) and meropenem-EDTA (10 μ g + 730 μ g), were placed 15 mm apart from each other and incubated at 37°C for 18 hours. A carbapenem non-susceptible isolate showing a ≥ 7 mm increase in the zone of inhibition with either imipenem-EDTA or meropenem-EDTA compared to the imipenem or meropenem disc alone was considered an MBLs producer. The EDTA solution was prepared by dissolving 18.9 g of EDTA in 100 ml of distilled water, sterilized in an autoclave after adjusting the pH to 8. Ten microliters of this solution were added to each imipenem and meropenem discs.

3.2.7.3.2. Double Discs Synergy Test (DDST)

The detection of MBLs was also performed using the DDST as described by Lee *et al.* (2003). The procedure is as follows: A 10- μ g imipenem disc is placed in the center of a MHA plate inoculated with a 0.5 McFarland dilution of the test isolate. An EDTA disc (1900 μ g), prepared by immersing 6-mm Whatman filter No. 1 discs (n=100) in an EDTA solution (190 mg of EDTA dissolved in 1 ml of distilled water), is then placed 15 mm away from the imipenem disc, center to center. The plate is incubated at 37°C

overnight. If the zone of inhibition around the imipenem disc is extended on the side nearest the EDTA disc, it indicates that the isolate is MBLs producer.

3.2.7.3.3. Modified Hodge Test (MHT)

The MHT was conducted as described by Lee *et al.* (2001). A MHA plate was inoculated overnight with a suspension of *E. coli* ATCC 25922, adjusted to a 0.5 McFarland standard. After a brief drying period of 3-5 minutes, a 10 µg imipenem disc was placed in the center of the plate. The *E. coli* test isolate was then streaked heavily from the edge of the disc toward the edge of the plate, and the plates were incubated overnight at 37°C. Four organisms were tested on the same plate with a single disc. The MHT result was considered positive if growth of *E. coli* ATCC 25922 was observed within the inhibition zone of the imipenem disc, leading to a distorted zone that indicates carbapenemase production. A negative MHT result was indicated by the absence of growth of *E. coli* ATCC 25922 alongside the test organism.

3.2.7.3.4. Lateral Flow Immunoassay (LFIA)

LFIA (Combo Test) is a multiplex immunochromatographic assay that uses monoclonal antibodies to rapidly detect KPC, IMP, NDM, VIM, and OXA-48 carbapenemases. This test can be performed using pure bacterial colonies, following the manufacturer's instructions as follow:

1. Scrape one loop of bacteria using a 1 µl inoculating disposable loop. Wash the bacteria into a tube containing dilution buffer. Repeat this step three times.
2. Remove the inoculating loop, cover the tube with the dropper, and mix well using a vortex mixer.
3. Add four drops of the processed sample extract into the sample well.
4. Observe the results within 15-20 minutes. Results observed after 20 minutes are considered invalid.

3.2.8. Solutions Used in Molecular Screening

According to Bartlett and Stirling (1998), the solutions for molecular screening were prepared as follows:

3.2.8.1. Tris-Borate-EDTA (TBE) Buffer

The working buffer (1X) was prepared by diluting the 10X stock solution. Specifically, 100 ml of TBE buffer (10X) was added to 900 ml of DW. This solution was stored at room temperature until it was used for agarose gel electrophoresis.

3.2.8.2. Tris-EDTA (TE) Buffer

The TE buffer was prepared at a concentration of 1X by diluting the stock TE solution with DW. This solution was stored at 4°C until it was needed for use with primers.

3.2.8.3. Loading Dye Solution

The loading dye was prepared by dissolving 0.25 g of bromophenol blue and 4 g of sucrose in 10 ml of DW. The total volume was then adjusted to 100 ml, and the solution was stored at 4°C until it was used.

3.2.8.4. Ethidium Bromide Solution

This solution was prepared by dissolving 0.05 g of ethidium bromide in 10 ml of DW to achieve a final concentration of 5 mg/ml. It was stored in a dark reagent bottle to protect it from light.

3.2.9. DNA Extraction

In this study, DNA was extracted from carbapenem resistant *E. coli* isolates following the instructions provided by a commercial extraction kit. The procedure is outlined below:

1. Up to 1×10^9 bacterial cells of overnight growth were transferred to a 1.5 ml microcentrifuge tube and centrifuged at 14000 rpm for 1 minute. The supernatant was then discarded.
2. Using a pipette, 180 μ l of GT buffer was added to the cell pellet.

3. Next, 20 μl of Proteinase K was added, and the mixture was incubated in a water bath at 60°C for at least 10 minutes, inverting the tube every 3 minutes.
4. Then, 200 μl of GB buffer was added to the sample, which was mixed by vortexing for 10 seconds. The sample was incubated at 70°C for at least 10 minutes, inverting the tube every 3 minutes during the incubation.
5. After that, 200 μl of absolute ethanol was added to the sample lysate, and the mixture was shaken vigorously.
6. The resulting mixture was transferred to the GD column and centrifuged for two minutes in a collecting tube. The collection tube was discarded and replaced.
7. Four hundred μl of W1 buffer was added to the GD column and centrifuged for 30 seconds. The collection tube was then replaced, and the flow-through was discarded.
8. Six hundred μl of wash buffer (W2) was added to the GD column and centrifuged for 30 seconds. The collection tube was replaced, and the column was centrifuged for an additional 3 minutes after each substitution.
9. Finally, 100 μl of pre-heated elution buffer at 70°C was added to the center of the column matrix. The column was allowed to stand for at least 3 minutes, and then centrifuged for 30 seconds to elute the purified DNA, and stored at -20°C.

3.2.10. DNA Concentration Estimation

The extracted genomic DNA is analyzed using a NanoDrop spectrophotometer to measure DNA concentration ($\text{ng}/\mu\text{l}$) and assess DNA purity by reading the absorbance at 260/280 and 260/230 nm wavelengths. The procedure is as follows:

1. To prepare the device, add 1 μl of sterile DW to the lens, ensuring that it does not touch the lens.
2. Next, add 1 μl of the DNA sample to the lens and read the absorbance at 260/280 and 260/230 nm.

An optical density of 1 corresponds to approximately 50 ng/ μl of double-stranded DNA. The acceptable ratio for DNA purity is between 1.8 ± 0.2 .

3.2.11. Primers Preparation

The oligonucleotide primers used in this study are listed in Table 3-8, including both forward and reverse primers. Following the supplier's recommendations, these primers were supplied in lyophilized form and were dissolved in nuclease-free water to achieve a final concentration of 100 picomoles/ μl . To prepare a working solution at a concentration of 10 pmol/ μl , 10 μl of this stock solution was resuspended in 90 μl of nuclease-free water. All primer stock solutions were stored in Eppendorf tubes and frozen at -20°C until needed.

3.2.12. Preparation of Polymerase Chain Reaction (PCR) Mixture

Preparation of the PCR mixture was performed with a final volume of 25 μl , following the Go Tag Green manufacturer's instructions (Table 3-10).

Table (3-10): Total Volume of PCR Reaction Mixture.

Reagent	Volume per PCR reaction
Go tag PCR green master mix	12.5 μl
Forward primer	2 μl
Reverse primer	2 μl
DNA template	4 μl
Nuclease free water	4.5 μl
Total volume	25 μl

3.2.13. PCR Thermocycling Conditions

The PCR thermocycling conditions were optimized based on their reference procedures. The specific program for each primer set is outlined in Table 3-11. The presence or absence of the PCR product was verified in all 40 carbapenem resistant *E. coli* isolates using PCR. All components for the

PCR were combined in a PCR tube and mixed under sterile conditions while placed on an ice bag.

Table (3-11): The Optimum Condition of *bla*, *Int1* and *Int2* Genes Detection.

Gene	Initial denaturation	Cycling conditions			Final extension	Cycles
		Denaturation	Annealing	Extension		
<i>bla</i> _{KPC}	95/15 min	94/30 sec	57/1 min	72/90 sec	72/10 min	30
<i>bla</i> _{NMC}	94/10 min	95/1min	52/30 sec	72/1min	72/6min	35
<i>bla</i> _{IMI}	94/5min	95/1min	52/30 sec	72/1min	72/5 min	30
<i>bla</i> _{GES}	93/3 min	93/1 min	55/1 min	72/1 min	72/7 min	40
<i>bla</i> _{NDM-1}	94/10 min	94/60 sec	60/30 sec	72/30 sec	72/10 min	35
<i>bla</i> _{IMP}	94/10 min	94/60 sec	60/30 sec	72/30 sec	72/10 min	35
<i>bla</i> _{VIM}	94/10 min	94/60 sec	60/30 sec	72/30 sec	72/10 min	35
<i>bla</i> _{GIM}	95/2 min	95/30 sec	59/30sec	72/80sec	72/5 min	30
<i>bla</i> _{SPM}	95/15 min	94/30 sec	60/60 sec	72/90 sec	72/5 min	30
<i>bla</i> _{SIM}	95/2 min	95/30 sec	58/30 sec	72/1min	72/5 min	30
<i>bla</i> _{OXA} (ESBL)	94/10 min	94/40 sec	60/40 sec	72/1 min	72/5 min	30
<i>bla</i> _{OXA-48}	94/5 min	94/1 min	50/1 min	72/1 min	72/10 min	35
<i>bla</i> _{OXA-51}	94/3min	94/30sec	57/30sec	72/36sec	72/5min	35
<i>Int1</i>	94/5min	94/30sec	55/30sec	72/1min	72/7min	35
<i>Int2</i>	94/5min	94/30sec	62/30sec	72/1min	72/10min	30

3.2.14. Agarose Gel Preparation

The gel electrophoresis procedures were performed according to the method outlined by Bartlett and Stirling (1998). An agarose gel was prepared using 100 ml of 1X TBE buffer by adding 1.5 g of agarose powder. The solution was boiled until clear, then cooled to 50°C, after which 0.5 µg/ml of ethidium bromide was added to the agarose solution. The agarose was poured into a pre-prepared gel tray and allowed to cool and solidify. The comb was then gently removed from the tray, creating wells for loading the DNA sample. The wells of the agarose gel were loaded with 5 microliters of the amplified PCR product, alongside a 5 µl DNA ladder for size comparison. The gel tray was placed in the electrophoresis chamber, and

enough 1X TBE buffer was added to cover the gel layer. The electrical current was applied at 70 volts for 1.5 to 2 hours. Finally, visualization of the PCR products was achieved using the E-graph documentation system. Positive results were determined when the DNA band sizes from the samples corresponded to the target size of the DNA product, as indicated by the ladder.

3.2.15. Sequencing of *bla*_{NDM-1} and *bla*_{OXA} Genes

The PCR products of the *bla*_{NDM-1} and *bla*_{OXA} genes were sent to Macrogen Company in Seoul, Korea, for sequencing. Chromatograms from the ABI sequence files were thoroughly examined to ensure that the annotations and variations were not a result of PCR or sequencing errors, using Finch TV software for analysis. The virtual locations and additional details of the obtained PCR fragments were determined by comparing the observed nucleic acid sequences of our local samples with those retrieved from the reference database using Basic Local Alignment Search Tool (BLAST software from National Center for Biotechnology Information (NCBI). Phylogenetic tree was constructed and the evolutionary distance calculated according to the method of Jukes and Cantor (1969), and the topology was inferred using the neighbor-joining method (Saitou and Nei 1987), based on bootstrap analysis of 1000 trees (using MEGA X software).

3.2.16. Statistical Analysis

Data analysis was conducted using SPSS version 24 (IBM Statistical Package for the Social Sciences, Chicago, IL, USA). The chi-square test was employed to identify any significant differences in resistance or prevalence where applicable. A *P*-Value of ≤ 0.05 was considered statistically significant.

Chapter Four

RESULTS

4. Results

4.1. Study Population and Bacterial Isolates

During the study period, a total of 2203 specimens were collected from various clinical sources: 908 specimens (41.22%) were from Al-Hussein Teaching Hospital, while 1295 specimens (58.78%) came from Al-Nasiriya Teaching Hospital. The specimens were categorized as follows: 814 (36.95%) urine, 516 (23.42%) blood, 495 (22.47%) sputum, 223 (10.12%) body fluids, 72 (3.27%) swabs from different regions, 58 (2.63%) wound and burn exudates, and 25 (1.13%) CSF (Figure 4-1).

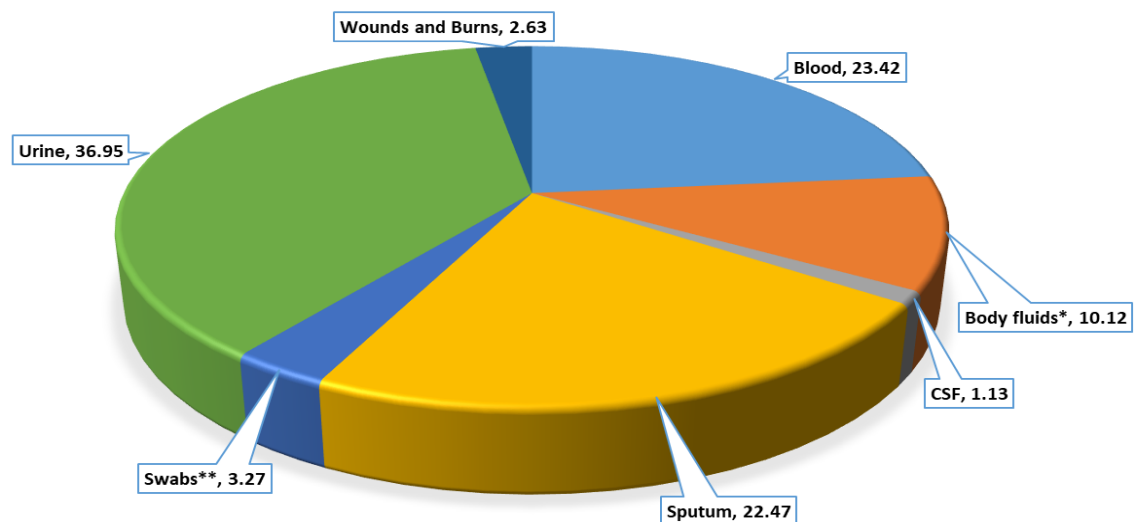


Figure (4-1): Percentages of Specimen's Distribution (N= 2203). *Body fluids include: ascites, pericardial, pleural, seminal, and bronchoalveolar lavage (BAL). **Swabs: are taken from various sources such as abscesses, urethra, ear, diabetic foot, nasal passages, and throat.

A total of 1212 bacterial isolates were recovered from various clinical specimens, representing 55.02% of the total tested, while 991 clinical specimens (44.98%) showed no bacterial growth. Among the patients with positive cultures, *E. coli* accounted for 15.35% (186 out of 1212) of all isolates, making it the second most commonly cultured bacteria after *Staphylococcus* species (306 isolates, 25.25%), which was the most frequently isolated bacteria. The distribution of other bacterial genera included 176 isolates (14.52%) of *Pseudomonas* species, 161 isolates

(13.28%) of *Streptococcus*, 119 isolates (9.82%) of *Klebsiella* species, 73 isolates (6.02%) of *Enterococcus* species, 66 isolates (5.45%) of *Acinetobacter* species, 31 isolates (2.56%) of *Proteus*, 20 isolates (1.65%) of *Moraxella* species, and 12 isolates (0.99%) of *Enterobacter* species. Additionally, 21 isolates (1.73%) were identified as belonging to other bacterial genera (Figure 4-2). *Candida* species, a type of yeast, appeared as a pure culture in 41 cases (3.38%). Mixed growths were excluded from this report.

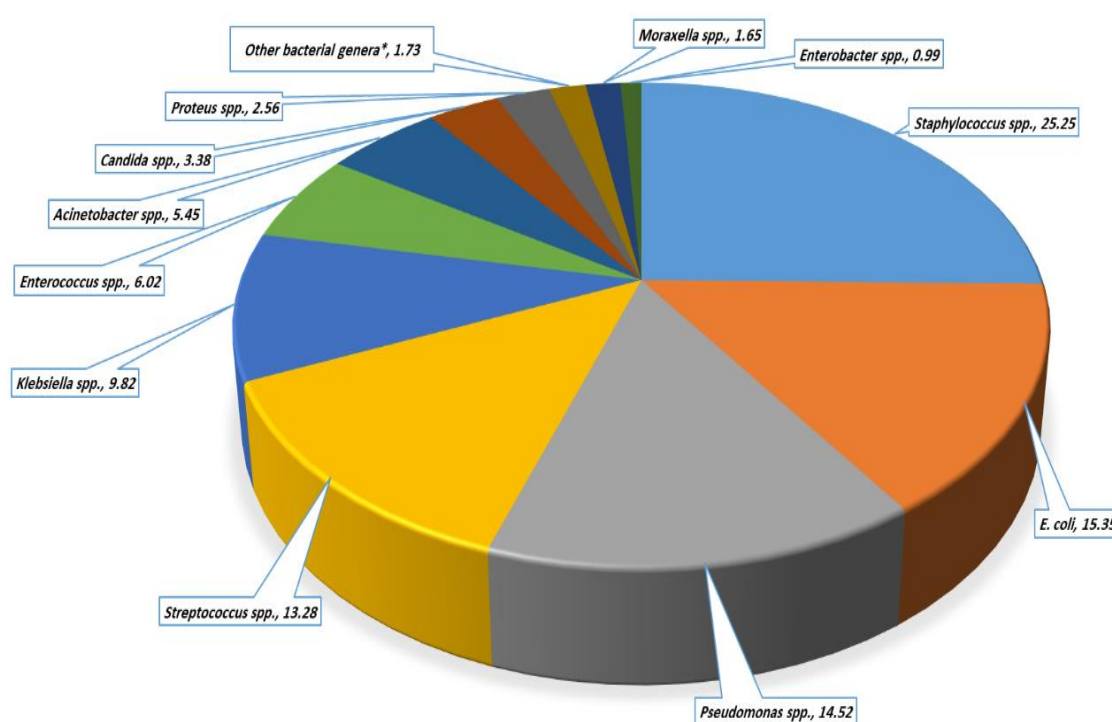


Figure (4-2): Percentages of Common Types of Bacteria Isolated from Different Specimens (N= 1212). *Other bacterial genera, which accounted for 21 isolates (1.73%), were the least dominant. This group includes *Burkholderia* spp. (6 isolates), *Serratia* spp. (4 isolates), *Achromobacter* spp. (2 isolates), and *Neisseria* spp. (2 isolates). Additionally, *Aeromonas* spp., *Alloiococcus* spp., *Leclercia* spp., *Listeria* spp., *Morganella* spp., *Pasteurella* spp., and *Providencia* spp. were each represented by a single isolate.

4.2. Identification and Prevalence of *E. coli* Isolates

Based on cultural, morphological characteristics, as well as microscopic and biochemical tests of bacterial isolates, a total of 186 isolates (8.44%) were identified as *E. coli* and confirmed using the VITEK-2 system. Of the *E. coli* isolates, 119 (63.98%) were obtained from urine specimens.

The remaining isolates were recovered from various specimens: sputum (36 isolates, 19.35%), body fluids (14 isolates, 7.53%), swabs from different sources (8 isolates, 4.30%), blood (4 isolates, 2.15%), wound and burn exudates (4 isolates, 2.15%), and CSF (1 isolate, 0.54%). There is a significant statistical association ($P<0.00$) between the type of specimen and the prevalence of *E. coli* infection, as illustrated in Table 4-1.

Table (4-1): The Prevalence of *E. coli* Isolates Obtained from Various Clinical Specimens in Thi-Qar Hospitals (N=2203).

Hospital	Type of Specimens	No. of Specimens (%)	No. of <i>E. coli</i> Isolates (%)	P-Value
Al-Hussein Teaching Hospital	Blood	171(18.83)	2 (1.17)	<0.00*
	Body fluids	48 (5.29)	8 (16.67)	
	CSF	17 (1.87)	1 (5.88)	
	Sputum	228 (25.11)	26 (11.40)	
	Swabs	28 (3.08)	4 (14.29)	
	Urine	399 (43.94)	74 (18.55)	
	Wounds and Burns	17 (1.87)	3 (17.65)	
Total		908 (100)	118 (13.00)	
Chi-Square	$X^2=34.23$, Df=6, $P\leq 0.05$			
Al-Nasiriyah Teaching Hospital	Blood	345 (26.64)	2 (0.58)	<0.00*
	Body fluids	175 (13.51)	6 (3.43)	
	CSF	8 (0.62)	0 (0.00)	
	Sputum	267 (20.62)	10 (3.75)	
	Swab	44 (3.40)	4 (9.09)	
	Urine	415 (32.05)	45 (10.84)	
	Wounds and Burns	41 (3.17)	1 (2.44)	
Total		1295 (100)	68 (5.25)	
Chi-Square	$X^2=46.00$, Df=6, $P\leq 0.05$			

* There is a significant difference.

According to Table 4-2, infections caused by *E. coli* were most frequently observed in Al-Hussein Teaching Hospital, which accounted for 118 cases (63.44%). In contrast, Al-Nasiriyah Teaching Hospital reported 68 cases (36.56%). This difference is statistically significant ($P<0.00$). The study population consisted of females, with 1130 (51.29%) female patients and 1073 (48.71%) male patients. Among the 186 *E. coli* isolates, 99 (53.23%) were obtained from female patients and 87 (46.77%) from male patients. The occurrence of *E. coli* isolates in relation to gender was found

to be statistically non-significant ($P=0.58$). The highest incidence of *E. coli* infections was noted in the age group above 60 years (64 cases, 34.41%), followed by the 21-40 year age group (50 cases, 26.88%). The relationship between age group and *E. coli* infection was found to be statistically significant ($P < 0.00$).

The majority of specimens were collected from inpatients (hospital-acquired infections) (1211 cases, 54.97%), while the remainder (992 cases, 45.03%) were obtained from outpatients (community-acquired infections). Among the patients with *E. coli* positive cultures, 101 (54.30%) were inpatients and 85 (45.70%) were outpatients, indicating a significant difference ($P = 0.01$). The demographic details of the patients infected with *E. coli* are summarized in Table 4-2.

Table (4-2): Demographic Characteristics of Patients with *E. coli* Infections (N=2203).

Patient Profile	Status	No. of Specimens (%)	No. of <i>E. coli</i> (%)
Age Group (Years)	<1	15 (0.68)	5 (2.69)
	1-20	380 (17.25)	27 (14.52)
	21-40	653 (29.64)	50 (26.88)
	41-60	519 (23.56)	40 (21.51)
	>60	636 (28.87)	64 (34.41)
	Total	2203 (100)	186 (8.44)
Chi-Square	$X^2=15.95$, Df=4, $P \leq 0.05$		$P\text{-value} < 0.00^*$
Gender	Female	1130 (51.29)	99 (53.23)
	Male	1073 (48.71)	87 (46.77)
	Total	2203 (100)	186 (8.44)
Chi-Square	$X^2= 0.30$, Df=1, $P \leq 0.05$		$P\text{-value}=0.58$
Hospital	Al-Hussein Teaching Hospital	908 (41.22)	118 (63.44)
	Al-Nasiriyah Teaching Hospital	1295 (58.78)	68 (36.56)
	Total	2203 (100)	186 (8.44)
Chi-Square	$X^2= 41.42$, Df=1, $P \leq 0.05$		$P\text{-value}<0.00^*$
Hospitalization	Inpatient	1211 (54.97)	101 (54.30)
	Outpatient	992 (45.03)	85 (45.70)
	Total	2203 (100)	186 (8.44)
Chi-Square	$X^2= 7.06$, Df=,1 $P \leq 0.05$		$P\text{-value}= 0.01^*$

* There is a significant difference.

4.3. Antibiotic Susceptibility of *E. coli* Isolates

The antibiotic susceptibility of 186 *E. coli* isolates were tested against 23 antibiotics, categorized into eleven classes based on their mechanism of action, as detailed in Table 4-3. The results of the study indicated that resistance to the ampicillin was 100% among all *E. coli* isolates. Regarding penicillins combined with β -lactamase inhibitors, resistance was observed in 84.41% of isolates for ampicillin-sulbactam and in 80.65% for amoxicillin-clavulanic acid. Among the third-generation cephalosporins, resistance to ceftazidime was the most prevalent at 83.87%, followed by cefotaxime at 79.57% and ceftriaxone at 73.12%. Resistance to the fourth-generation cephalosporin, cefepime, was noted at 67.74%. Additionally, resistance to the cephamycin, cefoxitin, was seen in 59.68% of the isolates. Notably, moderate resistance to aztreonam, was found in 40.86% of the isolates. In contrast, carbapenems emerged as the most effective class of β -lactam antibiotics, with only 11.29%, 11.83%, and 17.20% of isolates showing resistance to imipenem, meropenem, and doripenem, respectively. Regarding aminoglycosides, amikacin was the most potent, with an overall resistance rate of 26.88% among the isolates, followed by gentamicin at 37.10% and tobramycin at 59.14%. For the three fluoroquinolone antibiotics tested, the resistance rates were 72.04% for nalidixic acid, 51.61% for ciprofloxacin, and 50.54% for levofloxacin. The isolates also demonstrated significant resistance to tetracycline (60.75%) and doxycycline (58.06%). Additionally, resistance to trimethoprim was high at 66.67%, with an intermediate resistance rate of 7.53%. Concerning phenicols, 36.02% of isolates were resistant to chloramphenicol. In terms of susceptibility to nitrofurantoin, the majority of isolates (51.61%) displayed susceptibility, while 40.32% were resistant and 8.06% exhibited intermediate resistance.

Table (4-3): Antibiotic-Susceptibility of *E. coli* Isolates (N= 186).

Class of Antibiotics	Disc of Antibiotics	No. (%) of the Isolates:		
		Resistance	Intermediate	Susceptible
Penicillins	Ampicillin	186 (100.00)	0 (0.00)	0 (0.00)

Penicillins-β-Lactamase Inhibitors	Ampicillin-sulbactam	157 (84.41)	7 (3.76)	22 (11.83)
	Amoxicillin-Clavulanic acid	150 (80.65)	13 (6.99)	23 (12.37)
Cephalosporins	Ceftazidime	156 (83.87)	2 (1.08)	28 (15.05)
	Cefotaxime	148 (79.57)	7 (3.76)	31 (16.67)
	Ceftriaxone	136 (73.12)	4 (2.15)	46 (24.73)
	Cefepime	126 (67.74)	3 (1.61)	57 (30.65)
Cephameycins	Cefoxitin	111 (59.68)	9 (4.84)	66 (35.48)
Monobactam	Aztreonam	76 (40.86)	13 (6.99)	97 (52.15)
Carbapenems	Imipenem	21 (11.29)	11 (5.91)	154 (82.80)
	Meropenem	22 (11.83)	15 (8.06)	149 (80.11)
	Doripenem	32 (17.20)	8 (4.30)	146 (78.49)
Aminoglycosides	Tobramycin	110 (59.14)	7 (3.76)	69 (37.10)
	Amikacin	50 (26.88)	11 (5.91)	125 (67.20)
	Gentamicin	69 (37.10)	9 (4.84)	108 (58.06)
Quinolones	Nalidixic Acid	134 (72.04)	13 (6.99)	39 (20.97)
	Ciprofloxacin	96 (51.61)	10 (5.38)	80 (43.01)
	Levofloxacin	94 (50.54)	7 (3.76)	85 (45.70)
Tetracyclines	Tetracycline	113 (60.75)	7 (3.76)	66 (35.48)
	Doxycycline	108 (58.06)	10 (5.38)	68 (36.56)
Folate Pathway Inhibitor	Trimethoprim	124 (66.67)	14 (7.53)	48 (25.81)
Phenicol	Chloramphenicol	67 (36.02)	10 (5.38)	109 (58.60)
Nitrofurans	Nitrofurantoin	75 (40.32)	15 (8.06)	96 (51.61)

4.4. Clinical Data and Resistance Patterns of Carbapenem Resistant *E. coli* Isolates

In the current investigation, carbapenem non-susceptibility was defined as resistance or intermediate susceptibility to one or more of the three carbapenem antibiotics tested: imipenem, meropenem, and doripenem. Antibiotic susceptibility data for the 186 *E. coli* isolates indicated that 40 (21.51%) were non-susceptible. Among the 40 carbapenem non-susceptible isolates, 21 (52.50%) exhibited resistance to imipenem, 22 (55.00%) to meropenem, and 32 (80.00%) to doripenem. In terms of intermediate resistance, 11 (27.50%) of the non-susceptible isolates showed intermediate resistance to imipenem, while 15 (37.50%) and 8 (20.00%) exhibited intermediate susceptibility to meropenem and doripenem, respectively. Susceptibility to imipenem was observed in 8 (20.00%) isolates, whereas 3

(7.50%) were found to be susceptible to meropenem. Notably, no doripenem-susceptible isolates were identified among the carbapenem non-susceptible *E. coli* isolates (Figure 4-3).

According to table (4-4), the majority of the carbapenem resistant *E. coli* isolates were collected from urine (27 isolates, 67.50%), followed by sputum (6 isolates, 15.00%), body fluids (5 isolates, 12.50%), swabs (1 isolate, 2.50%), and CSF (1 isolate, 2.50%). No carbapenem resistant isolates were found in wound and burn exudates or blood specimens. Statistical analysis showed no significant association ($P=0.20$) between the type of specimens and the rate of carbapenem non-susceptibility.

Table (4-4): Distribution of Carbapenem Resistant *E. coli* Isolates from Different Clinical Specimens (N=40).

Type of Specimen	No. of Specimens	No. of <i>E. coli</i> Isolates (%)	No. of Carbapenem Non-Susceptible <i>E. coli</i> (%)
Blood	516	4 (0.18)	0 (0.00)
Body fluid	223	14 (0.64)	5 (12.50)
CSF	25	1 (0.05)	1 (2.50)
Sputum	495	36 (1.63)	6 (15.00)
Swab	72	8 (0.36)	1 (2.50)
Urine	814	119 (5.40)	27 (67.50)
Wounds and Burns	58	4 (0.18)	0 (0.00)
Total	2203	186 (8.44)	40 (21.51)
Chi-Square	$X^2= 8.50$, Df=6, $P\leq 0.05$		$P\text{-value}= 0.20$

* There is a significant difference.

The demographics of patients with carbapenem resistant *E. coli* infections enrolled in the study are summarized in Table (4-5). The highest prevalence of these infections was observed in patients over the age of 60, with 15 cases (37.50%). This was followed by the 41-60 age group, which had 14 cases (35.00%). In the 21-40 age group, there were 6 cases (15.00%), and in the 1-20 age group, there were 5 cases (12.50%). Notably, there were no infections reported in patients under the age of 1 year. The relationship between age and the profiles of carbapenem resistant *E. coli* infections was not found to be statistically significant ($P=0.07$). Additionally, infections

with carbapenem resistant *E. coli* were more prevalent in males (23 cases, 57.50%) compared to females (17 cases, 42.50%). However, there was no significant difference in the occurrence of *E. coli* isolates based on gender ($P=0.12$). In terms of hospital identification, 24 isolates (60.00%) were found at Al-Hussein Teaching Hospital, while 16 isolates (40.00%) were identified at Al-Nasiriyah Teaching Hospital. No statistical association was found between the hospitals and the prevalence of carbapenem resistant *E. coli* ($P=0.71$). Regarding hospitalization status, 25 isolates (62.50%) were obtained from hospitalized patients, while 15 isolates (37.50%) were collected from outpatients. There was no significant difference in this regard ($P=0.24$).

Table (4-5): Demographic Characteristics of Patients with Carbapenem Resistant *E. coli* Isolates (N=40).

Patient profile	Status	No. of <i>E. coli</i> (%)	No. of Carbapenem Resistant <i>E. coli</i> (%)
Age Group (Years)	<1	5 (2.69)	0 (0.00)
	1-20	27 (14.52)	5 (12.50)
	21-40	50 (26.88)	6 (15.00)
	41-60	40 (21.51)	14 (35.00)
	>60	64 (34.41)	15 (37.50)
	Total	186 (100.00)	40 (21.51)
Chi-Square	$X^2=8.65$, Df=4, $P \leq 0.05$		$P\text{-value}= 0.07$
Gender	Female	99 (53.23)	17 (42.50)
	Male	87 (46.77)	23 (57.50)
	Total	186 (100.00)	40 (21.51)
Chi-Square	$X^2=2.35$, Df=1, $P \leq 0.05$		$P\text{-value}=0.12$
Hospital	Al-Hussein Teaching Hospital	118 (63.44)	24 (60.00)
	Al-Nasiriyah Teaching Hospital	68 (36.56)	16 (40.00)
	Total	186 (100)	40 (21.51)
Chi-Square	$X^2=0.26$, Df=1, $P \leq 0.05$		$P\text{-value}=0.71$
Hospitalization	Inpatient	101 (54.30)	25 (24.75)
	Outpatient	85 (45.70)	15 (17.65)
	Total	186 (100.00)	40 (21.51)
Chi-Square	$X^2=5.80$, Df=,1 $P \leq 0.05$		$P\text{-value}= 0.24^*$

* There is a significant difference.

4.5. Antibiotic Susceptibility of Carbapenem Resistant *E. coli* Isolates

The 40 carbapenem resistant *E. coli* isolates were subjected to antibiotic susceptibility testing using the discs-diffusion method against 23 selected antibiotics. The percentage of antibiotic resistance among these isolates is illustrated in Figure (4-3).

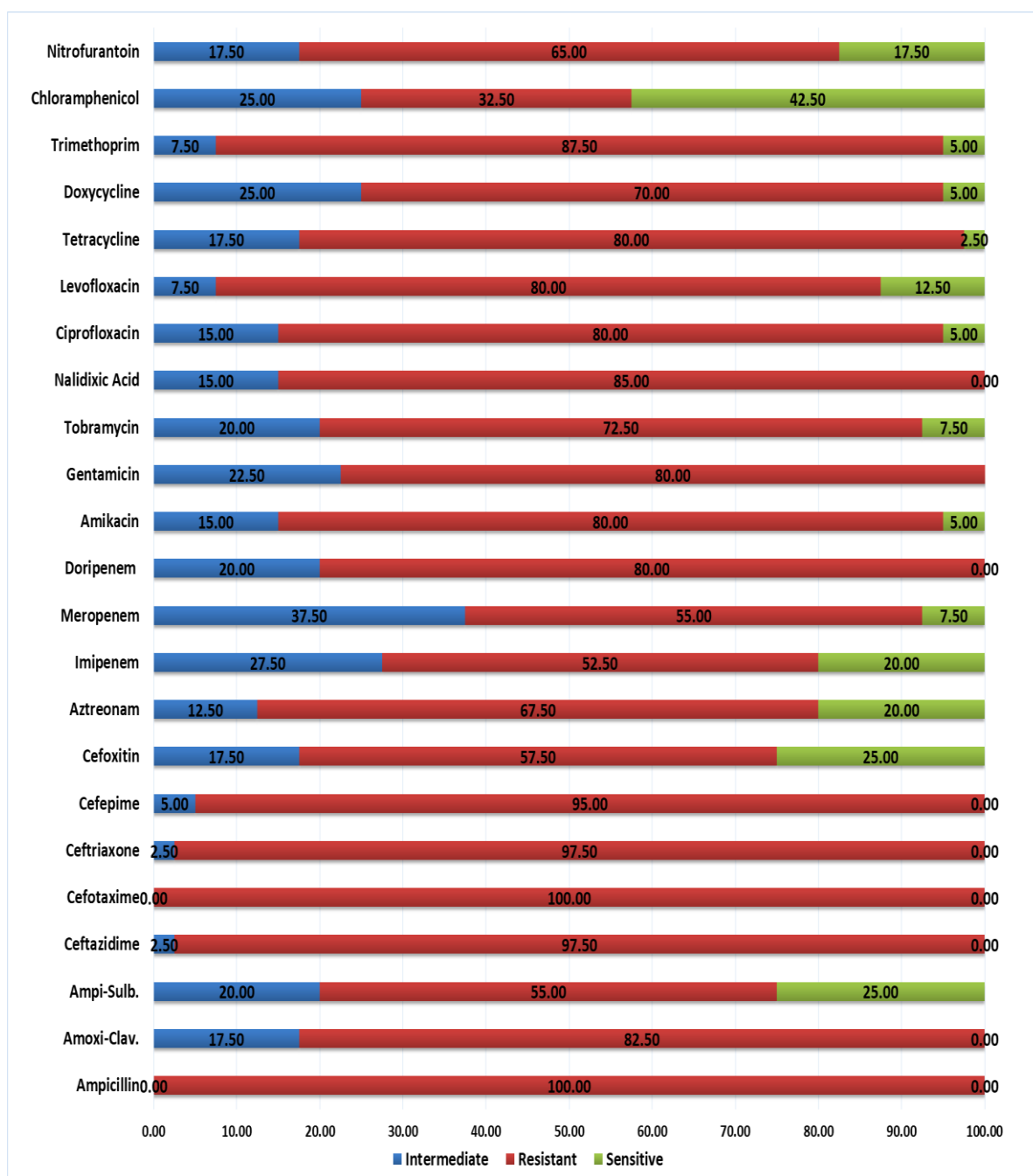


Figure (4-3): Discs Diffusion Test Results of Carbapenem Resistant *E. coli* Isolates (N= 40).

All isolates exhibited resistance to penicillin antibiotics, with a 100% resistance rate to ampicillin. Resistance to β -lactam/ β -lactamase inhibitor combinations was observed in 55.00% of the isolates for ampicillin-sulbactam and 82.50% for amoxicillin-clavulanic acid. A high percentage of isolates demonstrated resistance to third-generation cephalosporins, with 97.50% resistant to both ceftazidime and ceftriaxone. Additionally, all isolates were completely resistant to cefotaxime (100.00%). Furthermore, a significant majority of the isolates (95.00%) were resistant to the fourth-generation cephalosporin, cefepime. The overall antibiotic resistance patterns showed that 57.50% of the isolates were resistant to ceftazidime and 67.50% were resistant to the monobactam, aztreonam.

Among the carbapenem class antibiotics, resistance rates were noted as follows: 52.50% for imipenem, 55.00% for meropenem, and 80.00% for doripenem. In terms of aminoglycosides, resistance rates were 72.50% for tobramycin and 80.00% for both amikacin and gentamicin. Regarding quinolones, 85.00% of the isolates were found to be resistant to nalidixic acid, while 80.00% were resistant to both ciprofloxacin and levofloxacin. The isolates also displayed a high level of resistance (80.00%) to tetracycline and 70.00% to doxycycline. High frequencies of resistance were observed toward trimethoprim (87.50%) and nitrofurantoin (65.00%), while a moderate level of resistance to chloramphenicol was noted at 32.50%.

4.6. Multiple Antibiotic Resistance Phenotypes

According to the definition proposed by Magiorakos *et al.* (2012), multi-resistant isolates are classified as MDR, XDR, and PDR. The frequency of multi-resistance among the carbapenem resistant *E. coli* isolates recovered in this study is illustrated in Figure (4-4).

The current study found that the majority of the carbapenem resistant *E. coli* isolates, 27 in total (67.50%), exhibited XDR phenotypes, meaning they were non-susceptible to at least one agent in all but two of the eleven

antimicrobial classes tested. In contrast, 13 isolates (32.50%) demonstrated PDR phenotypes, characterized by non-susceptibility to all tested antimicrobial categories. Notably, no MDR isolates were identified, which are defined as those exhibiting resistance to at least one agent in three or more antimicrobial categories.

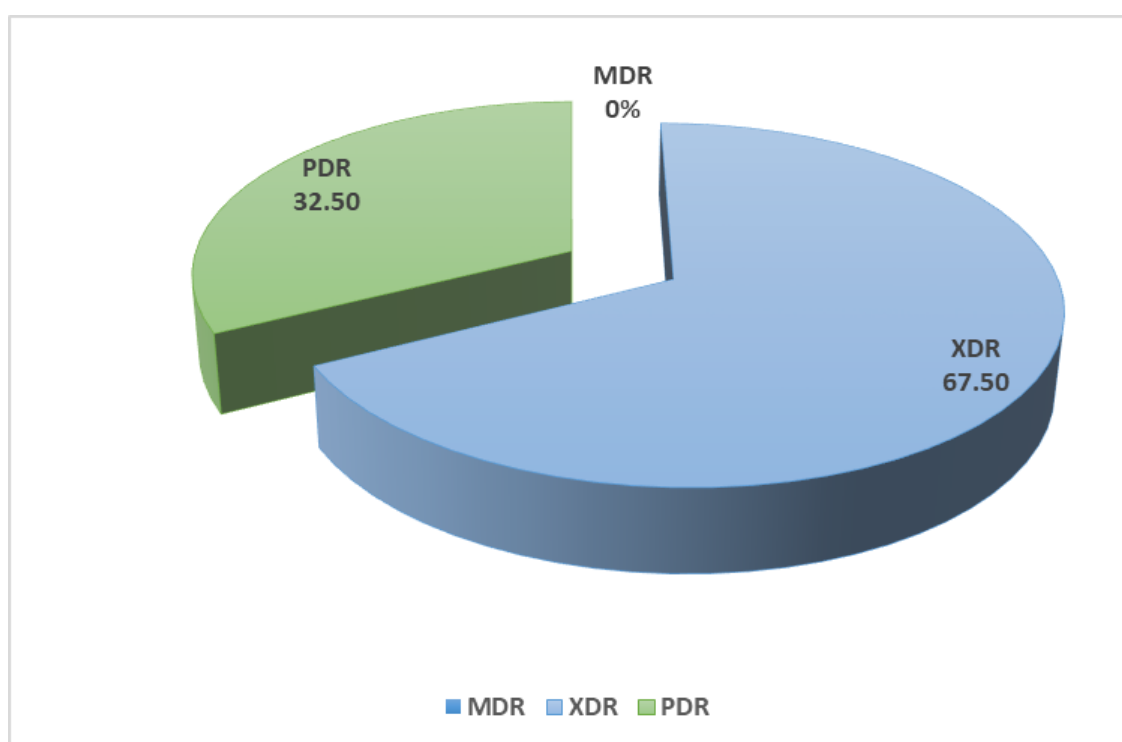


Figure (4-4): Multiple Antibiotic Resistant Phenotypes of Carbapenem Resistant *E. coli* Isolates (N=40).

4.7. Multiple Antibiotics Resistance (MAR) Index

In this study, the antibiogram was used to calculate the MAR indexes. The frequency of the MAR index is illustrated in Figure (4-5). Based on the current findings, the overall MAR index analysis shows that all carbapenem resistant *E. coli* isolates (40, 100%) had high MAR indexes, exceeding 0.2. Specifically, there were 2 isolates with a MAR index of 0.74 (EC-24 and EC-34) and another 2 isolates with a MAR index of 0.78 (EC-33 and EC-40). Additionally, 5 isolates (12.50%) displayed a MAR index of 0.83 (EC-2, EC-3, EC-5, EC-9, and EC-29) while 7 isolates (17.50%) had a MAR index of 0.87 (EC-1, EC-6, EC-13, EC-28, EC-32, EC-38, and EC-39). Furthermore,

7 isolates (17.50%) showed a MAR index of 0.91 (EC-4, EC-10, EC-11, EC-14, EC-25, EC-31, and EC-35) and 5 isolates (12.50%) had a MAR index of 0.96 (EC-7, EC-8, EC-17, EC-19, and EC-30). Notably, 12 isolates (30.00%) exhibited a MAR index of 1, indicating resistance to all 23 antibiotics tested. These isolates (EC-12, EC-15, EC-16, EC-18, EC-20, EC-21, EC-22, EC-23, EC-26, EC-27, EC-36, and EC-37).

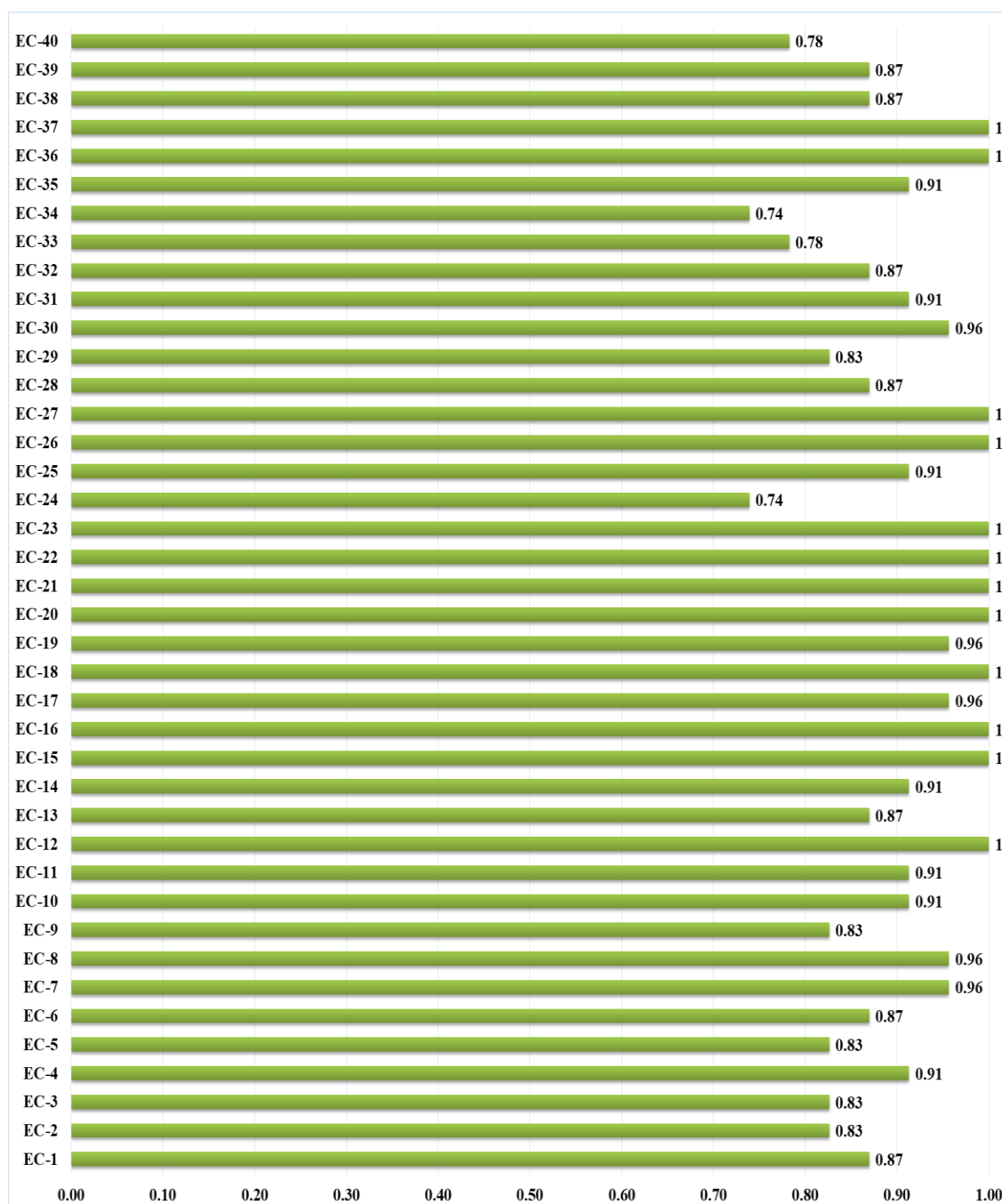


Figure (4-5): Multiple Antibiotic Resistances Indexes of Carbapenems Resistant *E. coli* Isolates (N=40).

4.8. Multiple Antibiotic Resistance Patterns

The *in-vitro* antibiotic susceptibility of all carbapenem resistant *E. coli* isolates revealed a total of 24 different antibiotic resistance patterns (Table 4-6). Analysis of the resistance patterns indicated that 2 isolates (5.00%) were resistant to both 17 and 18 antibiotics in different profiles. Additionally, 5 isolates (12.50%) were resistant to 19 antibiotics in various patterns. Furthermore, the profiles of isolates resistant to 20 and 21 antibiotics revealed 7 isolates (17.50%), exhibiting different resistance patterns. Finally, 5 isolates (12.50%) were found to be resistant to 22 antibiotics, presenting various resistance manners. Overall, the majority of these isolates (28, 70.00%) were classified as XDR. Conversely, the remaining 12 isolates (30.00%) were resistant pattern to all antibiotics tested (PDR).

Table (4-6): Antibiotic Resistance Profiles and Multi-Resistance Phenotypes of Carbapenem Resistant *E. coli* Isolates (N=40).

No. of patterns	Antibiotic-resistant pattern	No. of antibiotics (n= 23)	No. of classes (n= 11)	Type of resistance	No. (%) of isolates (n= 40)	Isolate Number
1	AM, AMC, CAZ, CTX, CRO, FEP, ATM, MRP, DOR, AK, CN, NA, CIP, LEV, TE, DO, F	17	9	XDR	2 (5.00)	EC-24
						EC-34
2	AM, AMC, AMS, CAZ, CTX, CRO, FEP, FOX, DOR, AK, CN, TOB, NA, CIP, TE, DO, TMP, F	18	10	XDR	1 (2.50)	EC-40
3	AM, AMC, AMS, CAZ, CTX, CRO, FEP, FOX, MRP, DOR, CN, TOB, NA, CIP, LEV, TE, DO, TMP	18	9	XDR	1 (2.50)	EC-33
4	AM, AMC, AMS, CAZ, CTX, CRO, FEP, IMP, DOR, AK, CN, TOB, NA, CIP, TE, DO, TMP, C, F	19	10	XDR	1 (2.50)	EC-29
5	AM, AMC, CAZ, CTX, CRO, FEP, ATM, MR, DOR, AK, CN, TOB, NA, CIP, LEV, TE, DO, TMP, C	19	10	XDR	2 (5.00)	EC-3
						EC-5
6	AM, AMC, CAZ, CTX, CRO, FEP, FOX, IMP, MRP, DOR, AK, CN, TOB, NA, CIP, TE, DO, TMP, F	19	10	XDR	1 (2.50)	EC-9
7	AM, AMC, AMS, CAZ, CTX, CRO, FEP, FOX, ATM, IMP, MRP, DOR, AK, NA, CIP, LEV, TE, DO, TMP	19	11	XDR	1 (2.50)	EC-2

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8	AM, AMC, AMS, CAZ, CTX, CRO, FEP, FOX, ATM, IMP, MRP, DOR, AK, CN, TOB, NA, LEV, TE, DO, TMP	20	11	XDR	1 (2.50)	EC-38
9	AM, AMC, AMS, CAZ, CTX, CRO, FEP, FOX, IMP, MRP, DOR, AK, CN, TOB, NA, CIP, TE, DO, TMP, C	20	10	XDR	1 (2.50)	EC-1
10	AM, AMC, AMS, CAZ, CTX, CRO, FEP, FOX, IMP, MRP, DOR, AK, CN, TOB, NA, LEV, TE, DO, TMP, F	20	10	XDR	1 (2.50)	EC-32
11	AM, AMC, CAZ, CTX, CRO, FEP, ATM, IMP, MRP, DOR, AK, CN, TOB, NA, CIP, LEV, TE, DO, TMP, F	20	10	XDR	2 (2.50)	EC-28
						EC-39
12	AM, AMC, CAZ, CTX, CRO, FEP, ATM, MRP, DOR, AK, CN, TOB, NA, CIP, LEV, TE, DO, TMP, C, F	20	11	XDR	1 (2.50)	EC-6
13	AM, AMC, CAZ, CTX, CRO, FEP, ATM, IMP, DOR, AK, CN, TOB, NA, CIP, LEV, TE, DO, TMP, C, F	20	11	XDR	1 (2.50)	EC-13
14	AM, AMC, AMS, CAZ, CTX, CRO, FEP, ATM, IMP, MRP, DOR, AK, CN, TOB, NA, CIP, LEV, TE, DO, TMP, F	21	10	XDR	1 (2.25)	EC-14
15	AM, AMC, AMS, CAZ, CTX, CRO, FEP, FOX, ATM, IMP, MRP, DOR, AK, CN, TOB, NA, CIP, LEV, DO, TMP, F	21	11	XDR	1 (2.50)	EC-11
16	AM, AMC, AMS, CAZ, CTX, CRO, FEP, FOX, ATM, IMP, MRP, DOR, AK, CN, TOB, NA, CIP, LEV, TE, DO, TMP	21	11	XDR	1 (2.50)	EC-10
17	AM, AMC, AMS, CAZ, CTX, CRO, FEP, FOX, ATM, IMP, MRP, DOR, AK, CN, TOB, NA, CIP, LEV, TE, TMP, F	21	11	XDR	1 (2.50)	EC-4
18	AM, AMC, AMS, CAZ, CTX, CRO, FEP, FOX, IMP, MRP, DOR, AK, TOB, NA, CIP, LEV, TE, DO, TMP, C, F	21	11	XDR	1 (2.50)	EC-25
19	AM, AMC, AMS, CAZ, CTX, CRO, FEP, FOX, IMP, MRP, DOR, CN, TOB, NA, CIP, LEV, TE, DO, TMP, C, F	21	11	XDR	1 (2.50)	EC-35
20	AM, AMC, CAZ, CTX, CRO, FEP, FOX, ATM, MRP, DOR, AK, CN, TOB, NA, CIP, LEV, TE, DO, TMP, C, F	21	12	XDR	1 (2.50)	EC-31
21	AM, AMC, AMS, CAZ, CTX, CRO, FEP, FOX, ATM, IMP, MRP, DOR, AK, CN, TOB, NA, CIP, LEV, TE, TMP, C, F	22	12	XDR	1 (2.50)	EC-8
22	AM, AMC, AMS, CAZ, CTX, CRO, FEP, FOX, ATM, IMP, MRP, DOR, AK, CN, TOB, NA, CIP, TE, DO, TMP, C, F	22	12	XDR	1 (2.50)	EC-17
23	AM, AMC, AMS, CAZ, CTX, CRO, FEP, FOX, ATM, IMP, MRP, DOR, AK, CN, TOB, NA, CIP, LEV, TE, DO, TMP, F	22	11	XDR	3 (7.50)	EC-7
						EC-19
						EC-30
24	AM, AMC, AMS, CAZ, CTX, CRO, FEP, FOX, ATM, IMP, MRP, DOR, AK, CN, TOB, NA, CIP, LEV, TE, DO, TMP, C, F	23	12	PDR	12 (30.00)	EC-12
						EC-15
						EC-16
						EC-18
						EC-20
						EC-21
						EC-22
						EC-23
						EC-26
						EC-27

						EC-36
						EC-37

AM: Ampicillin, AMC: Amoxicillin-Clavulanic acid, AMS: Ampicillin-Sulbactam, CAZ: Ceftazidime, CTX: Cefotaxime, CRO: Ceftriaxone, FEP: Cefepime, FOX: Cefoxitin, ATM: Aztreonam, IMP: Imipenem, MRP: Meropenem, DOR: Doripenem, AK: Amikacin, CN: Gentamicin, TOB: Tobramycin, NA: Nalidixic Acid, CIP: Ciprofloxacin, LEV: Levofloxacin, TE: Tetracycline, DO: Doxycycline, TMP: Trimethoprim, C: Chloramphenicol, and F: Nitrofurantoin.

4.9. Phenotypic Screening of B-Lactamases

4.9.1. Screening of ESBLs

Out of the 40 *E. coli* isolates tested, 100% showed resistance to at least one of the third-generation cephalosporins or aztreonam, indicating potential ESBLs production. Isolates indicating initial ESBL production underwent confirmatory testing using discs approximation methods. The confirmatory tests revealed that 10 of the isolates (25.00%) were indeed ESBLs producers (Figure 4-6, A). A statistical association was found between the initial and confirmatory ESBLs production ($P < 0.00$) (Table 4-7).

4.9.2. Screening of AmpC

The 40 *E. coli* isolates were also tested for the presence of AmpC β -lactamases. Initially, cefoxitin susceptibility was evaluated, and 28 isolates (70.00%) were resistant to cefoxitin, thereby considered possible AmpC (Plasmidic) producers. Thereafter, all isolates underwent testing with the CIAT to detect inducible (chromosomal) AmpC β -lactamases; none of the isolates tested positive for this test. A statistically significant relationship was observed between the initial and confirmatory AmpC production ($P < 0.00$) (Table 4-7).

4.9.3. Screening of Carbapenemases

All isolates demonstrating reduced susceptibility to carbapenems were further screened for carbapenemases production. Initially, DDST and CDT evaluated for the MBLs production. The result indicated that 11 isolates (27.50%) were classified as MBLs producers based on the results of both tests (Figure 4-6, B and C).

Additionally, the isolates were evaluated for carbapenemases production using the MHT, which detects class A carbapenemases or OXA-48. The results revealed that only 1 isolate (2.50%) was identified as a possible class A carbapenemase or OXA-48 producer, evidenced by the development of cloverleaf-like indentations (Figure 4-6, D). The production of carbapenemases by *E. coli* isolates was found to be statistically significant ($P < 0.00$) (Table 4-7).

A LFIA was also conducted for the rapid detection of KPC, IMP, NDM, VIM, and OXA-48 carbapenemase enzymes. The results of this assay indicated that 11 isolates (27.50%) were positive for NDM and 1 isolate (2.50%) was positive for OXA-48, while the remaining enzymes were not detected (Figure 4-6, E and F). A significant difference was observed between NDM and OXA-48 production ($P < 0.00$) (Table 4-7).

Table (4-7): Phenotypic Screening of β -Lactamase Enzymes in 40 Carbapenem Resistant *E. coli* Isolates (N=40).

Type of Test	No. of Positive Isolates (%)	Isolate No.	P-Value
Initial Screening of ESBLs Producers	40 (100.00)	All isolates	<0.00*
Confirmatory Screening of ESBLs Producers	10 (25.00)	EC-1, EC-9, EC-13, EC-14, EC-24, EC-28, EC-29, EC-34, EC-38, EC-39	
Chi-Square	X ² = 48.00, Df=1, P<0.05		
Initial Screening of AmpC (Plasmidic) Producers	28 (70.00)	EC-1, EC-2, EC-4, EC-7, EC-8, EC-9, EC-10, EC-11, EC-12, EC-14, EC-15, EC-16, EC-17, EC-18, EC-19, EC-20, EC-21, EC-22, EC-23, EC-26, EC-27, EC-30, EC-31, EC-32, EC-33, EC-36, EC-37, EC-40	<0.00*
Confirmatory Screening of AmpC (Chromosomal) producers	0 (0.00)	---	
Chi-Square	X ² = 43.08, Df=1, P≤0.05		
MBLs Producer by DDST	11 (27.50)	EC-1, EC-2, EC-3, EC-5, EC-6, EC-8, EC-10, EC-11, EC-12, EC-24, EC-34	<0.00*
MBL Producer by CDT	11 (27.50)	EC-1, EC-2, EC-3, EC-4, EC-7, EC-8, EC-10, EC-11, EC-12, EC-17, EC-18	

Class A or OXA-48 Producer by MHT		1 (2.50)	EC-27	
Chi-Square		X ² = 10.76, Df=2, P≤0.05		
LFIA	KPC	0 (0.00)	---	<0.00*
	IMP	0 (0.00)	---	
	NDM	11 (27.50)	EC-4, EC-7, EC-8, EC-10, EC-11, EC-13, EC-18, EC-19, EC-20, EC-23, EC-33	
	VIM	0 (0.00)	---	
	OXA-48	1 (2.50)	EC-27	
Chi-Square	X ² = 10.13, Df=1, P≤0.05 (NDM, OXA-48)			

* There is a significant difference.

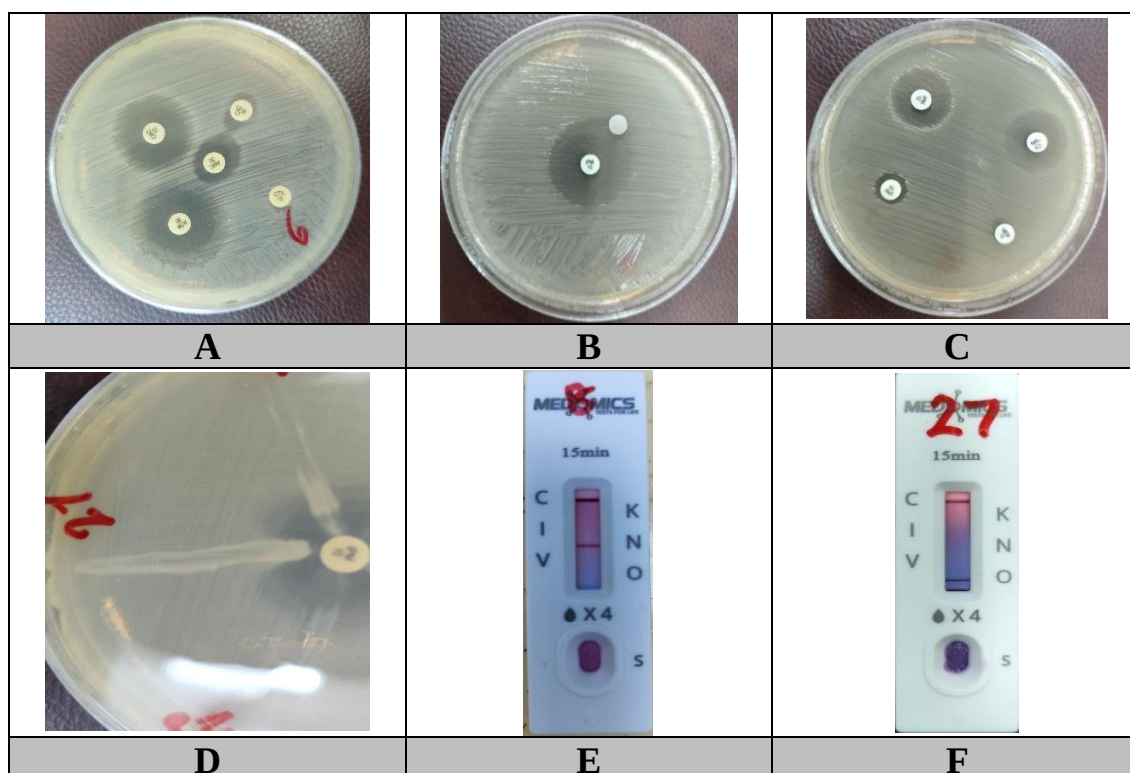


Figure (4-6): Phenotypic Screening of β -Lactamases. A: A confirmatory test for ESBLs revealed an augmentation between the central amoxicillin-clavulanic acid and aztreonam discs, confirming that the isolate produce ESBLs. B: The DDST yielded a positive result for MBLs, as the zone around the imipenem disc increased on the side closest to the EDTA disc, confirming the isolate as MBLs producer. C: The CDT also indicated a positive result for MBLs, showing a ≥ 5 mm increase in inhibition zones with imipenem-EDTA and meropenem-EDTA compared to imipenem or meropenem alone. D: The MHT was positive, as evidenced by the observation of cloverleaf-like indentation, indicating carbapenem-hydrolyzing activity. E and F: A LFIA showed positive results for NDM and OXA-48.

4.10. Genotypic Screening of Carbapenemases

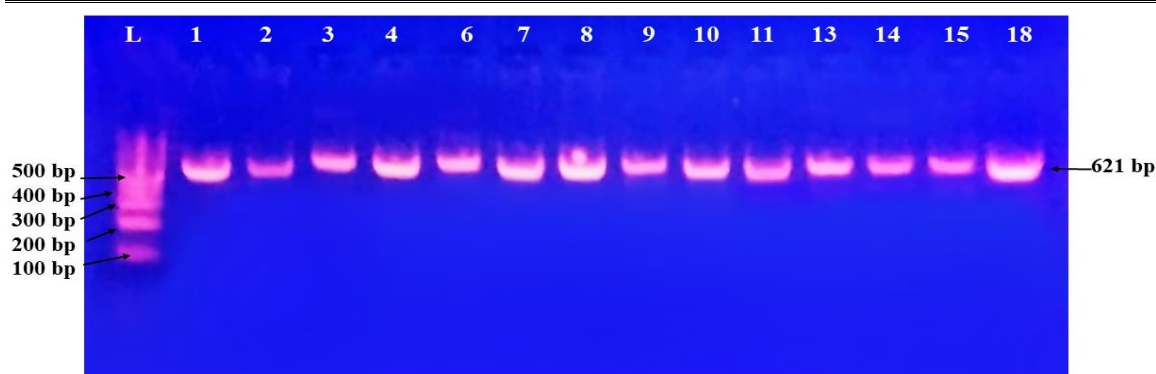
All 40 clinical isolates of carbapenem resistant *E. coli* isolates were screened for the potential gene determinants encoding carbapenemases by

PCR during this investigation. The PCR products revealed that carbapenemase genes were heterogeneously distributed among 34 isolates (85.00%), with some of these isolates harboring more than one carbapenemase gene. Meanwhile, 6 isolates (15.00%) (EC-16, EC-17, EC-31, EC-32, EC-36, and EC-38) did not carry any of the tested carbapenemase genes. Overall, the *bla*_{NDM-1} was the most predominant, identified in 24 isolates (60.00%) (Figure 4-7). This was followed by *bla*_{OXA}, found in 16 isolates (40.00%) (Figure 4-8), *bla*_{OXA-48} in 6 isolates (15.00%) (Figure 4-9), *bla*_{OXA-51} in 2 isolates (5.00%) (Figure 4-10), and *bla*_{VIM} in 2 isolates (5.00%) (Figure 4-11). These genes were either present individually in a single bacterial isolate or co-located with more than one gene in another isolate. The remaining carbapenemase genes (*bla*_{KPC}, *bla*_{NMC}, *bla*_{IMI}, *bla*_{GES}, *bla*_{SPM}, *bla*_{GIM}, and *bla*_{SIM}) were not detected in this study. The carbapenemase genes found in each positive isolate are detailed in Table (4-8).

Table (4-8): Detailed Results of the Carbapenemases and *bla*_{OXA} (ESBL) Genes in Carbapenems Resistant *E. coli* Isolates (N=40).

Type of <i>bla</i> gene	No. of Isolates (%)	Isolate No.	Antibiotic Resistance to carbapenems			β-Lactamase Phenotypic Screening		Carbapenemase Phenotypic Screening				MAR Index	Multiple Antibiotic Resistance Phenotype
			IMP	MRP	DOR	ESBL	AmpC	CDT	DDST	MHT	LFIA		
<i>bla</i> _{VIM}	2 (5.00)	5	S	IN	IN	-	-	+	-	-	-	0.83	XDR
		10	R	R	R	-	-	+	+	-	NDM	0.91	XDR
<i>bla</i> _{NDM-1}	24 (60.00)	1	IN	R	R	+	-	+	+	-	-	0.87	XDR
		2	IN	R	R	-	-	+	+	-	-	0.83	XDR
		3	S	IN	R	-	-	+	+	-	-	0.83	XDR
		4	R	R	R	-	-	-	+	-	NDM	0.91	XDR
		6	S	IN	R	-	-	+	-	-	-	0.87	XDR
		7	R	R	R	-	-	-	+	-	NDM	0.96	XDR
		8	R	R	R	-	-	+	+	-	NDM	0.96	XDR
		9	R	IN	IN	+	-	-	-	-	-	0.83	XDR
		10	R	R	R	-	-	+	+	-	NDM	0.91	XDR
		11	R	R	R	-	-	+	+	-	NDM	0.91	XDR
		13	IN	S	IN	+	-	-	-	-	NDM	0.87	XDR

		14	IN	IN	R	+	-	-	-	-	-	0.91	XDR
		15	R	R	R	-	-	-	-	-	-	1.00	PDR
		18	R	R	R	-	-	-	+	-	NDM	1.00	PDR
		19	R	R	R	-	-	-	-	-	NDM	0.96	XDR
		23	R	R	R	-	-	-	-	-	NDM	1.00	PDR
		24	S	IN	IN	+	-	+	-	-	-	0.74	XDR
		25	R	IN	R	-	-	-	-	-	-	0.91	XDR
		26	R	R	R	-	-	-	-	-	-	1	PDR
		29	IN	S	IN	+	-	-	-	-	-	0.83	XDR
		30	IN	R	R	-	-	-	-	-	-	0.96	XDR
		33	S	IN	R	+	-	-	-	-	NDM	0.78	XDR
		35	R	IN	R	-	-	-	-	-	-	0.91	XDR
		37	R	R	R	-	-	-	-	-	-	1.00	PDR
bla OXA (ESB L)	16 (40.0 0)	1	IN	R	R	+	-	+	+	-	-	0.87	XDR
		2	IN	R	R	-	-	+	+	-	-	0.83	XDR
		3	S	IN	R	-	-	+	+	-	-	0.83	XDR
		4	R	R	R	-	-	-	+	-	NDM	0.91	XDR
		5	S	IN	IN	-	-	+	-	-	-	0.83	XDR
		10	R	R	R	-	-	+	+	-	NDM	0.91	XDR
		11	R	R	R	-	-	+	+	-	NDM	0.91	XDR
		12	R	R	R	-	-	+	+	-	-	1.00	PDR
		13	IN	S	IN	+	-	-	-	-	NDM	0.87	XDR
		14	IN	IN	R	+	-	-	-	-	-	0.91	XDR
		15	R	R	R	-	-	-	-	-	-	1.00	PDR
		19	R	R	R	-	-	-	-	-	NDM	0.96	XDR
		20	R	R	R	-	-	-	-	-	NDM	1.00	PDR
		33	S	IN	R	+	-	-	-	-	NDM	0.78	XDR
		34	S	IN	IN	+	-	+	-	-	-	0.74	XDR
		39	IN	IN	R	+	-	-	-	-	-	0.87	XDR
bla OXA-48	6 (15.0 0)	12	R	R	R	-	-	+	+	-	-	1.00	PDR
		20	R	R	R	-	-	-	-	-	NDM	1.00	PDR
		21	R	IN	R	-	-	-	-	-	-	1.00	PDR
		22	IN	R	R	-	-	-	-	-	-	1.00	PDR
		27	R	R	R	-	-	-	-	+	OXA-48	1.00	PDR
		40	S	S	IN	-	-	-	-	-	-	0.78	XDR
bla OXA-51	2 (5.00)	1	IN	R	R	+	-	+	+	-	-	0.87	XDR
		28	IN	R	R	+	-	-	-	-	-	0.87	XDR



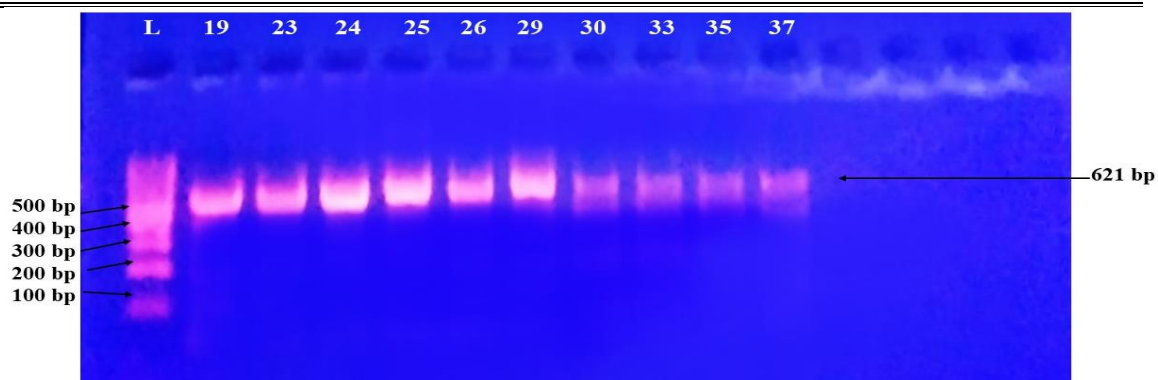


Figure (4-7): Electrophoresis of the PCR Products of *bla*_{NDM-1} (621 bp) from *E. coli* Isolates. Line (L): DNA Ladder (100 bp). Lines (1-37): Positive Results of The *bla*_{NDM-1}.

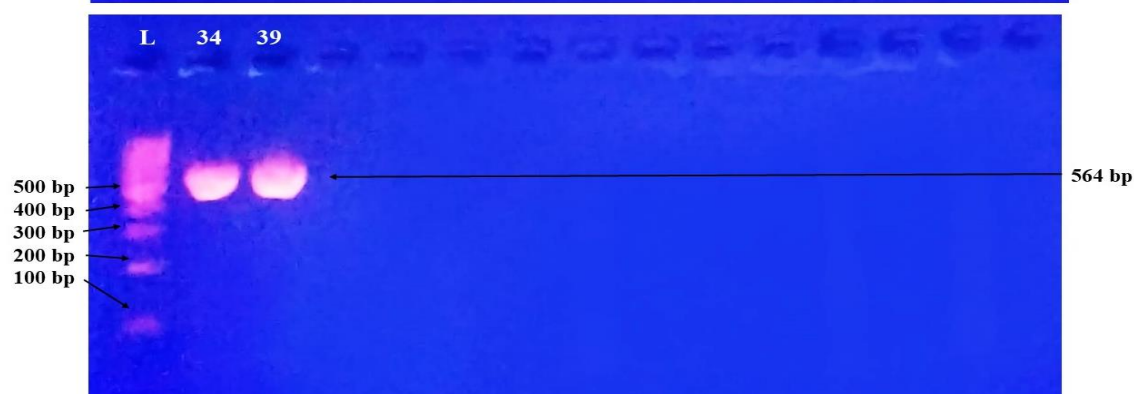
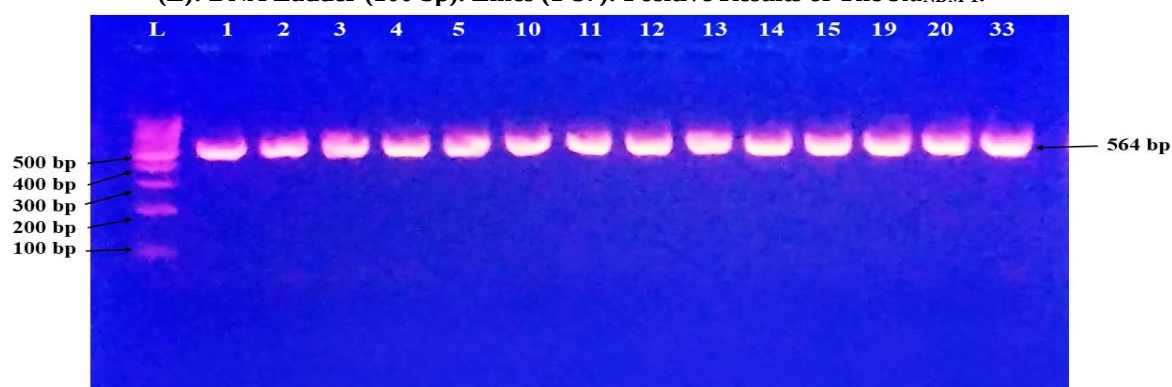


Figure 4-8: Electrophoresis of the PCR Products of *bla*_{OXA} (ESBL) (564 bp) from *E. coli* Isolates. Line (L): DNA Ladder (100 bp). Lines (1-39): Positive Results of the *bla*_{OXA}.

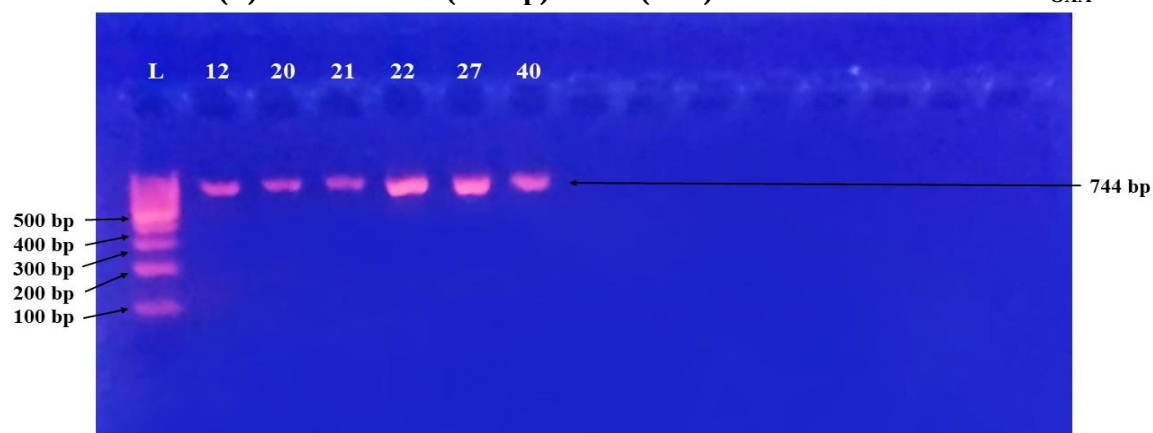


Figure 4-9: Electrophoresis of the PCR Products of *bla*_{OXA-48} (744 bp) from *E. coli* Isolates. Line (L): DNA Ladder (100 bp). Lines (12-40): Positive Results of The *bla*_{OXA-48}.



Figure 4-10: Electrophoresis of the PCR Products of *bla*_{OXA-51} (353 bp) from *E. coli* Isolates. Line (L): DNA Ladder (100 bp). Lines (5-10): Positive Results of the *bla*_{OXA-51}.

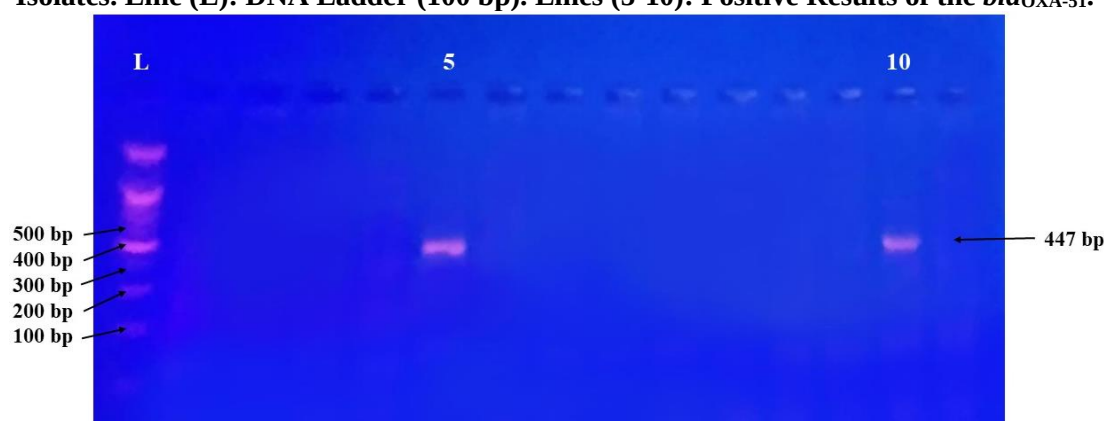


Figure 4-11: Electrophoresis of the PCR Products of *bla*_{VIM} (447 bp) from *E. coli* Isolates. Line (L): DNA Ladder (100 bp). Lines (5-13): Positive Results of the *bla*_{VIM}.

Out of 34 bacterial isolates that contained carbapenemase genes, 18 (52.94%) carried more than one carbapenemase gene (Table 4-9). A combination of two genes, *bla*_{VIM} and *bla*_{NDM-1}, was identified in 1 isolate (5.56%). The co-occurrence of *bla*_{VIM} with *bla*_{OXA} genes was observed in 2 isolates (11.11%). Notably, 11 isolates (61.11%) that tested positive for *bla*_{NDM-1} also carried the *bla*_{OXA}. Additionally, a combination of *bla*_{NDM-1} and *bla*_{OXA-51} was found in 1 isolate (5.56%). The pairing of *bla*_{OXA} with *bla*_{OXA-48} and *bla*_{OXA-51} was present in 2 (11.11%) and 1 (5.56%) isolates, respectively. The individual genes were found alone in the following isolates: *bla*_{NDM-1} in 13 (38.24%), *bla*_{OXA} in 2 (5.88%), *bla*_{OXA-48} in 4 (11.76%), and *bla*_{OXA-51} in 1 (2.94%).

Table (4-9): Co-Production Patterns of Carbapenemase and ESBL *bla*_{OXA} Genes in Carbapenem Resistant *E. coli* Isolates (N=18).

Pattern of combination	No. (%) of isolates	Isolate No.
<i>bla</i> _{VIM} and <i>bla</i> _{NDM-1}	1 (5.56)	EC-10
<i>bla</i> _{VIM} and <i>bla</i> _{OXA}	2 (11.11)	EC-5, EC-10
<i>bla</i> _{NDM-1} and <i>bla</i> _{OXA}	11 (61.11)	EC-1, EC-2, EC-3, EC-4, EC-10, EC-11, EC-13, EC-14, EC-15, EC-19, EC-33
<i>bla</i> _{NDM-1} and <i>bla</i> _{OXA-51}	1 (5.56)	EC-1
<i>bla</i> _{OXA} and <i>bla</i> _{OXA-48}	2 (11.11)	EC-12, EC-20
<i>bla</i> _{OXA} and <i>bla</i> _{OXA-51}	1 (5.56)	EC-1

4.11. Demographic Distributions of Carbapenemase Genes Carrying Isolates

An analysis of the various risk factors associated with carbapenemase production in *E. coli* revealed that out of 50 carbapenemase genes, 34 (68.00%) were associated with urine specimens, 7 (14.00%) with body fluids, 6 (12.00%) with sputum, 2 (4.00%) with swabs, and 1 (2.00%) from CSF. Additionally, carbapenemase gene production was more frequent among inpatients, with 36 isolates (72.00%), compared to 14 isolates (28.00%) from outpatients. When analyzing the gender distribution of the carbapenemase genes, it was found that 27 isolates (54.00%) were recovered from male patients, while 23 isolates (46.00%) came from female patients. The mean age of the patients was 55.08 ± 23.82 years, with a median of 54 years and an age range of 9 to 95 years. Most of the patients (32, 64.00%) were hospitalized at Al-Hussein Teaching Hospital, while 18 patients (36.00%) were treated at Al-Nasiriyah Teaching Hospital (Table 4-10).

Table 4-10: Demographic Distributions and Genotypic Screening of Carbapenemases Genes Carrying Isolates.

Parameters		Carbapenemase gene (%)					Total (%)
		<i>bla</i> _{NDM-1}	<i>bla</i> _{OXA}	<i>bla</i> _{OXA-48}	<i>bla</i> _{OXA-51}	<i>bla</i> _{VIM}	
Age (Mean $\pm$ S.D)		55.54 $\pm$ 23.94	52 $\pm$ 26.84	51.33 $\pm$ 18.01	63 $\pm$ 14.14	77.5 $\pm$ 24.75	55.08 $\pm$ 23.82
Gender	Male	15 (62.50)	9 (56.25)	2 (33.33)	0 (0.00)	1 (50.00)	27 (54.00)
	Female	9 (37.50)	7 (43.75)	4 (66.67)	2 (100.00)	1 (50.00)	23 (46.00)

Specimen	Body fluids	2 (8.33)	2 (12.50)	2 (33.33)	0 (0.00)	1 (50.00)	7 (14.00)
	CSF	0 (0.00)	1 (6.25)	0 (0.00)	0 (0.00)	0 (0.00)	1 (2.00)
	Sputum	4 (16.67)	1 (6.25)	1 (16.67)	0 (0.00)	0 (0.00)	6 (12.00)
	Swabs	1 (4.17)	1 (6.25)	0 (0.00)	0 (0.00)	0 (0.00)	2 (4.00)
	Urine	17 (70.83)	11 (68.75)	3 (50.00)	2 (100.00)	1 (50.00)	34 (68.00)
Hospital	HTH	15 (62.50)	13 (81.25)	1 (16.67)	1 (50.00)	2 (100.00)	32 (64.00)
	NTH	9 (37.50)	3 (18.75)	5 (83.33)	1 (50.00)	0 (0.00)	18 (36.00)
Hospitalization	Inpatients	18 (75.00)	12 (75.00)	2 (33.33)	2 (100.00)	2 (100.00)	36 (72.00)
	Outpatient	6 (25.00)	4 (25.00)	4 (66.67)	0 (0.00)	0 (0.00)	14 (28.00)

4.12. Association between Phenotypic and Genotypic Screening of Carbapenemase Genes

Out of 11 isolates that tested positive for DDST, results indicated that 10 (90.91%) of them carried MBL genes. This included 1 (9.09%) isolate with the *bla_{VIM}* gene and 9 (81.82%) isolates with the *bla_{NDM-1}* genes. Whereas, 16 (61.54%) of the isolates with positive MBL genes, confirmed by PCR, were not detected phenotypically. From the CDT, which produced 11 positive results, PCR analysis revealed that 10 (81.82%) of the carbapenem resistant *E. coli* isolates possessed MBL genes, specifically 2 (18.18%) with *bla_{VIM}* and 8 (72.73%) with *bla_{NDM-1}*. However, 1 (9.09%) isolate that tested positive in the CDT did not have detectable MBL genes via PCR. Furthermore, results from the MHT showed that only one isolate tested positive, which was confirmed by PCR to contain the *bla_{OXA-48}* gene (EC-27). Notably, 5 (83.33%) isolates had a positive result for the *bla_{OXA-48}* gene but were not detected phenotypically by this method. In the LFIA, among the 11 NDM-positive isolates, 10 (90.91%) were confirmed to have the *bla_{NDM-1}* genes through PCR. Additionally, one isolate yielded a positive result for the OXA-48 enzyme, which was also confirmed to have the *bla_{OXA-48}* gene by PCR (EC-27). In contrast, 5 (83.33%) additional isolates that

tested positive by PCR were not detected phenotypically by this assay. Regarding the ESBL *bla*_{OXA}, 7 isolates (63.64%) and 8 isolates (72.73%) showed positive phenotypic results through the DDST and the CDT, respectively. In contrast, 1 isolate (9.09%) with the *bla*_{OXA-51} gene demonstrated positive phenotypic results via both the DDST and CDT. However, the MHT did not detect the presence of either the *bla*_{OXA} or *bla*_{OXA-51} genes.

Among the ESBL-producing *E. coli* isolates, 7 (70.00%) of these isolates carried the *bla*_{NDM-1}, while 6 (60.00%) contained the *bla*_{OXA}. All isolates with the *bla*_{OXA-51} gene demonstrated phenotypic ESBLs production. Notably, isolates that produced the *bla*_{VIM} or *bla*_{OXA-48} genes did not exhibit ESBLs production. It is important to mention that because inducible AmpC productions cannot be observed phenotypically, none of the carbapenemase-producing *E. coli* isolates were found to produce AmpC.

Table (4-11): Phenotypic and Genotypic Screening of Carbapenemase Genes.

Phenotypic Method		No. of Positive Isolates (%)	No. of Carbapenemase Gene (%)				
			<i>bla</i> _{NDM-1}	<i>bla</i> _{VIM}	<i>bla</i> _{OXA}	<i>bla</i> _{OXA-48}	<i>bla</i> _{OXA-51}
DDST		11 (27.50)	9 (81.82)	1 (9.09)	7 (63.64)	1 (9.09)	1 (9.09)
CDT		11 (27.50)	8 (72.73)	2 (18.18)	8 (72.73)	1 (9.09)	1 (9.09)
MHT		1 (2.50)	0 (0.00)	0 (0.00)	0 (0.00)	1 (100.00)	0 (0.00)
LFIA	NDM	11 (27.50)	10 (90.91)	1 (9.09)	7 (63.64)	1 (9.09)	0 (0.00)
	OXA-48	1 (2.50)	0 (0.00)	0 (0.00)	0 (0.00)	1 (100.00)	0 (0.00)
ESBLs		10 (25.00)	7 (70.00)	0 (0.00)	6 (60.00)	0 (0.00)	2 (20.00)
Inducible AmpC		0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)

4.13. Frequency of Class 1 and Class 2 Integrons

Class 1 and class 2 integrons in the carbapenem resistant *E. coli* isolates were screened by PCR. The present study revealed that class 1 integrons was common among them; all isolates 40 (100.00%) gave a positive result for the *intI1* gene (Figure 4-12), whereas class 2 integron was found in 13 (32.50%) of the isolates tested (Figure 4-13).

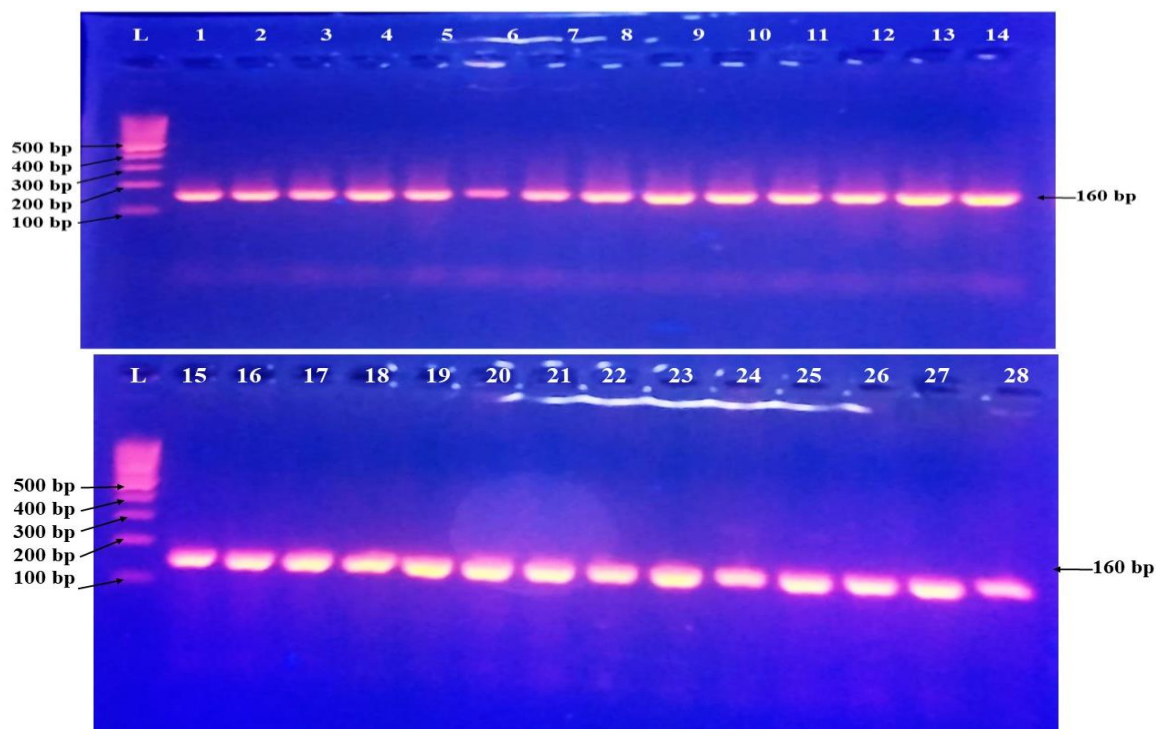


Figure (4-12): Electrophoresis of the PCR Products of *IntI1* (160 bp) from *E. coli* Isolates. Line (L): DNA Ladder (100 bp). Lines (1-28): Positive Results of *IntI1*.

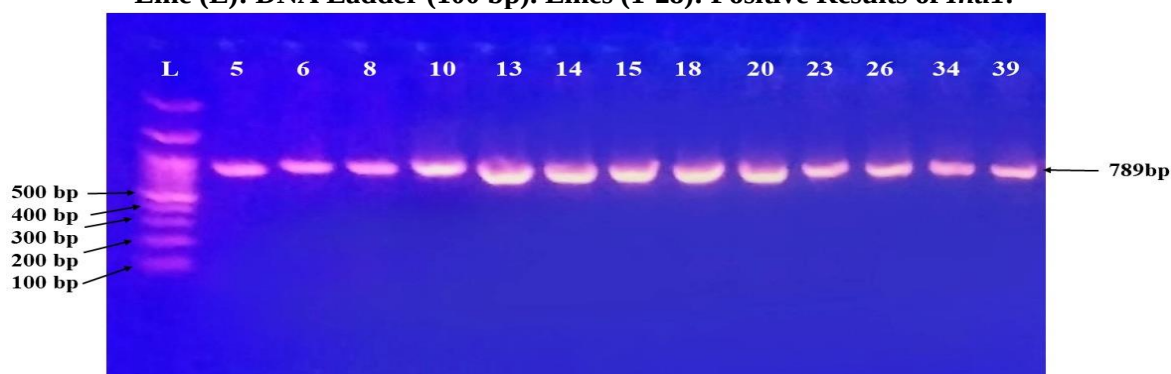


Figure (4-13): Electrophoresis of the PCR Products of *IntI2* (789 bp) from *E. coli* Isolates. Line (L): DNA Ladder (100 bp). Lines (5-39): Positive Results of *IntI2*.

The genes *bla*_{NDM-1}, *bla*_{OXA}, *bla*_{OXA-48}, *bla*_{OXA-51}, and *bla*_{VIM} were all detected in combination with integron class 1. In contrast, the occurrence of

these genes among isolates with class 2 integrons was as follows: 9 (37.50%) carried *bla*_{NDM-1}, 2 (100.00%) carried *bla*_{VIM}, 7 (43.75%) carried *bla*_{OXA}, 2 (33.33%) carried *bla*_{OXA-48}, and 0 (0.00%) carried *bla*_{OXA-51} (Table 4-12). Furthermore, the distribution of integrons in combination with multiple resistance phenotypes showed that all carbapenem resistant *E. coli* isolates positive for integron class 1 exhibited a rate of 70.00% for XDR and 30.00% for PDR. In comparison, isolates positive for integron class 2 showed XDR and PDR rates of 28.57% and 41.66%, respectively.

Table (4-12): Relationship Between Integrons, Carbapenemase Genes, and Multiple Resistance Patterns.

Integron Class	No. of Positive Isolates (%)	No. of Carbapenemase Gene (%)					Multiple Antibiotic Resistance Patterns	
		<i>bla</i> _{NDM-1}	<i>bla</i> _{VIM}	<i>bla</i> _{OXA}	<i>bla</i> _{OXA-48}	<i>bla</i> _{OXA-51}	XDR	PDR
Class 1	40 (100.00)	24 (100.00)	2 (100.00)	16 (100.00)	6 (100.00)	2 (100.00)	28 (70.00)	12 (30.00)
Class 2	13 (32.50)	9 (37.50)	2 (100.00)	7 (43.75)	2 (33.33)	0 (0.00)	8 (28.57)	5 (41.66)

4.14. Sequencing of *bla*_{NDM-1} and *bla*_{OXA} genes

The PCR products of *bla*_{NDM-1} and *bla*_{OXA} positive isolates were sequenced. The sequenced genes were submitted to the DNA Data Bank of Japan (DDBJ). The results of sequencing revealed that out of 22 *bla*_{NDM-1} genes of *E. coli* isolates were found to be identical to the *bla*_{NDM-1} variant and assigned accession numbers from LC856650 to LC856671, as shown in Figure (4-12).

The phylogenetic tree of *bla*_{NDM-1} gene isolates shows that most local isolates cluster together, indicating high genetic similarity and possible common source or horizontal gene transfer. Some isolates such as LC856659 and LC856661 appear on separate branches, suggesting minor genetic variations.

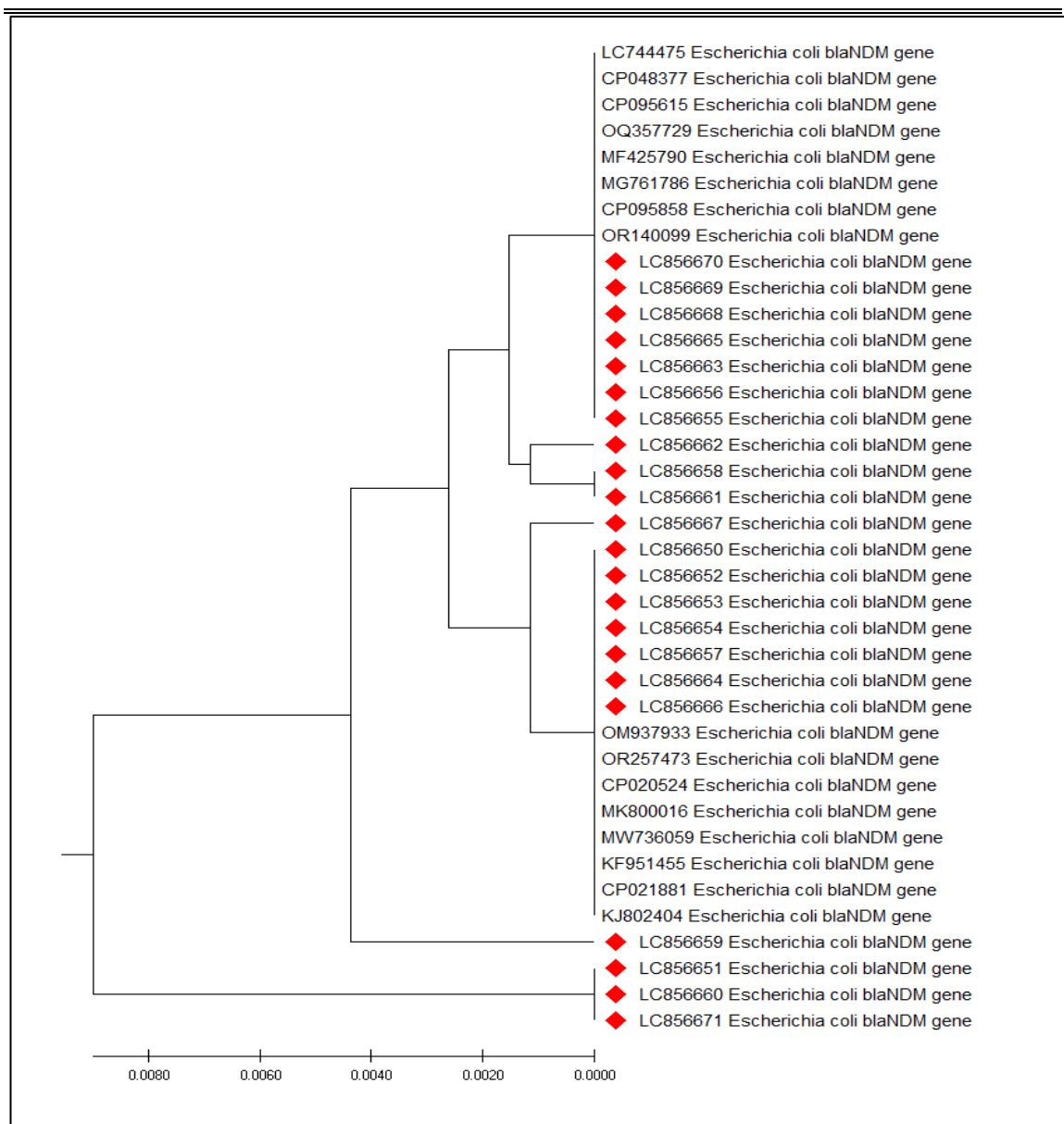


Figure (4-12): Phylogenetic Tree for *bla*_{NDM-1} Genes of *E. coli* Constructed by Maximum Likelihood Method with Nucleotide Sequences of 16 Reference Isolates from Gene Bank.

Current Isolates are indicated with Red Triangle.

The sequencing results of the 11 isolates harboring ESBLs *bla*_{OXA} gene revealed identity to *bla*_{OXA-1} variant, and assigned accession numbers from LC856639 to LC856649 and Figure (4-13).

The phylogenetic tree of *bla*_{OXA-1} gene isolates demonstrates that most local isolates form a tight cluster, indicating local gene stability and likely plasmid-mediated spread. The clear separation from global sequences also suggests unique local molecular characteristics.

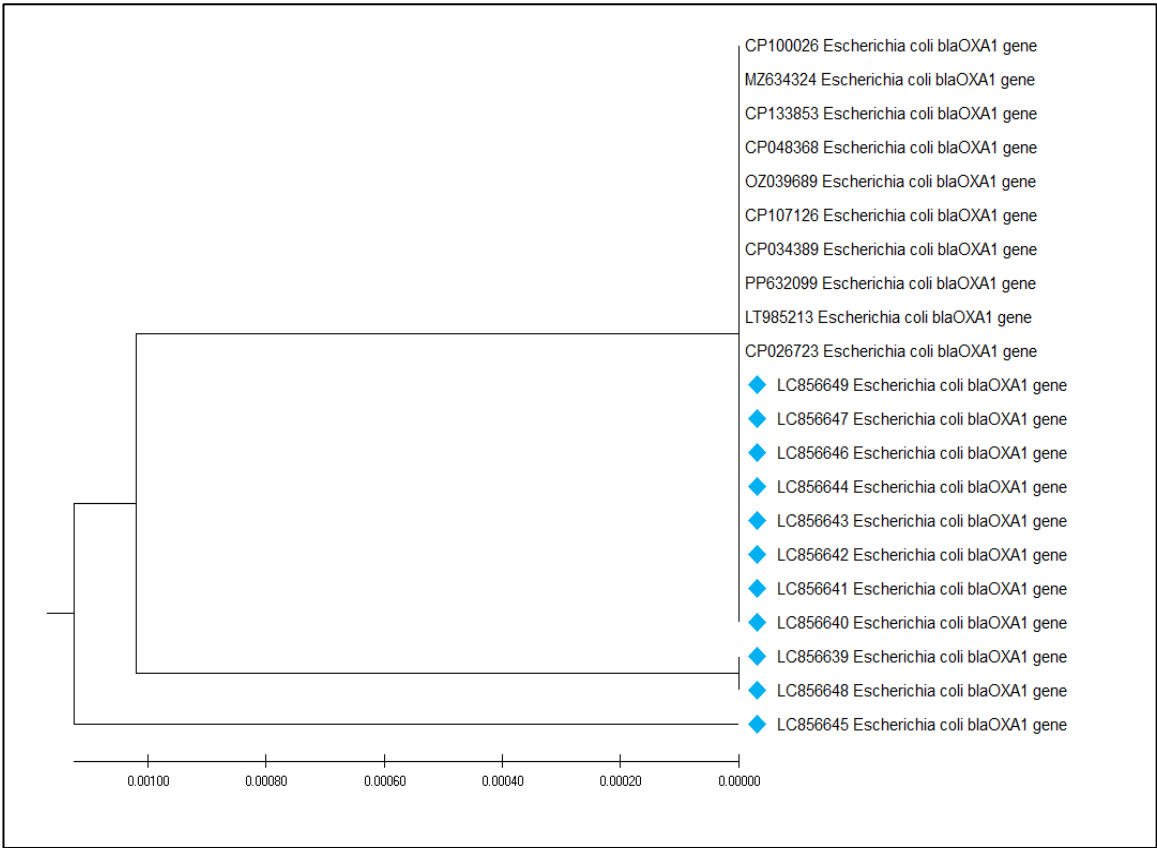


Figure (4-13): Phylogenetic Tree for *bla*_{OXA-1} Genes of *E. coli* Isolates Constructed by Maximum Likelihood Method with Nucleotide Sequences of 10 Reference Isolates from Gene Bank. Current Isolates are indicated with Blue Rhombus.

Chapter Five

DISCUSSION

5. Discussion

5.1. Finding of Clinical *E. coli* Isolates

Escherichia coli is a common commensal bacterium found in the gastrointestinal tract of both children and adults. It has frequently been utilized in studies examining the incidence of antibiotic resistance among commensal bacteria (Oluyeye *et al.*, 2015). In recent years, it has become widely recognized that the normal flora plays a significant role in the emergence and spread of antimicrobial resistance in pathogens (Pokharel *et al.*, 2023). This urgent issue requires action to prevent new resistant strains and control their spread among humans and animals (Rosa *et al.*, 2014).

While studies on carbapenems resistant bacteria exist in Iraq, research on carbapenems resistant *E. coli* isolates in Thi-Qar province is limited. This study, the first of its kind in the region, aims to evaluate the frequency, susceptibility, phenotypic, and molecular characteristics of these isolates in the region. During the surveillance period, of 2203 specimens, 908 (41.22%) were collected from Al-Hussein Teaching Hospital, while 1295 (58.78%) were sourced from Al-Nasiriyah Teaching Hospital (Table 4-1). The difference in the number of specimens collected from the two hospitals is attributed to Al-Nasiriyah Teaching Hospital, a newly established facility located centrally in Thi-Qar province, which offers a wider range of medical services. In contrast, Al-Hussein Teaching Hospital is an older institution that does not provide all medical services.

Among these specimens, 1212 (55.02%) were positive for bacterial growth, and 991 (44.98%) were negative. The percentage of positive bacterial infections is fairly consistent with a previous study conducted by Fadhil and Hadi (2024) in Babylon province, which found that out of 315 specimens, 210 (67%) exhibited positive bacterial growth, while 105 (33%) were negative. The results from this study indicate a higher incidence of

positive bacterial growth compared to findings in Karbala, where Mohammed *et al.* (2014) reported that, out of 1637 cultured specimens, only 317 specimens (19.36%) displayed positive bacterial growth. In contrast, a study in Misan City reported a much higher rate, with 87.5% (175 out of 200) of specimens showing positive bacterial cultures, while 12.5% (25 out of 200) exhibited no growth (Al-Lami and Al-Maliky, 2022). This ratio of no growth results from those patients who probably were suffering from a viral infection, immunological disorder, or other conditions.

In this study, the VITEK-2 system was utilized to verify the identification of *E. coli* at the species level and to reduce variability in biochemical test results. The results showed a prevalence rate of *E. coli* in Thi-Qar hospitals at 186, making up 15.35% of the total, as illustrated in Figure 4-2, making it the second most commonly cultured bacteria compared to other bacterial genera. A prior study in Thi-Qar by Abd Alameer *et al.* (2015) reported 90 *E. coli* isolates from 318 clinical specimens, resulting in a prevalence of 28.33%. Al-Sadawy and Hussein (2016) found that *E. coli* comprised 24.62% of bacteria isolated from Al-Hussain Teaching Hospital. Similarly, Jabur and Abed (2020) found *E. coli* in 100 out of 430 specimens from Thi-Qar Hospital. Various regions in Iraq have reported differing rates of *E. coli*. In Kirkuk, Fakhraddin and Ali (2022) recorded *E. coli* at 35.6%. Mahdi *et al.* (2020) in Karbala also reported *E. coli* (23.9%) among other bacteria. In Baghdad, Salman *et al.* (2022) found that 56% of isolates were *E. coli*, while in Basrah, Jasim *et al.* (2022) reported a similar prevalence. Various global studies have reported similar findings. In India, *E. coli* was identified in 20.47% of the 1050 specimens tested (Navdeep *et al.*, 2023). In a study conducted in Singapore, *E. coli* was found in 48.9% of 387 cases of Enterobacteriaceae (Tan *et al.*, 2009). Additionally, an analysis from the United Arab Emirates (UAE) revealed a higher prevalence, with *E. coli* present in 63.8% of the cases (Al-Zarouni *et al.*, 2008). Furthermore, in Iran,

Hassanzadeh *et al.*, (2009) found that the most prevalent isolates were *Pseudomonas* spp. (39.2%) and *E. coli* (23.8%). In Syria found that the leading cause of UTIs in children (63%) was *E. coli* (Abbood *et al.*, 2022). The high prevalence of *E. coli* infections in this study is partially due to its status as a normal component of bowel flora, facilitating easy transmission, especially with poor personal hygiene.

The present study found that the primary source of *E. coli* isolation was urine (63.98%), followed by sputum (19.35%), body fluids (7.53%), swabs (4.30%), blood and wound secretions (2.15% each), and CSF (0.54%) (Table 4-1). Many studies have been documented a high prevalence of *E. coli* in various clinical specimens. For instance, a study by Abd Alameer *et al.* (2015) conducted in Thi-Qar hospitals found that out of 90 *E. coli* isolates, 1 out of 42 (2.38%) came from burns, 7 out of 25 (28%) from high vaginal swabs, 11 out of 41 (26.82%) from wound infections, 18 out of 33 (54.54%) from diarrheal infections, and 38 out of 67 (56.71%) from UTIs. Another study in Thi-Qar by Abbas (2015) revealed that, out of 105 specimens collected from patients, only 22 specimens demonstrated growth of *E. coli*: 10 (58.8%) from urine, 2 (11.7%) from wounds, 5 (29.4%) from stool, and 5 (22.7%) from vaginal swabs. The results published by Abdul-Husin, (2021) in the Karbala province include that 72% of all *E. coli* isolates were found to be the cause of UTIs, followed by wound swabs 24%. Another study in Karbala, the isolation rate of *E. coli* from urine was high, with 62/121 positive cases (51.2%), while the rate was lower for blood-positive cases (43/196, 15.1%) (Mohammed *et al.*, 2014). In Duhok province, the majority of *E. coli* isolates were obtained from urine specimens (418/454, 92.2%), followed by wounds (18, 3.9%), cervical specimens (7, 1.5%), blood (4, 0.9%), semen (3, 0.7%), ascitic fluid (2, 0.4%), and CSF (2, 0.4%) (Naqid *et al.*, 2020). Furthermore, *E. coli* is widely acknowledged as the leading cause of infections globally. This is supported by a study conducted by Xu *et al.*

(2023), which found that the majority of *E. coli* isolates came from urine specimens (67.9%). Other specimen types included sputum (11.0%), pus (7.2%), blood (5.7%), and wound secretions (3.0%). In India Navdeep *et al.* (2023) revealed that most of *E. coli* isolates were identified from urine 116 (53.95) followed by blood 32 (14.88%), sputum 21 (9.76), pus 17 (7.90%), pleural fluid 11 (5.11%), CSF 8 (3.72), ascitic fluid and synovial fluid 4 (1.86%, for each), and BAL fluid 2 (0.93%). Body fluid infections are mostly caused by *E. coli* (23.86%), as reported by Rouf and Nazir (2019). El-Baz *et al.* (2022) in Egypt found that 28.7% of all *E. coli* isolates obtained during their study were swabbed from wounds, throat, and vagina. The differences in specimen types reflect how infections develop based on the anatomical area, with urine specimens being the most likely to yield positive *E. coli* findings due to the ease of developing UTIs as opposed to infections in other sites like the CSF specimens.

The occurrence of clinical isolates of *E. coli* showed a slight increase in females (53.23%) compared to males (46.77%) (Table 4-2). This finding aligns with other studies conducted in various regions of Iraq. A previous three-year survey in Baghdad reported that *E. coli* was insignificantly higher in females (196/918, 21.4%) than in males (20/108, 18.5%) during the years 2017-2018 (Assafi *et al.* 2022). In Duhok province, Naqid *et al.* (2020) found that *E. coli* isolates were more prevalent in females (70.7%) than in males (29.3%). In contrast, a survey in Babylon revealed that 85% of females had *E. coli* compared to 15% of males (Fadhil and Hadi, 2024). These findings contrast slightly with those reported by Jabur and Abed (2020), who found that *E. coli* isolates in Thi-Qar hospitals were more common among males (53.00%) than females (47.00%). Another study in Thi-Qar indicated a slightly higher incidence in males (53.84%) than in females (46.16%) among patients infected with *E. coli* (Al-Sadawy and Hussein, 2016). Al-Benwan and Jamal (2023) also found that females were the predominant hospital

visitors in Kuwait, in which 1020 (76%) and 322 (24%) of *E. coli* isolates were obtained from females and males, respectively. The higher prevalence of this microorganism in females may be attributed to the shorter urethra in females, which reduces the distance bacteria must travel to reach the bladder. Additionally, sexual activities can increase the likelihood of bacterial inoculation into the bladder (Magliano *et al.*, 2012; Czajkowski *et al.*, 2021).

The patients' ages were categorized into five groups. The highest incidence of *E. coli* infection was observed in the group of patients above 61 years old, with 64 cases (34.41%) (Table 4-2). Various studies have reported different prevalence rates of *E. coli* infection among age groups. Similarly, Navdeep *et al.* (2023) reported that the age distribution 61-70 years age group was dominating with 24.65% followed by the 31-40 years with 15.34% and then 41-50 years with 14.88% which was having *E. coli* infection. This finding consistent with a study in Erbil city by Ahmed and Ganjo (2019) who found that maximum number of isolates were found in 61-80 years of age group. Abdul-Husin (2021) found that in Karbala province, the highest incidence (33.33%) occurred in the 31-45 year age group. Assafi *et al.* (2022) reported that the highest rate of *E. coli* infection was found in individuals aged 20 to 39 years (98 patients, 47%), while the incidence significantly decreased among those aged 40 years and older, reaching its lowest in elderly patients aged 70-74 years (2 cases, 1%). In Iran, research conducted by Tajbakhsh *et al.* (2015) indicated that the 30-39-year age group had the highest infection rate at 54.78%. Nagoba *et al.* (2008) revealed that the maximum number of *E. coli* isolates were found in 41-50 (79.31%) year's age group. Amin *et al.* (2009) shown that the dominating age group was 21-40 years age group. Several factors may explain the higher infection rates in older adults, who are particularly vulnerable to *E. coli* infections due to a declining immune response and increased chronic health conditions. The next highest incidence was in the 21-40 age group, likely

influenced by lifestyle factors. The 41-60 age group showed a decrease in incidence compared to younger adults, reflecting low health risks. The 1-20 age group had 27 cases (14.52%), likely due to enhanced hygiene practices. Lastly, infants under 1 year had the lowest incidence (5, 2.69%), possibly could not be accurately evaluated due to insufficient specimen sizes.

The current study found that *E. coli* is a major contributor to hospital-acquired infections, with 101 cases (54.30%) involving inpatients and 85 cases (45.70%) involving outpatients. A survey in teaching hospitals in Nepal reported a 34.4% prevalence of bacteria causing nosocomial infections (Sah *et al.*, 2014). Additionally, a study by Shrestha *et al.* (2018) showed that *E. coli* represented 28.30% of cases in critical care units. *E. coli* is a common cause of severe infections in hospitalized patients, particularly in low and middle-income countries where healthcare resources are limited (Livermore, 2012; Braz *et al.*, 2020). Hospitalization and *E. coli* infections can also facilitate bacterial transmission between patients, leading to increased healthcare costs due to extensive treatments and extended stays (Shahida *et al.*, 2016).

5.2. Antimicrobial Susceptibilities of the *E. coli* Isolates

The findings revealed a variables susceptibility rates to the antimicrobial used (Table 4-3). Notably, all *E. coli* isolates demonstrated significant ampicillin resistance, consistent with previous studies conducted in Thi-Qar by Farhood *et al.* (2024) and in Karbala by Taqi (2023), in Al-Diwaniy by Al-Mayahi (2013) as well as in Baghdad by Abbas *et al.* (2024), which all reported 100% resistance to ampicillin. A prior study in Thi-Qar hospitals by Abd Alameer *et al.* (2015) found slightly lower rate with 90% of clinical isolates were resistant to ampicillin. Many other regions around the world have also reported high ampicillin resistance rates (Celebi *et al.*, 2007; Olowe *et al.*, 2008; Uma *et al.*, 2009). The high levels of resistance to ampicillin suggests a widespread occurrence of β -lactamases production,

particularly TEM-1 and SHV-1 enzymes, which hydrolyze the β -lactam ring, a phenomenon consistently noted in *E. coli* globally, often attributed to the overuse of penicillins (Bush and Bradford, 2020).

Regarding the intravenous formulation ampicillin-sulbactam, the current study reported a high resistance rate of 84.41%. This resistance rate is slightly lower than the findings reported by Halim (2024) in Babylon and Al-Muthana province by Kareem (2023), who documented resistance rates of 95.5% and 98% for *E. coli* isolates, respectively. In contrast, Kacmaz and Sultan (2007) found that only 16.39% of *E. coli* isolates were resistant to ampicillin-sulbactam. Furthermore, Waltner-Toews *et al.* (2011) reported that 31.1% of *E. coli* isolates showed resistant responses to ampicillin-sulbactam. Resistance to the oral formulation, amoxicillin-clavulanic acid, was found to be 80.65%. A similar result of 80% was reported from Thi-Qar hospitals (Farhood *et al.*, 2024). Higher rates were recorded in Kirkuk and Babylon provinces, studies indicated that *E. coli* isolates exhibited 100% resistance to amoxicillin-clavulanic acid (Fakhraddin and Ali, 2022; Al-Hasnawy *et al.*, 2019). These results are relatively consistent with a study in Iran, which reported a resistance rate of 86.11% (Khoshvaght *et al.*, 2014). Additionally, Poovendran *et al.* (2013) found that 95% of *E. coli* isolates were resistant to amoxicillin-clavulanic acid. In contrast, Wuna *et al.* (2023) reported a moderate resistance rate of 31.8% among *E. coli* isolates, while Ya'aba *et al.* (2020) noted a moderate resistance level of 61.22% to amoxicillin-clavulanic acid. The present data showed a low effectiveness of penicillin- β -lactamase inhibitor combinations for treatment of *E. coli*, suggesting the dominance of ESBLs enzymes, such as CTX-M variants, which are resistant to inhibition by β -lactamase inhibitors (Al-Muhanna *et al.*, 2020).

Regarding resistance to third and fourth-generation cephalosporins, the following resistance rates were observed in the present study: ceftazidime

(83.87%), cefotaxime (79.57%), ceftriaxone (73.12%), and cefepime (67.74%). There was also a moderate resistance to ceftazidime at 59.68%. The results of this study reveal slight differences when compared to a previous study conducted in Babylon Hospitals, which reported resistance rates of 71.5% for ceftazidime, 73.5% for ceftriaxone, 86% for cefepime, and 70.5% for ceftazidime (Halim, 2024). In another study in Al-Qadisiya province, the resistance rates for third-generation cephalosporins were noted as follows: ceftazidime (81.6%), cefotaxime (74.9%), and ceftriaxone (74.9%), while the fourth-generation cephalosporin, cefepime, had a reported resistance rate of 74.9%. Additionally, many isolates showed resistance to ceftazidime at a rate of 56.0% (Al-Mayahi and Almohana, 2015). In Baghdad, a previous study demonstrated high resistance rates in *E. coli* to both third- and fourth-generation cephalosporins, with resistance rates of 92.92% for cefotaxime, 92.04% for ceftazidime, 90.27% for ceftriaxone, and 84.96% for cefepime (Al-Hasani *et al.*, 2023). Abbas *et al.* (2024) also reported substantial resistance in *E. coli*, with rates of 77% for ceftriaxone, 70% for ceftazidime, and lower rates of 40% for cefepime and 30% for ceftazidime. In Karbala province a study observed resistance rates of 62.5% for ceftazidime and 70.84% for ceftriaxone, while cefepime demonstrated the lowest resistance rate at 29.16% (Taqi, 2023). In Thi-Qar province, a previous study reported lower resistance rates for ceftazidime (72.22%), cefotaxime (67.77%), ceftriaxone (70.00%), and cefepime (47.77%) (Abd Alameer *et al.*, 2015). Conversely, Jabur and Abed (2020) reported that all isolates in Thi-Qar were 100% resistant to ceftazidime, 95.5% resistant to ceftriaxone, 84.9% resistant to cefepime, and 54.6% resistant to cefotaxime, which is higher than the findings of this study. Overall, this study suggests that *E. coli* resistance is higher than the globally reported rates. Ghaima *et al.* (2018) demonstrated that patients with UTs caused by *E. coli* exhibited resistance rates of 42.7% for ceftriaxone, 52.2% for ceftazidime, and 33% for cefepime. Furthermore

Jahani *et al.* (2017) noted that 65.5% of isolates were resistant to ceftazidime. This mirrors findings from an Iraqi study where 78% of *E. coli* isolates were ESBL-producers, linked to the unregulated use of cephalosporins in clinical settings (Al-Muhanna *et al.*, 2020). These enzymes hydrolyze cephalosporins, but cephameycin, cefoxitin, is not hydrolyzed, while AmpC β -lactamases (e.g., CMY-2) may contribute to its resistance (Jacoby, 2009). The lower resistance to cefepime may reflect its stability against some ESBLs, though rising resistance signals plasmid-mediated AmpC overexpression (Tan *et al.*, 2009). Such resistance is alarming in Iraq, where cephalosporins are empirically prescribed for urinary and gastrointestinal infections (Khudhair *et al.*, 2021).

In the case of aztreonam a resistance rate of 40.86% was observed. A similar finding in Baghdad reported that 40% of *E. coli* isolates were resistant to aztreonam (Khalaf and Flayyih, 2024). A slightly higher resistance rate of 55.88% was reported in Karbala (Mohammed *et al.*, 2014). In Thi-Qar, a previous study found that 67.77% of *E. coli* was resistant to aztreonam (Abd Alameer *et al.*, 2015). Additionally, in Babylon province, a recent study reported resistance rates of 76.5% among *E. coli* isolates (Halim, 2024). The high resistance of *E. coli* to aztreonam indicates the presence of ESBLs and AmpC β -lactamases (Samaha-Kfoury and Araj, 2003). These enzymes not only confer resistance to aztreonam but also to various other β -lactams. Additionally, other mechanisms such as porin alterations, efflux pumps, and modified target sites contribute to this resistance, making them a significant concern in the treatment of resistant *E. coli* infections (Cantón and Coque, 2021; Sinha *et al.*, 2022).

Regarding the carbapenem antibiotics, their efficacy remains relatively high, with resistance rates ranging from 11% to 17%. This is consistent with their designation as last-resort agents. However, even low levels of resistance to carbapenems are concerning because carbapenems

resistant Enterobacteriaceae are identified as priority pathogens by WHO due to their potential for rapid dissemination and public health impact (Tacconelli *et al.*, 2018). The importance of monitoring resistance patterns is underscored by literature documenting the prevalence of these antibiotics in different geographical regions. In Iraq, previous research indicates that the level of resistance to carbapenems differs across the country's regions. In Babylon province, a study showed that imipenem and meropenem were effective against the majority of *E. coli* isolates, with resistance rates of 11.90% for each drug, which aligns with our findings (Al-Hasnawy *et al.*, 2019). Another study conducted in Babylon hospitals reported resistance rates of 11.5% for imipenem and 15.5% for meropenem (Halim, 2024). In Baghdad province, a study noted that 10% and 7% of *E. coli* isolates were resistant to imipenem and ertapenem, respectively (Abbas *et al.*, 2024). A study in Najaf province found resistance rates of 9.6% for imipenem and 25% for meropenem (Al-Hilali, 2015). Conversely, in Karbala province, a study reported slightly higher resistance rates, with both imipenem and meropenem showing 16.67% resistance (Taqi, 2023). On the other hand, a study conducted in Thi-Qar, a much lower resistance rate of 3.33% for imipenem was reported, which contrasts with our results (Abd Alameer *et al.*, 2015). However, Jabur and Abed (2020) reported a resistance rate of 27.3% for imipenem in Thi-Qar. Additionally, another study in Thi-Qar province found that 60% of *E. coli* isolates from patients with colorectal carcinoma were resistant to imipenem, while all isolates exhibited 100% resistance to meropenem, which also contradicts our findings (Alalwany *et al.*, 2024). Our findings are consistent with those of Sharma *et al.* (2016) in India, who reported resistance rates for imipenem of 11.86% in 2012 and 11.36% in 2014. In contrast, another study in India reported higher resistance rates of 24.7% for imipenem and 37.6% for meropenem (Malik *et al.*, 2021). Notably, doripenem showed the highest resistance rate, consistent with

previous studies, such as the research conducted by Souli *et al.* (2020), which identified patterns of higher resistance rates to doripenem across multiple hospital settings in Europe. Similarly to the findings observed in the study by Paul *et al.* (2021) that reported resistance rates of 70% against doripenem in their patient cohort.

For aminoglycosides, the observed resistance rates were 59.14% for tobramycin, 26.88% for amikacin, and 37.10% for gentamicin. This trend mirrors regional studies, which suggest that amikacin retains its potency due to its limited use compared to gentamicin and tobramycin (Hassan *et al.*, 2019). These findings are in line with previous research that analyzed data from approximately 100 studies on antimicrobial-resistant *E. coli* in Iraq. That earlier study indicated that gentamicin had the highest resistance rate at 41.65%, while amikacin exhibited the lowest resistance rate at 17.3% (Al-Dahmoshi *et al.*, 2022). Another study revealed that only 29% of isolates were resistant to amikacin, whereas the resistance rates for gentamicin and tobramycin were 36% and 54.5%, respectively, in hospitals in Babylon (Halim, 2024). In contrast, Abbas *et al.* (2024) reported a moderate resistance rate of 47% for gentamicin and a low resistance rate of 7% for amikacin. Mohammed *et al.* (2022a) found that 24% of *E. coli* isolates in Zakho city were resistant to amikacin, while 74.35% showed resistance to gentamicin. In Wasit province, a study documented that 46.1% of isolates were resistant to gentamicin and 9.8% to amikacin (Al-Mayahie *et al.*, 2016). A study conducted in Erbil city reported that 25.15% of *E. coli* were resistant to amikacin, while 75% were resistant to gentamicin (Mansoor *et al.*, 2015). In Karbala province, a study reported a high resistance rate of 78.57% for gentamicin, while amikacin's resistance rate was low at 21.42% (Taqi, 2023). Lower resistance rates were noted in Misan, where 11.1% of *E. coli* were resistant to both amikacin and gentamicin (Alquraishi *et al.*, 2018). The susceptibility rates in this study align with those reported in Turkey, where

susceptibility rates for amikacin, gentamicin, and tobramycin against *E. coli* isolates from various clinical specimens were 39.6%, 35.9%, and 32.1%, respectively (Kucukates and Kocazeybek, 2002). Conversely, a study in Chinese hospitals reported resistance rates of 7% for amikacin and 63% for gentamicin (Cao *et al.*, 2011). The resistance of *E. coli* isolates to aminoglycosides is primarily attributed to the presence of aminoglycoside-modifying enzymes, which reduce the effectiveness of these antibiotics (Zhao *et al.*, 2023). The emergence of these enzymes has been closely linked to the overuse and misuse of aminoglycosides in healthcare settings (Ghosh *et al.*, 2022).

The resistance rates for three quinolone antibiotics were observed as follows: 72.04% of isolates were resistant to nalidixic acid, 51.61% to ciprofloxacin, and 50.54% to levofloxacin. These findings are consistent with a previous study conducted in Babylon hospitals, which reported that 51% of *E. coli* isolates were resistant to nalidixic acid, while 44% and 41% were resistant to ciprofloxacin and levofloxacin, respectively (Halim, 2024). Another study in Babylon province reported resistance rates of 64.28% for both nalidixic acid and ciprofloxacin, and 50% for levofloxacin (Al-Hasnawy *et al.*, 2019). In Fallujah hospitals, a study indicated a ciprofloxacin resistance rate of 59.7% and a levofloxacin resistance rate of 64.1% (Ghaffoori and Suleiman, 2022). In Thi-Qar province, a study found low resistance rates for fluoroquinolones, with 25.55% of *E. coli* isolates resistant to ciprofloxacin and 24.44% to norfloxacin, which contradicts the findings of the current study (Abd Alameer *et al.*, 2015). Additionally, resistance to nalidixic acid in this context is comparable to results from Sharma *et al.* (2013) and Wagle *et al.* (2018), who reported resistance rates of 78.9% and 71.5%, respectively. Ciprofloxacin resistance is in line with global studies by Khoshvaght *et al.* (2014), who noted resistance rates of 47%. In Egypt, a study indicated a high prevalence of quinolone resistance among *E. coli*

isolates, especially from urine specimens, with resistance rates of 58.95% for ciprofloxacin and 57.46% for levofloxacin (Abdel-Rhman *et al.*, 2021). Another study reported even higher resistance rates: 80% for nalidixic acid, 67% for levofloxacin, and 73% for ciprofloxacin (Yakout and Ali, 2022). Additionally, the present study's results are higher than those reported in Nigeria, where a ciprofloxacin resistance rate of 27% was noted (Ekwealor *et al.*, 2016). Resistance to this class of antimicrobials is primarily caused by mutations in the DNA gyrase (*gyrA*) and topoisomerase IV genes, which reduce the binding efficacy of the drugs. Additionally, plasmid-mediated quinolone resistance (PMQR) genes are also commonly found. Al-Dahmoshi *et al.* (2016) reported that 40% of Iraqi *E. coli* isolates carried the *qnrB* gene, which worsens resistance even with limited clinical use of these antibiotics.

The high resistance rates observed for tetracycline (60.75%) and doxycycline (58.06%) among the isolates are concerning. Resistance to tetracycline typically arises from several mechanisms, including the acquisition of resistance genes through mobile genetic elements, active efflux pumps that expel the drug from bacterial cells, and enzymatic inactivation of the antibiotic (Aarestrup, 2005; O'Brien *et al.*, 2011). This trend could be indicative of increased antibiotic misuse or over-prescription, which often leads to selective pressure favoring resistant strains (Laxminarayan *et al.*, 2013). These findings align with prior studies conducted in the Babylon province, where tetracycline resistance rates of 60% and 55% were reported, respectively (Alhusayni *et al.*, 2022; Halim, 2024). Moreover, Al-Hasani *et al.*, (2023) documented a concerning resistance rate of 80.53% for tetracycline among their isolates, while an investigation in Karbala province indicated an 80% resistance rate (Omran *et al.*, 2023). Research by Sakhi (2019) in Thi-Qar revealed that *E. coli* isolates had the highest tetracycline resistance rate at 78%. A study in the Babylon province indicated a higher doxycycline resistance rate of 83.33%

(Al-Hasnawy *et al.*, 2019). In contrast, a study in the Kurdistan Region reported a lower tetracycline resistance rate of 48.51% for tetracycline (Assafi *et al.*, 2022). In research conducted in Egypt, a resistance rate of 64.4% to tetracycline among *E. coli* isolates was found (Abdelhamid and Abozahra, 2017). Similarly, previous studies such as Sabir *et al.* (2014) noted a doxycycline resistance rate of 66.6%, while Shatalov (2015) reported an even higher resistance rate of 78.4%.

The observed resistance rate of 66.67% to trimethoprim among *E. coli* isolates raises significant concerns about the effectiveness of these antimicrobial, which is commonly prescribed for UTIs, respiratory tract infections, and various other bacterial infections. Local research noted a resistance rate of approximately 70% among *E. coli* isolates in patients with UTIs (Labi *et al.*, 2023). Similarly, a study found that *E. coli* isolates from hospitals in Babylon exhibited a resistance rate of 62.5% (Halim, 2024). In studies conducted in Thi-Qar, the resistance rate to trimethoprim was reported to be 50% (Farhood *et al.*, 2024), while other study reported a much higher resistance level of 96.1% (Jabur and Abed 2020). Furthermore, research by Mohammed and Ibrahim (2022) revealed a high resistance rate of 82% in *E. coli* isolates, surpassing the current findings. In contrast, a study conducted in Baghdad province reported a lower resistance rate of 36% (Khalaf and Flayyih, 2024). However, this finding contradicts the reports of previous studies that noted a complete resistance rate of 100% (Aslani *et al.*, 2011; Amin and Sirous, 2018). Niazi *et al.* (2023) indicated that alarming levels of resistance to trimethoprim have been reported across various regions, often exceeding 60%. The primary cause of trimethoprim resistance in Gram-negative bacteria is plasmid-borne dihydrofolate reductase (*dfr*) genes, typically found in class I integrons. These genes are commonly present in clinical isolates from Iraq (Khudhair *et al.*, 2021).

This study found that 36.02% of the *E. coli* isolates exhibited resistance to chloramphenicol, indicating a moderate yet concerning level of resistance to this bactericidal agent. This level of resistance is consistent with trends observed in other studies that have reported varying rates of chloramphenicol resistance among *E. coli*. For example, a study conducted in Najaf province reported a resistance rate of 34.2% (Hadi, 2008), while Al-Hasani *et al.* (2023) found that 37.17% of isolates were resistant to this antibiotic. In Nigeria, a study reported a moderate resistance rate of 36.5%, which is also in agreement with our findings (Najim *et al.*, 2019). In contrast, lower resistance rates of 14.44% and 19% were reported in Thi-Qar and Babylon hospitals, respectively (Abd Alameer *et al.*, 2015; Halim, 2024). Furthermore, there have also been report of high susceptibility to chloramphenicol, with 81.8% of isolates showing sensitivity in Karbala province (Mohammed *et al.*, 2014). The resistance in *E. coli* is primarily due to two mechanisms: enzymatic inactivation via chloramphenicol acetyltransferases (CATs) and efflux pumps expressed by genes such as *cmlA*, *floR*, or *fexA* (Schwarz *et al.*, 2016; Hasan and Al-Harmoosh, 2020). A study from Iraq detected the *catA1* and *floR* genes in 28% and 34% of chloramphenicol-resistant *E. coli* isolates, respectively (Al-Rubaye *et al.*, 2023).

The present study indicates that nitrofurantoin remains relatively effective against *E. coli* infections, exhibiting a resistance rate of 40.32%. This effectiveness is largely due to its unique mechanism, which generates toxic intermediates that damage DNA. Resistance to nitrofurantoin is typically requires mutations in multiple genes, such as *nfsA* and *ribE*. Salih *et al.* (2023) confirmed its efficacy against MDR *E. coli* in Iraq, recommending its use as a first-line therapy for UTIs. This finding aligns with studies conducted in the Baghdad and Babylon provinces, where resistance rates were reported at 45.3% and 42.85%, respectively

(Mohammed *et al.*, 2022b; Al-Hasnawy *et al.*, 2019). Slightly lower resistance rates were observed in the Thi-Qar and Karbala provinces, with 32.22% and 36% of *E. coli* isolates resistant to nitrofurantoin, respectively (Abd Alameer *et al.*, 2015; Omran *et al.*, 2023). In contrast, a study from Zakho city reported a significantly higher resistance rate of 82.4% (Mohammed *et al.*, 2022a).

5.3. Dissemination and Resistance Pattern of Carbapenems resistant *E. coli* Isolates

The epidemiology of carbapenems resistant bacterial isolates in Iraq remains largely unknown due to a lack of hospital reports on carbapenem susceptibility rates and limited published data on their spread. Consequently, the proliferation of these bacteria poses a significant threat to public health, necessitating focused efforts on detection and infection control strategies. To our knowledge, this study is the first to demonstrate the prevalence of carbapenems resistant *E. coli* clinical isolates in Thi-Qar hospitals.

In this study, 40 (21.62%) of *E. coli* isolates were found to be resistant to carbapenem, as determined by the discs diffusion method (Table 4-4). This finding corresponds with the global increase in carbapenems resistant Gram-negative bacteria. The Centers for Disease Control and Prevention (CDC) reported slightly higher prevalence rates for the Middle East from 2008 to 2013, indicating a resistance rate of 26.75% (Peirano *et al.*, 2014). Other countries have reported carbapenem resistance rates of 18.4% in Iran (Jomehzadeh *et al.*, 2022), 19.78% in Egypt (Abdelraheem *et al.*, 2023), 22.8% in Uganda (Ssekatawa *et al.*, 2021), 24% in Tanzania (Mushi *et al.*, 2014), 31.77% in India (Pawar *et al.*, 2018), and 37% in Lebanon (Dagher *et al.*, 2018). Additionally, a national laboratory surveillance study in the Netherlands from 2014 to 2018 found that among 892 carbapenemase-

producing Enterobacterales, 264 (30%) of *E. coli* isolates were carbapenems resistant (van der Zwaluw *et al.*, 2020).

Several studies conducted in cities in Iraq have reported varying findings. In the Basrah province, one study revealed that out of 294 isolates, 61 (29.5%) were identified as carbapenems resistant Gram-negative bacteria (Abas and Al-Hamdani, 2017). Another study in Basrah involving cancer patients suspected of having UTIs found that out of 102 *E. coli* isolates, 22 (21.57%) were carbapenems resistant (Albadery *et al.*, 2023). Recent studies in Baghdad and the Kurdistan region showed that approximately 30% of *E. coli* isolates were resistant to carbapenems (Marwa and Ibrahim, 2024; Haji *et al.*, 2021). Furthermore, a survey across hospitals in various cities in Iraq found that 18 (14%) of 128 *E. coli* isolates were resistant to carbapenems (Hashem *et al.*, 2023). Lastly, data from Erbil city indicated that 13.5% of *E. coli* isolates were carbapenems resistant (Ahmed *et al.*, 2019).

The demographic data of carbapenems resistant *E. coli* isolates in the present study revealed that the majority of isolates were collected from urine specimens (67.5%), followed by sputum (15%), body fluids (12.5%), and one isolate (2.5%) each from swabs and CSF (Table 4-4). These results are consistent with global trends, where UTIs remain the predominant cause of infections associated with MDR *E. coli*. For example, a study in India reported that the majority of carbapenems resistant *E. coli* isolates were derived from urine (23/48, 47.92%), followed by pus (7/48, 14.58%), tracheal aspirates (6/48, 12.5%), stool (5/48, 10.42%), blood (2/48, 4.17%), and other sources (4/48, 8.33%) (Jaggi *et al.*, 2019). Similarly, a study in Iran found that most carbapenems resistant isolates were retrieved from urine (58.33%), followed by wounds (25%), and with blood and sputum each accounting for 8.33% (Jomehzadeh *et al.*, 2022). A study in Uganda found that carbapenems resistant *E. coli* were predominantly isolated from anal swabs (31.1%), followed by urine (24.1%), wound/pus swabs (17.7%),

tracheal aspirates (16.7%), sputum (15.8%), blood (14.3%), and vaginal swabs (11%) (Ssekatawa *et al.*, 2021). In Iraq, a study found that the most common source of carbapenems resistant *E. coli* isolates was urine specimens, while only 2.7% originated from swab specimens, in Erbil city (Heshu *et al.*, 2023). Another study in Erbil observed that urine specimens (65.3%) were the predominant source of carbapenems resistant *E. coli* isolates; pus swabs were the second most common source (15.3%), followed by sputum (10.2%), blood (6.1%), and other specimens like body fluids (3.1%) (Ahmed and Ganjo, 2019). In Basrah province, a study reported 61 clinical isolates of carbapenems resistant *E. coli* collected from seven sources: burns (19 isolates, 31.14%), urine (12 isolates, 19.67%), blood (8 isolates, 13.11%), swabs (10 isolates, 16.39%), wounds (7 isolates, 11.47%), tissue (4 isolates, 6.55%), and sputum (1 isolate, 1.63%) (Abas and Al-Hamdani, 2017). The low incidence of carbapenems resistant *E. coli* in blood and wound specimens in this study may reflect lower sampling rates or more prompt clinical interventions in such severe infections.

The prevalence of carbapenems resistant *E. coli* infections shows notable age-related trends. The highest incidence, at 37.5%, was found in patients over 60 years old, with a corresponding study in China reporting that 60% of infected individuals were in this age group (Zeng *et al.*, 2024). In Iraq, a study found that carbapenems resistant *E. coli* infections were particularly prevalent among older patients, aligning with our findings (Al-Hasan *et al.*, 2020). Older adults are at greater risk for MDR infections due to declining immune function, increased comorbidities, and more exposure to healthcare settings (Wong *et al.*, 2020; Rottier *et al.*, 2019). The second highest prevalence, at 35%, was observed in the 41-60 age group, who share similar vulnerabilities. In contrast, the prevalence dropped to 15% in the 21-40 age group and 12.5% in those aged 1-20, suggesting that younger populations generally have stronger immune systems and less exposure to

antibiotics (Bradford, 2017). Notably, there were no cases reported among infants under one year, likely due to the protective effects of maternal antibodies (Mizrahi *et al.*, 2020).

The gender distribution of infections caused by carbapenems resistant *E. coli* isolates shows a slightly higher prevalence among males (57.5%) compared to females (42.5%). This trend is consistent with emerging findings in the literature on AMR, which indicate that males are often at a greater risk for MDR infections. For example, a study by Jaggi *et al.* (2019) found a similar rate of carbapenem resistance in males (27/48 isolates, 56.25%) compared to females (21/48 isolates, 43.75%). In Iraq, a study by Al-Bayati *et al.* (2023) reported similar trends, noting that male patients exhibited higher rates of carbapenems resistant *E. coli*. Conversely, Taqi and Hadi (2023) in Karbala province found no significant relationship between sex and infection rates, with both genders exhibiting an equal rate. Furthermore, research by Al-Sa'ady *et al.* (2020) discovered that, out of 11 carbapenems resistant *E. coli* isolates, 5 (45.45%) were from males and 6 (54.55%) were from females.

The results of this study also indicate a significant difference in the origin of carbapenems resistant *E. coli* isolates, with 62.50% obtained from hospitalized patients, compared to outpatients (37.5%). This non-selective approach to patient populations may have contributed to the higher occurrence of carbapenems resistant *E. coli* observed in this study. This finding aligns with previous research, which reported that the rate of carbapenem resistance was significantly higher among inpatients (35/48, 72.92%) than outpatients (13/48, 27.08%) (Jaggi *et al.*, 2019). Additionally, Abas and Al-Hamdani (2017) noted that most carbapenems resistant isolates were collected from inpatients, with only 1.6% sourced from outpatients in Basrah. A study from Iran highlighted that 70% of carbapenems resistant *E. coli* infections occurred in hospitalized patients, further linking resistance to

healthcare-associated risks (Solgi *et al.*, 2023). Lee *et al.* (2023) observed a notable correlation between the high incidence of carbapenems resistant Enterobacteriaceae and the consumption of broader-spectrum antibiotics in hospitals. The hospital environment has also been documented as a reservoir and source of carbapenems resistant infections, contributing to several outbreaks (Tofteland *et al.*, 2013; Decraene *et al.*, 2018). However, many studies have raised concerns about the potential risk of community transmission of carbapenems resistant *E. coli* (Gupta *et al.*, 2011; Van Duin *et al.*, 2013). A study by Bohl *et al.* (2023) emphasizes the alarming increase in carbapenem resistance among *E. coli* isolates in both community and hospital settings, underscoring the role of plasmid-mediated resistance genes in transmission.

5.4. Antibiotic Susceptibility of Carbapenems resistant *E. coli* Isolates

The current investigation reveals high resistance rates among carbapenems resistant *E. coli* isolates, with 80% resistant to doripenem, 55% to meropenem, and 52.5% to imipenem. Susceptibility testing further indicates that resistance to various antimicrobial agents is notably high (Figure 4-3). These findings align with global trends showing increasing carbapenem resistance in Enterobacteriaceae, often attributed to the spread of carbapenemase genes. These genes enable bacteria to hydrolyze nearly all β -lactam classes and frequently confer resistance to structurally unrelated antimicrobial agents. This phenomenon occurs because plasmids that carry carbapenemase genes typically also encompass determinants of resistance to multiple antibiotic classes, which contributes to the high levels of MDR observed in carbapenems resistant isolates (Nordmann *et al.*, 2012; Morrissey *et al.*, 2014). These findings are consistent with research conducted in Karbala province, which demonstrated that bacteria developing

resistance to carbapenems also exhibited resistance to most antibiotics (Mahdi *et al.*, 2020). Moreover, the observed resistance patterns extend beyond carbapenems, with 100% resistance to ampicillin and cefotaxime, 97.5% to ceftazidime and ceftriaxone, 95% to cefepime, and 57.5% to ceftiofur (Figure 4-3). The high resistance rates of carbapenems resistant isolates against penicillins and cephalosporins are not surprising, as carbapenemases are known to confer resistance to all β -lactam antibiotics. This extensive β -lactam resistance likely reflects the co-production of ESBLs or AmpC β -lactamases alongside carbapenemases, which is common in MDR *E. coli* (Rawat *et al.*, 2017). This concurs with reports documented high resistance rates of carbapenems resistant isolates to β -lactam antibiotics (Olowo-okere *et al.*, 2019; Pawar *et al.*, 2020).

Additionally, our results indicate that the majority of isolates (55.0% and 82.5%) were resistant to ampicillin-sulbactam and amoxicillin-clavulanic acid, respectively. Recently, there have been increasing reports of resistance among *E. coli* isolates against β -lactam/ β -lactamase inhibitors. Likely, due to *E. coli* producing carbapenemase or AmpC β -lactamases are not inhibited by the presence of clavulanic acid (Krishnamurthy *et al.*, 2013).

Although aztreonam is effective against bacteria producing class B carbapenemases, our study found that 67.5% of carbapenems resistant isolates were resistant to aztreonam, similar to findings from other studies (Prevotat *et al.*, 2010; Singh *et al.*, 2017). This is in agreement with a study in Karbala province, which found that the antimicrobial resistance rate of *E. coli* to aztreonam was 79.16% among carbapenems resistant *E. coli* isolates (Abdulameer, 2023). This suggests the presence of additional resistance genes, such as ESBLs, AmpC β -lactamases, or class A carbapenemases, which are frequently co-produced by NDM-producing isolates (Tamma *et al.*, 2022).

Several studies have shown that aminoglycosides retain potent bactericidal activity and could serve as a good alternative treatment against some, but not all, carbapenems resistant *E. coli* isolates (Lee *et al.*, 2012; Bai *et al.*, 2013). The high resistance rates to aminoglycosides (72.5-80%) suggest the presence of plasmid-mediated resistance genes, such as *aac(6')-Ib-cr*, which are often co-carried with ESBL or carbapenemase genes (Fàbrega *et al.*, 2009). This variant has the ability to inactivate aminoglycosides (Robicsek *et al.*, 2006; Warburg *et al.*, 2009). These findings reflect trends in Iraq, where Al-Mayahie *et al.* (2016) reported that 78% of *E. coli* isolates from hospitals were ESBL-positive, revealing significant co-resistance to aminoglycosides. A study by Pawar *et al.* (2020), reported that 54.7% and 82.9% of carbapenems resistant Enterobacteriaceae are resistant to Amikacin and gentamicin, respectively.

The present results indicate that a significant majority of isolates showed resistance to quinolones and fluoroquinolones, including nalidixic acid (85.00%) and both ciprofloxacin and levofloxacin (80.00% each). This resistance to nalidixic acid may be linked to the excessive use of quinolones as empirical treatment of UTIs. Additionally, several studies have documented that the overuse of fluoroquinolones is a risk factor for infections caused by carbapenems resistant *E. coli* isolates (Schwaber *et al.*, 2008; Gasink *et al.*, 2006). This aligns with findings from Karbala province that reported a 75% resistance rate to ciprofloxacin and 62.5% for both gentamicin and tobramycin among carbapenems resistant *E. coli* (Abdulameer, 2023). Carbapenems resistant strains predominantly carry genes, such as *qnr*, which are responsible for resistance to fluoroquinolones on the same plasmids (Bratu *et al.*, 2005; Fàbrega *et al.*, 2009). This situation is critical, as it further limits the effectiveness of quinolones in treating these highly drug-resistant isolates. Various studies have reported similarly high

resistance rates among carbapenems resistant *E. coli* strains against fluoroquinolones (Mathers *et al.*, 2013; Giwa *et al.*, 2018; Miao *et al.*, 2019).

The rates of resistance to tetracycline and doxycycline (70% and 80%, respectively) in this study. Susceptibility to these agents is independent of the presence or type of carbapenemase, likely driven by selective pressure from the overuse of antibiotics in both clinical and veterinary settings (Aslam *et al.*, 2018; Johnston *et al.*, 2020). Moreover, this resistance can occur due to genes encoding ribosomal protection mechanisms or efflux pumps that actively transport antibiotics out of the cell (Speer *et al.*, 1992).

The high rate of trimethoprim resistance (87.50%) observed in this study aligns with findings from Pawar *et al.* (2020), who reported a high level of resistance among carbapenems resistant *E. coli* against trimethoprim (73.5%). In Iraq, a study by Abdulameer (2023) found a resistance rate of 66.67% to trimethoprim/sulfamethoxazole in Karbala province. This high rate of resistance might result from plasmid-borne dihydrofolate reductase (*dfr*) genes, which are commonly found as gene cassettes in class 1 integrons (Ahmed *et al.*, 2007). Although the mechanism of cross-resistance remains unclear, one possible explanation is the co-transmission of carbapenemases and trimethoprim-sulfamethoxazole resistance genes within the same conjugative plasmids (Frye and Jackson, 2013; Estrada *et al.*, 2016). Another contributing factor is the selective pressure resulting from the extensive use of trimethoprim in treating uncomplicated UTIs and as prophylaxis.

Although nitrofurantoin has dual bacteriostatic and bactericidal properties, that do not exhibit co-resistance with more commonly prescribed antibiotics (Hooton, 2003). The present study indicated a high resistance rate of 65% nitrofurantoin in carbapenems resistant isolates, aligning with high levels of resistance against UPEC reported worldwide, including in Iraq (Fayroz-Ali, 2012; Al-Mayahi, 2013; Mollick *et al.*, 2016; Shakhatreh *et al.*, 2019).

In this investigation, chloramphenicol was identified as the most effective antibiotic for treating infections caused by carbapenems resistant *E. coli*, exhibiting a resistance percentage of 32.50%. The low resistance rate against chloramphenicol is likely attributable to its infrequent use in treating infections caused by *E. coli* (Abdulameer, 2023).

Iraq is a developing country where antibiotics are sold over the counter. Over time, certain antibiotics gain more frequent use than others do without susceptibility testing, potentially leading to variability in resistance rates. The challenges in managing these infections may arise from delays in initiating effective therapy, the limited pharmacological options available, and the fact that patients with carbapenems resistant bacterial infections are often critically ill. Generally, physicians depend on *in vitro* antimicrobial susceptibility results to determine the best therapy for their patients. Empirical combination therapy for patients infected with carbapenems resistant organisms is typically based on local resistance patterns. However, definitive therapy should be guided by the determination of *in vitro* antimicrobial susceptibility profiles, especially when considering combination treatments for these pathogens. This is crucial since only a limited number of drugs remain effective, such as colistin, fosfomycin, tigecycline, aminoglycosides, and newer β -lactams (Falagas *et al.*, 2014; Rodríguez-Baño *et al.*, 2015).

5.5. Multiple Antibiotic Resistance Phenotypes, Patterns, and MAR Index

A significant majority (67.50%) of the isolates were found to be XDR, while the remaining 32.50% exhibited potential PDR phenotypes (Figure 4-4). These alarmingly high rates of XDR and PDR among carbapenems resistant *E. coli* isolates in Thi-Qar hospitals are concerning. Various researches has confirmed similar trends; for example, Tacconelli *et al.*

(2018) reported that XDR and PDR strains are increasingly dominant in various regions worldwide, often outnumbering MDR variants. This situation is alarming because infections caused by XDR isolates leave physicians with only one or two antibiotic treatment options, which can lead to increased mortality and morbidity (Giske *et al.*, 2008; Dehbanipour *et al.*, 2016). The high prevalence of XDR isolates in this study corresponds with findings from other regions, such as Iran, where 58% of carbapenems resistant *E. coli* displayed XDR phenotypes (Solgi *et al.*, 2017). Similarly, a meta-analysis indicated that XDR *E. coli* rates in South Asia exceed 50%, often associated with empirical antibiotic overuse and insufficient infection control (Kiran *et al.*, 2024). A study involving Syrian refugees also found XDR *E. coli* in 63% of isolates, which was attributed to disrupted healthcare and misuse of antibiotics (Abbara *et al.*, 2022).

The most significant finding of the present study was that 32.5% of carbapenems resistant isolates showed resistance to all tested antibiotics, categorizing them as possible PDR. Although infections caused by PDR Enterobacteriaceae are still relatively rare in Europe and other countries, they have been linked to high mortality rates (Souli *et al.*, 2008; Yang *et al.*, 2015; Mahmoud *et al.*, 2016). In a prior study involving 28 patients with PDR infections in Greece, the attributable mortality was 33.3% (Falagas *et al.*, 2008). The high rate of PDR observed in this study exceeds global averages, where PDR *E. coli* remains rare but is increasingly reported in intensive care units (Karaiskos *et al.*, 2019). In contrast, a study conducted in Babylon hospitals found that only 1.5% of *E. coli* isolates were PDR (Halim, 2024).

Interestingly, the absence of MDR phenotypes among the carbapenems resistant *E. coli* isolates is noteworthy. This finding suggests that resistance mechanisms may be evolving toward more complex patterns, highlighting the need for vigilant monitoring. This may be due to plasmids carrying genes for carbapenem resistance, which often also harbor additional

resistance genes for quinolones, aminoglycosides, chloramphenicol, sulfonamides, tetracyclines, and trimethoprim, thus enabling bacteria to become XDR or possibly PDR (Velkov *et al.*, 2015; Navon-Venezia *et al.*, 2017).

In this study all 40 isolates exhibited MAR indices >0.2 , with 30% demonstrating pan-drug resistance (PDR; MAR index=1), reflecting resistance to all 23 antibiotics tested (Figure 4-5). A MAR index resistance to >0.2 antibiotics indicates that bacteria originate from an environment where antibiotics are freely available (Riaz *et al.*, 2011; Veloo *et al.*, 2022). This confirmed that there was widespread use of antibiotics and high selective pressure in Thi-Qar hospitals as result of exposed to numerous antibiotics. This phenomenon is particularly stark in Iraq, where healthcare infrastructure challenges and antibiotic misuse contribute to the escalation of bacterial resistance (Al-Juboury *et al.*, 2022). This results slightly resemble with study conducted by Halim (2024) who found that all tested *E. coli* isolates exhibited MAR (> 0.2). The mean MAR index ranges from (0.23-1) for these isolates. The occurrence for *E. coli* isolates with a MAR index of 0.66 was high and then with an index of 0.52, while MAR index of 1 were found in 3 (10.34%) of *E. coli* isolates. Al-Mamouri (2019) in Najaf and Babylon Hospitals found that the MAR index analysis showed that all isolates had high MAR index values (>0.2). A significant proportion of isolates exhibited MAR indices of 0.7. Notably, 1 (1.7%) isolate with a MAR index of 1 showed resistance to all tested antibiotics (PDR). A study from Turkey reported similar findings with MAR values exceeding 0.9 in carbapenems resistant *E. coli* (Yilmaz *et al.*, 2020). Furthermore, the categorization of 30% of isolates as XDR. Notably, the 30% PDR prevalence exceeds rates reported in neighboring regions, such as Iran of 15% *E. coli* were PDR (Mohammadi *et al.*, 2021). It is critical to note that the high MAR indexes, particularly among isolates categorized as PDR, denote a desperate

situation where clinical treatment options are minimal. Such patterns resonate with previous research suggesting that infections from PDR strains lead to longer hospital stays, increased medical costs, and elevated mortality rates (Elghazawy *et al.*, 2022).

In this study, analysis of antibiotic resistance phenotype patterns revealed that most of the isolates were resistant to at least 20 different antibiotics, with some displaying resistance to as many as 23 (Tables 4-6). The diverse range of resistance patterns, including those seen in XDR and PDR isolates, suggests a potential for horizontal gene transfer among *E. coli* populations. Lu *et al.* (2023) emphasize that such genetic exchanges can promote the rapid spread of resistance determinants, which complicates treatment options even further and this likely contributes to the resistance observed in our local isolates.

5.6. Phenotypic Screening of β -Lactamases

Rapid detection of β -lactamases using methods with a short turnaround time is essential for early recognition and timely implementation of control measures in hospitals. In Iraqi hospitals, routine screening is primarily conducted using the discs diffusion test, with no confirmatory testing in place.

The detection of ESBL-producing isolates is critically important for all Iraqi hospitals and the community for several reasons. Firstly, these isolates are likely more prevalent than currently recognized due to difficulties in detection using traditional methods. Secondly, ESBLs pose a significant threat to current β -lactam therapy (Perez *et al.*, 2007). In this study, the initial screening for ESBLs showed that all carbapenems resistant *E. coli* isolates were identified as potential ESBL producers. Phenotypic confirmation of ESBLs production showed that 25% of all isolates were confirmed to be ESBL producers (Table 4-7). The phenotypic data generated in this study, using these screening tests, indicates significantly high rates of

ESBL producers in Thi-Qar hospitals, consistent with previous findings reported by other researchers in Iraq. In a study by Hadi (2008), it was reported that 15.8% of *E. coli* isolates from patients with UTIs in Najaf were ESBL producers using discs approximation test. Al-Hilali (2010) found that 13.6% of enteropathogenic *E. coli* isolates in Najaf were ESBL producers using the same test. In contrast, this study show lower rates than those reported by Al-Hilli (2010), who indicated that 45% of *E. coli* isolates obtained from Hilla hospitals were ESBL producers. The discs approximation test remains a reliable, convenient, and inexpensive method for screening ESBLs. However, the interpretation of this test can be subjective, and sensitivity may be reduced when ESBLs activity is very low, leading to wide inhibition zones around the cephalosporin and aztreonam discs (Vercauteren *et al.*, 1997). It is noteworthy that there are numerous reports where initial ESBL screening tests are positive, but confirmatory tests return negative results due to the coexistence of both ESBLs and AmpC β -lactamases (Fayroz-Ali, 2012; Rahamathulla and Harish, 2016; Al-Hasnawi, 2020). These negative results are likely when AmpC activity is greater than ESBLs activity, which can lead to erroneous outcomes (Yan *et al.*, 2004). Furthermore, several studies have also shown that confirmatory tests fail to detect some ESBL-producing strains in Gram-negative bacteria (Coudron *et al.*, 1997; Al-Muhannak, 2010).

The ability to detect AmpC is crucial in hospitals to enhance the clinical management of infections and provide reliable epidemiological data. Reduced susceptibility to cefoxitin in Enterobacteriaceae may initially indicate AmpC (Plasmidic) activity, but confirmation through additional testing is necessary due to the absence of CLSI guidelines for its detection. Results of initial screening test from Table (4-7) indicate a high rate of cefoxitin-resistant *E. coli* isolates (70.0%), which is higher than the 55% reported in Hilla city (Al-Hilli, 2010) but comparable to the 71.2% found in

Najaf province (Al-Muhannak, 2010). This increased resistance may be attributed to several factors, including overexpression of the chromosomal AmpC genes, acquisition of a plasmidic AmpC gene, porin or permeability mutations, or a combination of these factors (Mulvey *et al.*, 2005; Al-Sehlawi, 2012). Additionally, the CIAT was utilized to confirm the presence of inducible (Chromosomal) AmpC β -lactamases among carbapenems resistant *E. coli* isolates. This is based on the strong inducing effect of imipenem on these enzymes, which causes antagonism with ceftazidime. However, the current study did not find documented evidence of CIAT's utility for this purpose. There is a lack of published data regarding the prevalence of inducible AmpC β -lactamases in Thi-Qar. Al-Hilli (2010) found that 2.5% of *E. coli* isolates from Marjan Hospital in Hilla were phenotypically confirmed as inducible AmpC producers, while a study in Najaf reported that 1.9% of *K. pneumoniae* isolates produced inducible AmpC β -lactamases (Al-Sehlawi, 2012). Typically, chromosomal AmpC β -lactamases are inducible, while plasmid-mediated AmpC enzymes are not (Fortineau *et al.*, 2001). The findings of the present study suggest that chromosomally AmpC are rare or possibly presence of plasmidic AmpC β -lactamases among the carbapenems resistant *E. coli* isolates in Thi-Qar.

Carbapenemase production can confer specific phenotypic characteristics to organisms, influenced by species, type of enzyme produced, presence of additional resistance mechanisms, and the level of expression (Landman *et al.*, 2009). Specific tests can help identify carbapenemase activity phenotypically. However, detecting carbapenemases can be challenging; for some carbapenemase-producing bacteria, the minimum inhibitory concentration of carbapenems may be high but still fall within the susceptible range as defined by CLSI criteria (CLSI, 2010; Elizabeth and Vincent, 2010). Clinical isolates rarely exhibit a resistance phenotype solely attributable to carbapenemase expression, as they often co-

express other mechanisms of resistance (Nordmann *et al.*, 2012). Several screening methods using metal-chelating agents, such as EDTA and thiol-based compounds like 2-mercaptopropionic acid, have been developed to detect MBL-producing organisms (Walsh *et al.*, 2002; Yong *et al.*, 2002).

Various studies have reported that the imipenem-EDTA combination is the most sensitive method for detecting MBLs in Enterobacteriaceae (Queenan and Bush, 2007; Jacoby, 2009). One significant finding was that 27.50% of various carbapenems resistant isolates were identified as MBL producers through the DDST and CDT (Table 4-7). This results align with those obtained by Lee *et al.* (2006), who reported a 25% prevalence of MBLs using DDST. In contrast, our findings differed from two Indian studies; Uma *et al.* (2009) reported 70% MBLs, while Gupta *et al.* (2006) found 75%. Regarding the CDT, our results were slightly lower than those reported by Franklin *et al.* (2006), who had a 16% detection rate. In Mosul, a study reported that among 393 isolates, only 29 isolates (7.4%) were found to be MBL producing bacteria by CDT (Al-Hasso, 2023). Elbadawi *et al.* (2021) also reported a rate for MBLs of 50.9% from hospitalized patients with Enterobacteriaceae infection in Sudan, while Loqman *et al.* (2021) found that 25.9% of carbapenems resistant Enterobacteriaceae were positive for MBLs with CDT. Moreover, in the studies of Bhat *et al.* (2013) in India and Hussein *et al.* (2018) in Wasit city they found that 37% and 31%, respectively of *Pseudomonas* clinical isolates were MBLs positive by DDST. Zhang *et al.* (2022) identified two main issues with these methods. The first issue is the occurrence of false-positive results; among 14 non-carbapenemase-producing strains, 3 were misclassified as positive, leading to a reduced specificity of 79% for carbapenemase detection. The second issue is the inability to detect IMP-type carbapenemases accurately, resulting in a low negative predictive value of only 55% for carbapenemase detection and a sensitivity of 88% for MBLs detection. Moreover, the limitation of

detected genes via PCR/sequencing could lead to false positives from rarer carbapenemases (such as SME, NMC, GES) being present in these strains (Bonnin *et al.*, 2020). This failure to detect carbapenemases may arise from inadequate gene expression as these methods relies on the MBLs being well expressed and inhibited by EDTA (Zhang *et al.*, 2022).

All carbapenems resistant isolates were also screened phenotypically using the MHT. This test relies on the ability of carbapenemase producers to reduce the local concentration of carbapenems, allowing the growth of a carbapenem susceptible *E. coli* (ATCC 25922) isolate around the streak line near the carbapenem disc, characterized by a cloverleaf appearance (Lee *et al.*, 2001). The current study revealed that the vast majority of enrolled carbapenems resistant *E. coli* were negative in the MHT, with only one isolate (2.5%) testing positive, which was subsequently identified as an OXA-48 producer by LFIA and PCR (EC-27). Although the MHT is a simple and inexpensive test for detecting carbapenemases, it has limitations, including poor sensitivity for identifying MBL-producing isolates and reduced specificity due to false positives among ESBL producers or AmpC overexpression combined with outer membrane porin loss (Zhong *et al.*, 2020; Kumudunie *et al.*, 2021). Previous studies indicating that the MHT has difficult to detect the activity of MBLs because they are anchored to the outer membrane of Gram-negative bacteria (Castanheira *et al.*, 2011; Armin *et al.*, 2021). The MHT has demonstrated higher sensitivity for detecting KPC and OXA-48-producing isolates (Genc *et al.*, 2016; Nordmann *et al.*, 2012b). Therefore, this test may be beneficial for identifying KPC and OXA-48 producers, particularly in areas where these carbapenemases are prevalent. However, it is worth noting that the MHT's limited performance has been recognized for several years; consequently, it was removed from the CLSI recommendations as a phenotypic method for detecting carbapenemases in

2018 (CLSI, 2018). Additionally, a study suggested that DDST could detect more carbapenemase-producing *E. coli* isolates than MHT (Yan *et al.*, 2004).

Several LFIAs have been recently developed that can provide accurate results from cultured isolates within 15 minutes; however, they generally detect only one or a few of the most epidemiologically significant carbapenemases (Boutal *et al.*, 2018). In this investigation evaluated the effectiveness of the LFIA (Combo test) in detecting carbapenemase enzymes, specifically KPC, VIM, IMP, NDM, and OXA-producing isolates. The findings from LFIA revealed a significant prevalence of carbapenemase enzymes, with 27.5% of isolates testing positive for NDM and 2.5% positive for OXA-48. KPC, IMP, and VIM were not detected (Tables 4-7). Notably, there are no published studies on the use of this assay for detecting carbapenemase-producing *E. coli* isolates in Thi-Qar or other provinces of Iraq. The detection of NDM using the LFIA demonstrates its utility as a valuable tool for identifying carbapenemases, enabling timely adjustments to treatment strategies in clinical settings. This is consistent with the validation by Tijet *et al.* (2016), who confirmed LFIA's specificity of over 95% for NDM in clinical isolates. However, the assay's limited ability to detect other enzymes (e.g., OXA-48, KPC, VIM, IMP) highlights a critical limitation in regions where multiple carbapenemase co-production occurs. Additionally, the phenotypic detection of OXA-48 can be challenging because of its weaker hydrolytic activity against carbapenems, which may lead to underestimation in such assay (Boutal *et al.*, 2018). The LFIA's monoclonal antibodies may also exhibit variable sensitivity for OXA-48 subtypes, as evaluated by Dortet *et al.* (2022). The absence of KPC detection aligns with its rarity in Iraq and the Middle East, where other MBLs (e.g., NDM, VIM) are more prevalent (Khudhair *et al.*, 2021). The lack of IMP in this study may reflect the regional resistance gene pools. For instance, a surveillance study in Baghdad found no IMP producers among carbapenems

resistant isolates, whereas NDM was detected in 19% of cases (Al-Kadmy *et al.*, 2023).

5.7. Genotypic Screening of Carbapenemase Genes

Molecular techniques are regarded as reference methods for identifying carbapenemase genes due to their higher sensitivity (96%-100%) and specificity (97%-100%) compared to phenotypic methods (Doyle *et al.*, 2012; Moloney *et al.*, 2019; Deldar Abad Paskeh *et al.*, 2020). To date, there have been no reports specifically examining the dissemination of carbapenemase genes among *E. coli* isolates in Thi-Qar hospitals. The findings indicate that the primary mechanism underlying carbapenems resistant *E. coli* in Thi-Qar hospitals was the presence of carbapenemase genes. Based on the PCR assay, 34 (85.00%) of the isolates carried at least one carbapenemase gene (Table 4-8). The results highlighted the prevalence of class B genes (*bla*_{NDM-1} and *bla*_{VIM}), followed by class D genes (*bla*_{OXA}, *bla*_{OXA-48}, and *bla*_{OXA-51}).

Whereas, no *E. coli* isolates possessing class A carbapenemases (*bla*_{KPC}, *bla*_{NMC}, *bla*_{IMI}, and *bla*_{GES}) were observed. In this class, the gene *bla*_{NMC} was first identified in *E. cloacae* and is primarily associated with this genus, although there are sporadic reports of its presence in other species (Nordmann *et al.*, 1993). Similarly, variants of the *bla*_{IMI} gene are often found encoded in the chromosomes of environmental *Enterobacter* species. These genes lack the efficient plasmid mobilization mechanisms that characterize epidemic resistance genes such as *bla*_{KPC}, *bla*_{NDM} and *bla*_{OXA-48} (Feng *et al.*, 2016; Tijet *et al.*, 2016). The detection of *bla*_{IMI} in clinical *E. coli* isolates is uncommon worldwide, and there is no evidence of association with mobile elements (Queenan and Bush, 2007; Jean *et al.*, 2023), which may explain their absence in this study. Although *bla*_{KPC} carbapenemases are the most clinically prevalent among class A carbapenemases that are encoded by plasmids. KPC enzymes are most frequently detected in *K.*

pneumoniae, they are increasingly being identified in *E. coli*. The first report of KPC in *E. coli* appeared in 2006 (Kuskucu *et al.*, 2016). Several studies conducted in Iraq have shown that the *bla*_{KPC} is absent in clinical *E. coli* isolates (Al-Muhanna *et al.*, 2017; Albayaty *et al.*, 2021; Al-Fatlawi *et al.*, 2023); consistent with our findings. Comparable to previous global reports, the *bla*_{KPC} was the least detected among carbapenemase-producing *E. coli*, even in some studies none of *bla*_{KPC} was detected (Rimrang *et al.*, 2012; Zeighami *et al.*, 2015; Gheitani *et al.*, 2018; Tavakoly *et al.*, 2018; Masseron *et al.*, 2019). The first report of KPC-producing *E. coli* in Iraq describes in Al-Diwaniya city, which found among 39 carbapenems resistant *E. coli* isolates 6 (15.38) were KPC positive (Al-Mayahi, 2013). In recent study in Wasit city found that among 38 UPEC, 6 (15.7%) were *bla*_{KPC} positive (Al-Mayahie, 2022). A study in Turkey reported that among 24 carbapenems resistant *E. coli* isolates, only two were found to be positive for KPC, and both were isolated from the same patient (Kuskucu *et al.*, 2016). Additionally, the frequency of KPC in Spain and Germany was reported at 1.7% and 28.8%, respectively, among *E. coli* isolates (Kaase *et al.*, 2016; Ortega *et al.*, 2016). A probable explanation for the no detection of the *bla*_{KPC} among carbapenems resistant isolates in this study might be that these isolates contain other classes of carbapenemase.

Although GES enzymes are also plasmid-encoded members of class A carbapenemases (Bedenic *et al.*, 2014), they are less common on a global scale but have been reported in diverse geographical regions and bacterial species, including *E. coli*, *P. aeruginosa*, and *A. baumannii* (Al-Agamy *et al.*, 2014; Naas *et al.*, 2016; Findlay *et al.*, 2022). Surveillance for the *bla*_{GES} gene, particularly the carbapenemase-producing variants, is not as frequently reported in Iraqi studies as it is for KPC, NDM, or OXA-48. While some studies examining ESBLs have occasionally detected *bla*_{GES}, often in the context of ESBLs, reports confirming the presence of carbapenemase-

producing *bla*_{GES} variants in clinical *E. coli* remain scarce in Iraq (Al-Kadmy *et al.*, 2023). The absence of such findings in this study further contributes to the limited data suggesting that *bla*_{GES} is currently not a significant factor driving carbapenem resistance in *E. coli* in Iraq.

The findings of this study underscore a significant public health concern regarding the prevalence of MBLs genes among carbapenems resistant *E. coli* isolates in Thi-Qar. Our findings shows that isolates carrying the *bla*_{NDM-1} constituted 60% of the *E. coli* isolates studied (Table 4-8). Globally, the prominence of *bla*_{NDM} in *E. coli* has been well-documented, particularly in endemic regions. For instance, a study conducted in India reported that of 312 carbapenems resistant Gram-negative bacteria tested, 90.3% exhibited carbapenem resistance, with *bla*_{NDM} being the dominant carbapenemase, accounting for 63% of cases (Garg *et al.*, 2019). Other study in North India, involving 62 isolates from international patients revealing that 23 (37.10%) exhibited resistance to carbapenems. Among these 23 carbapenems resistant isolates, 13 (56.52%) were from Iraqi patients. Of the isolates from Iraq, 6 (46.15%) contained the *bla*_{NDM} gene (Jaggi *et al.*, 2019). In Thailand, a multicenter study covering 11 provinces from 2012 to 2017 reported that 64% (493/766) of carbapenems resistant Enterobacteriaceae were found to have the *bla*_{NDM} (Takeuchi *et al.*, 2022). In Nepal, among 250 clinical isolates of *E. coli*, 38 were resistant to carbapenems; all (100%) of these isolates carried the *bla*_{NDM} gene, indicating a concerning prevalence rate (Shrestha *et al.*, 2017). In Pakistan, *bla*_{NDM} genes were detected in 52 out of 113 clinical isolates of *E. coli* from pediatric patients, representing 46% of isolates (Nosheen *et al.*, 2021). Furthermore, a study from Bangladesh reported that 50% of carbapenems resistant uropathogens contained the *bla*_{NDM} gene (Begum and Shamsuzzaman, 2016). The spread of *bla*_{NDM} is also evident in other geographical regions, such as Russia, where 71.92% of carbapenems resistant *E. coli* isolates were found to possess the

*bla*_{NDM} gene, which accounted for two-thirds of all carbapenemases (Kuzmenkov *et al.*, 2021). Consistent findings have emerged from Saudi Arabia, with study indicating that among 142 of *E. coli* from diabetic patients 109 (76.7%) carried the *bla*_{NDM} gene (Alzahrani *et al.*, 2022). Similarly, an analysis at an Egyptian cancer center revealed that *bla*_{NDM} was the most common carbapenemases, found in 68.88% of 135 Enterobacteriaceae isolates (Tawfick *et al.*, 2020). Amladi *et al.* (2019) noted that among 18 *E. coli* isolates, the most frequently detected gene was *bla*_{NDM}, present in 68.5% of the specimens. Zanganeh *et al.* (2021) found that out of 12 carbapenems resistant *E. coli* isolates, 9 (75.0%) contained the *bla*_{NDM} genes.

Lower results were observed in the following studies. In the SMART global surveillance program, the *bla*_{NDM} gene was identified as one of the most common carbapenemase-encoding genes, accounting for 19.42% of carbapenemase positivity (Karlowsky *et al.*, 2017). In Switzerland and Germany, NDM-producing *E. coli* isolates comprised 18% (22 /122) and 20% (14/71), respectively, of the identified carbapenemase-producing Enterobacteriaceae (Chakraborty *et al.*, 2021). In Korea, an analysis of 3675 carbapenems resistant Enterobacteriaceae strains collected from 2017 to 2018, the composition was as follows: 57.06% *K. pneumoniae*, 18.72% *E. coli*, 7.97% *E. cloacae* complex, and 2.94% *C. freundii*, of which *bla*_{NDM} accounted for 21.3% (Kim *et al.*, 2021). In China, 31% of 1105 tested carbapenems resistant Enterobacteriaceae strains were positive for *bla*_{NDM} (Zhang *et al.*, 2017). In Taiwan, from 2016 to 2018, *bla*_{NDM} was reported as the predominant carbapenemases (39.1%) among *E. coli* isolates (Huang *et al.*, 2021). In Kuwait, the prevalence of *bla*_{NDM}-producing *E. coli* was found to be 19% (Jamal *et al.*, 2016). Conversely, among 50 carbapenems resistant UPEC isolates in Iran, no *bla*_{NDM} genes were reported (Nojoomi and Ghasemian, 2017).

Estimating the current magnitude of the NDM-producing *E. coli* epidemic in Iraq is difficult due to varying rates of gene prevalence reported in different studies and the lack of national surveillance. The first report of NDM-producing *E. coli* in Baghdad was published in 2017, indicating a prevalence of the *bla*_{NDM} gene at 40% (Hussein, 2017). The high rate of *bla*_{NDM-1} observed in our finding study aligns with a systematic review on carbapenem resistance in Iraq, which reported the highest rates of *bla*_{NDM} at 67.27% in Baghdad, while 50% for both Waist and Kurdistan region (Hasan *et al.*, 2023). Additionally, a study examining carbapenems resistant *E. coli* isolates across six hospitals in Basrah found an even higher prevalence of the *bla*_{NDM} gene at 77.77% (Abas and Al-Hamdani, 2017). On the other hand, a study conducted in Mosul city reported a lower rate, with *bla*_{NDM} found in only 16.3% of the isolates (8 out of 49) (Al-Hasso, 2023). In Karbala city, among 24 *E. coli* specimens tested, 16.67% were attributed to *bla*_{NDM} (Taqi, 2023). Additionally, a study in Erbil city found that 12.2% of *E. coli* isolates tested positive for the *bla*_{NDM} (Ahmed *et al.*, 2023). In contrast, a study by Al-Sa'ady *et al.* (2020) analyzed 300 isolates and found that 11 (3.66%) were carbapenemase-producing *E. coli*; however, none of these carried the NDM gene.

NDM-positive Enterobacteriaceae found in both human and animal gastrointestinal tracts may serve as environmental reservoirs and sources for the dissemination of *bla*_{NDM} (Ruppe *et al.*, 2014; Wang *et al.*, 2017). The increase in NDM-producing *E. coli* isolates noted in this study is likely due to the horizontal gene transfer of the *bla*_{NDM}, which is carried on mobile genetic elements, which are vital vectors in disseminating *bla*_{NDM} variants across various geographic regions and among diverse hosts (Ho *et al.*, 2018; Wu *et al.*, 2019). Zong *et al.* (2021) suggested that the capacity to accumulate resistance genes, the interaction with plasmid resistance-encoding genes, and selective pressure could contribute to the spread of carbapenems resistant

Enterobacteriaceae, which explains the significant variation of enzyme genes across different countries and regions.

The present study identified *bla*_{VIM} as one of MBLs present. Although *bla*_{VIM} is prevalent and has been reported from numerous geographic locations, our findings reveal that only 5.00% of carbapenems resistant *E. coli* isolates in our study harbored the *bla*_{VIM} gene (Table 4-8). This finding is consistent with similar observations from Baghdad, which reported 5% positivity among 40 *E. coli* isolates sourced from the Tigris River (Mahdi *et al.*, 2023). A study in Morocco found that 5.73% of 112 Enterobacteriaceae identified as carbapenemase producers were positive for *bla*_{VIM} (Loqman *et al.*, 2021). In contrast, higher prevalence rates of the *bla*_{VIM} gene have been documented in various studies. For example, in Basrah, 13 (59.1%) out of 22 *E. coli* isolates from cancer patients were found to possess the *bla*_{VIM} gene (Albadery *et al.*, 2023). Similarly, in Baghdad, 12 (24%) out of 50 *E. coli* isolates tested positive for the *bla*_{VIM} (Hammadi *et al.*, 2015). Another report indicated that *bla*_{VIM} was detected in 23 (54.7%) of 42 Gram-negative bacteria in Baghdad, including 6 (26.9%) of *E. coli* (Hasoon and Hamed, 2019). In Mosul, *bla*_{VIM} was detected in 1 (16.66%) of *E. coli* isolates among 6 VIM-producing Gram-negative bacilli (Al-Hasso *et al.*, 2023). In Kuwait, 3 (27.27%) VIM-positive *E. coli* were reported from Enterobacteriaceae isolates (Jamal *et al.*, 2013). Furthermore, in Iran, out of 28 *E. coli* isolates from fecal specimens of immunocompromised children, 7 (50%) of the 14 carbapenems resistant isolates tested positive for *bla*_{VIM} (Almasian Tehrani *et al.*, 2023). A survey in Germany conducted from 2012 to 2013 reported that among 73 carbapenems resistant Gram-negative bacteria, only one (14.29%) of the seven *E. coli* isolates tested positive for *bla*_{VIM} (Hauri *et al.*, 2014). A study in Spain revealed that 27 (22.3%) out of 121 carbapenemase-producing *E. coli* isolates were identified as *bla*_{VIM} positive (Ortega *et al.*, 2016). In Nigeria, the *bla*_{VIM} gene constituted 11.1% of 27 *E. coli* isolates

linked to foodborne illness cases (Nwike *et al.*, 2023). Slightly lower prevalence rates have been observed in different studies; for instance, a study in Iran indicated a *bla*_{VIM} prevalence of 3% among *E. coli* isolates from septicemic patients (Eslami, 2018). Conversely, some studies have reported an absence of the *bla*_{VIM} gene among tested *E. coli* isolates. For instance, in Iraq, Al-Sa'ady *et al.* (2020) found that none of the 11 carbapenemase-producing *E. coli* isolates contained the *bla*_{VIM}. Similarly, Hammadi *et al.* (2015) reported a complete absence of the *bla*_{VIM} gene in 28 *E. coli* isolates. Recent studies in Thi-Qar and Baghdad have also noted the absence of *bla*_{VIM} amplification in carbapenems resistant *E. coli* isolates (Hassan *et al.*, 2025; Mhawesh *et al.*, 2021). In Jordan, a study found that all UPEC isolates were negative for *bla*_{VIM} (Nairoukh *et al.*, 2018).

In the current study, the notable absence of other MBL genes, specifically *bla*_{IMP}, *bla*_{SPM}, *bla*_{GIM}, and *bla*_{SIM}, among carbapenems resistant *E. coli* isolates. The geographical prevalence of these MBLs is a critical aspect; for instance, while *bla*_{IMP}, which was first discovered in Japan in 1990 (Watanabe *et al.*, 1991), has been widely reported, its distribution remains somewhat restricted, particularly in regions such as the Asia-Pacific (Zavascki *et al.*, 2010). Other genes like *bla*_{SPM}, *bla*_{GIM}, and *bla*_{SIM} are much rarer in both *E. coli* and across bacterial species in general. Specifically, *bla*_{SPM} has mostly been reported in Brazil, *bla*_{GIM} sporadically in Germany, and *bla*_{SIM} is exceedingly rare on a global scale (Pitout *et al.*, 2015; Kazmierczak *et al.*, 2022; Gales *et al.*, 2022). While MBLs like *bla*_{NDM} and *bla*_{VIM} demonstrate high plasmid promiscuity and adaptability across various Enterobacterales species, including *E. coli*, the successful establishment and spread of rarer MBLs such as SPM, GIM, and SIM may be hindered by factors related to plasmid fitness, gene expression efficiency, or stability within the specific genetic background of *E. coli* (Mairi *et al.*, 2018). These genes may be better adapted to or more commonly found on plasmids

associated with other bacterial hosts like *P. aeruginosa* (for SPM, GIM, SIM). Globally, antimicrobial resistance is characterized by NDM and KPC as the predominant carbapenemases in Enterobacterales, with OXA-48-like enzymes also being significant. This context further emphasizes the comparatively minimal relevance of the aforementioned MBLs (Kazmierczak *et al.*, 2022). Comparative studies reveal varying prevalence rates of MBL genes across different regions. For example, a study from Ahvaz, Iran, indicated that out of 12 carbapenems resistant *E. coli* isolates, 11 possessed MBL genes, including *bla*_{IMP}, while none had *bla*_{SIM}, *bla*_{SPM}, or *bla*_{GIM} (Zangane Matin *et al.*, 2021). Conversely, research conducted in Qom, Iran, did not identify *bla*_{IMP} in *E. coli* resistant to imipenem, which aligns with our findings (Shams *et al.*, 2018). Similarly, a study in Seoul, South Korea, evaluated 50 carbapenemase-producing *E. coli* strains, finding only one strain with *bla*_{NDM}, while other carbapenemase genes, including *bla*_{IMP}, *bla*_{VIM}, *bla*_{SPM}, *bla*_{GIM}, *bla*_{SIM}, and *bla*_{KPC}, were absent (Shin *et al.*, 2021). In China, from 2010 to 2015, only one of 207 *E. coli* strains carried the *bla*_{KPC}, while MBL genes not detected (Li *et al.*, 2021). In Pakistan, a high prevalence of MBL-producing *E. coli* was reported, with only two strains identified with the *bla*_{IMP} gene out of 145 tested isolates (Nahid *et al.*, 2013).

In the context of Iraq, a study conducted in Baghdad found no amplification of *bla*_{IMP} in any of the 11 carbapenems resistant *E. coli* isolates (Mhawesh *et al.*, 2021). Recent findings from Thi-Qar reported five XDR *E. coli* isolates resistant to imipenem and meropenem, all harboring the *bla*_{NDM} gene, while other MBL genes (*bla*_{GIM}, *bla*_{SIM}, *bla*_{SPM}, and *bla*_{IMP}) were absent (Hassan *et al.*, 2025). Conversely, in Basrah, although *bla*_{VIM} (59.1%) was present in the majority of *E. coli* isolates from cancer patients, *bla*_{IMP} (22.73%) and *bla*_{SPM} (18.18%) were also found (Albadery *et al.*, 2023). The presence of these genes is particularly evident in *P. aeruginosa* in a study

that indicated 100% of isolates carried the *bla*_{VIM} gene, with a significant proportion also possessing *bla*_{GIM} and *bla*_{SPM} genes, while *bla*_{IMP} was not detected among carbapenems resistant isolates (Kareem *et al.*, 2025). In hospitals in Sulaimaniyah, *bla*_{IMP} was found in 16.6% of carbapenems resistant *P. aeruginosa* strains, and *bla*_{SIM} was detected in a smaller subset; however, there was no presence of *bla*_{SPM} or *bla*_{GIM} (Omer *et al.*, 2024). In Thi-Qar, among 20 *P. aeruginosa* isolates, the *bla*_{SPM} gene was found in 2 isolates (7.4%). No PCR products were observed for the *bla*_{IMP}, *bla*_{GIM}, and *bla*_{SIM} genes (Abid Al-Aziz *et al.*, 2020).

The current investigations highlight the presence of various oxacillinases genes, notably *bla*_{OXA}, *bla*_{OXA-48}, and *bla*_{OXA-51}, in carbapenems resistant *E. coli* isolates. The results indicate that *bla*_{OXA} (ESBL) is the second most prevalent gene, detected in 40% of carbapenems resistant *E. coli* isolates (Table 4-8). Although *bla*_{OXA} is not strictly classified as a carbapenemase, its contribution to enhanced carbapenem resistance is noteworthy. It is often found on large plasmids alongside genes for ESBLs (e.g., *bla*_{CTX-M}), AmpC β -lactamases (e.g., *bla*_{CMY}), and crucially, carbapenemases (e.g., *bla*_{NDM}, *bla*_{OXA-48}) or genes that confer permeability defects (Khan *et al.*, 2017; Findlay *et al.*, 2021). When *bla*_{OXA} is present in conjunction with these genes, the combined effect can significantly increase the minimum inhibitory concentration of carbapenems, potentially reaching levels of resistance (Findlay *et al.*, 2021). Moreover, the presence of a true carbapenemase in conjunction with *bla*_{OXA} would definitively confer resistance, suggesting that its detection may serve as an indicator for these plasmids carrying multiple resistance determinants. Additionally, studies from Lebanon (Alousi *et al.*, 2023) and Turkey (Gür *et al.*, 2022) found *bla*_{OXA} alongside *bla*_{NDM} or *bla*_{OXA-48} in carbapenems resistant Enterobacteriaceae, suggesting that *bla*_{OXA} may complement the resistance profiles. Recent study conducted in Iran found a significant correlation

between the presence of the *bla*_{OXA} and resistance to imipenem in *E. coli* isolates. Among 126 PCR-tested isolates, 10 (7.9%) harbored *bla*_{OXA}, while no other OXA types (OXA-2, OXA-10, and OXA-48), as well as *bla*_{NDM} or *bla*_{VIM} genes, were detected (Sadeghi *et al.*, 2023). A local research in Iraq has echoed these findings, where a study in Babylon and Karbala provinces found that all carbapenems resistant *K. pneumoniae* were positive for *bla*_{OXA} (Abbas, 2021). Several studies in Iraq have reported varying prevalence rates of the *bla*_{OXA}. For instance, Fayroz-Ali (2012) identified *bla*_{OXA} in 40 out of 51 *E. coli* isolates (62.5%). A study conducted in Najaf province, 19 out of 20 isolates of *E. coli* (95%) were found to have the *bla*_{OXA} (Hasnawi and Alhilo, 2025). In another study in Al-Najaf among 29 *E. coli* isolates revealed that 26 (89.65%) tested positive for *bla*_{OXA} (Doush and Tuwaij, 2023). Conversely, Al-Muhannak (2010) reported that only 17 (27.4%) of the 62 potential ESBL-producing clinical enteric isolates carried the *bla*_{OXA}. In Thi-Qar, the *bla*_{OXA} gene was detected in 5 (19.2%) out of 26 ESBL producers (Lhwak, 2017). In Wasit province, 8 (17.8%) out of 45 *E. coli* isolates from pediatric patients with UTIs were found to carry the *bla*_{OXA} (Nasser *et al.*, 2022). Global reports also highlight varying prevalence rates: Brazil reported a rate of 60% (Flores *et al.*, 2016), Portugal had a rate of 55% (Oliveira *et al.*, 2019) and Saudi Arabia showed 54.8% (Al-Agamy *et al.*, 2018). Notably, lower prevalence rates were observed In India, Chaudhary and Payasi (2014) found that out of 49 UPEC, only 5 (10.2%) included the *bla*_{OXA}. Similarly, Alizade *et al.* (2015) reported that 17.2% of UPEC in Kerman, Iran, were *bla*_{OXA} producers. In the United Kingdom, among 32 MDR *E. coli* strains, the *bla*_{OXA} was present in 12 isolates (34.2%) (Ibrahim *et al.*, 2023).

This study identified the *bla*_{OXA-48} in 15% of carbapenems resistant *E. coli* isolates, which encodes a carbapenem-hydrolyzing enzyme. This finding raises considerable concern due to its challenging detection and its

association with treatment failures. This finding supports existing research recognizing OXA-48 as a notable contributor to carbapenem resistance across various regions. For instance, a study in northern Iran reported a prevalence of *bla*_{OXA-48} in UPEC isolates at 15.3% (Paskeh *et al.*, 2020). Similarly, Damavandi *et al.* (2016) reported rates of 11% among hospitalized patients and 6% in outpatients in Iran. Higher rates of prevalence was noted in Peirano *et al.* (2014) that found out of 116 *E. coli* isolates, 30 (26%) tested positive for *bla*_{OXA-48}. Chaudhary and Payasi (2014) reported a prevalence of 32.6% among 49 *E. coli* isolates in India. In an extensive analysis conducted at an Egyptian Cancer Center, *bla*_{OXA-48} was identified as the most common carbapenemase, found in 32.59% of 135 Enterobacteriaceae isolates (Tawfick *et al.*, 2020). In India, Singh *et al.* (2023) reported that, among 500 studied isolates, 199 (39.8%) were positive for *bla*_{OXA-48}, with 38.9% are *E. coli* and 41.08% are *K. pneumoniae*. In Pakistan, Gondal *et al.* (2022) reported that the *bla*_{OXA-48} was the second most prevalent carbapenemase gene found in *E. coli*, with a prevalence rate of 27% (28 out of 104 isolates). A study in North India analyzed 62 isolates from international patients, identifying 23 carbapenems resistant *E. coli* isolates. Among these, 13 (56.52%) were from Iraqi patients, including 7 (53.84%) that carried the *bla*_{OXA-48} (Jaggi *et al.*, 2019). In contrast, a study conducted in China revealed a lower occurrence of *bla*_{OXA-48}, found in 7.3% (68/935) of Enterobacteriaceae (Han *et al.*, 2020). In Iran, Nojoomi and Ghasemian (2017) documented an 8% prevalence of *bla*_{OXA-48} in 50 imipenem-resistant *E. coli* isolates. In Iraq, there is significant variability in the rates of *bla*_{OXA-48} across different regions. A systematic review on carbapenem resistance in Iraq highlighted a high rates of the *bla*_{OXA-48} in specific locations, with Baghdad reporting a prevalence of 73.8% and Wasit showing 57.8% of *E. coli* isolates (Hasan *et al.*, 2023). In Basrah province, *bla*_{OXA-48} was detected in 28 out of 61 (45.9%) carbapenems resistant gram-negative bacteria,

including 6 (9.83%) *E. coli* (Abas and Al-Hamdani, 2017). Mhawesh *et al.* (2023) reported a lower prevalence of *bla*_{OXA-48}, finding it in only 3% of 35 carbapenems resistant and 5 carbapenem susceptible *E. coli* isolates. In Duhok city, *bla*_{OXA-48} was identified in 6 (12%) of 123 carbapenems resistant *K. pneumoniae* isolates (Al-Hassan *et al.*, 2020). Notably, in Najaf hospitals, the *bla*_{OXA-48} was found to be the most prevalent carbapenemase gene, detected in 9 (41%) of *K. pneumoniae* isolates (Al-Hasnawi, 2020). Meanwhile, a study from Baghdad noted that out of 27 *P. aeruginosa* isolates, 26 (96%) were confirmed as *bla*_{OXA-48} producers, and all tested *K. pneumoniae* isolates (100%) were positive for this gene (Al-Ouqaili *et al.*, 2018).

Unlike most OXA genes, *bla*_{OXA-48} is not found in a gene cassette within integrons (Naas and Nordmann, 1999; Poirel *et al.*, 2004). However, the spread of *bla*_{OXA-48} is attributed to successful global clone dissemination and horizontal gene transfer (Poirel *et al.*, 2012; Pitout *et al.*, 2019). Nowadays, OXA-48 is considered one of the “Big Five” carbapenemases (*bla*_{NDM}, *bla*_{KPC}, *bla*_{OXA-48}, *bla*_{VIM}, and *bla*_{IMP}) and is a significant concern in clinical settings worldwide. Surveillance studies employing molecular methodologies for identifying carbapenemases have shown that OXA-48 β -lactamases rank as the second or third most common carbapenemases among global Enterobacterales (de Jonge *et al.*, 2016; Karlowisky *et al.*, 2017; Boyd *et al.*, 2022; Peirano *et al.*, 2022).

The last class D β -lactamase gene examined in this investigation was the carbapenem-hydrolyzing *bla*_{OXA-51}. Although the *bla*_{OXA-51} is primarily considered to be chromosomally encoded and intrinsic to *A. baumannii* (Merkier and Centrón 2006; Patel and Bonomo, 2011). However, several reports indicate that *bla*_{OXA-51} have been mobilized and are spreading to non-*baumannii* species of *Acinetobacter* through conjugative plasmids (Lee *et al.*, 2012). Recent studies have also shown that *bla*_{OXA-51} can be found in

some Enterobacteriaceae species and *P. aeruginosa* (Leski *et al.*, 2013; Chaudhary *et al.*, 2014), largely due to the gene's frequently linked insertion sequence, ISAbal that may function as a promoter. Beyond facilitating gene transfer, ISAbal is also associated with carbapenem resistance, as it can induce overexpression of intrinsic *bla*_{OXA-51} by 50 fold (Evans *et al.*, 2013). Budak *et al.* (2012) were the first to identify OXA-51 in non-*A. baumannii* species. In their study, a patient infected with MDR *A. baumannii* was treated with an extended-spectrum β -lactam and β -lactamase inhibitor combination. However, two weeks later, an ertapenem-resistant *K. pneumoniae* isolate was discovered in the same patient. PCR and sequencing revealed the presence of both *bla*_{OXA-51} and *bla*_{CTX-M-15} β -lactamase genes. Plasmid analysis indicated two plasmids were present, measuring 40 kb and 1.2 kb respectively. Their study suggests that the primary reason for carbapenem resistance in this case was the *bla*_{OXA-51}. Genetic events such as recombination, co-integration, and transposition may insert these genes into the chromosome of *K. pneumoniae* (Budak *et al.*, 2013).

In the current investigation, only two (5%) of the carbapenems resistant *E. coli* isolates harbored the *bla*_{OXA-51}. Globally, few studies have assessed the prevalence of *bla*_{OXA-51} in *E. coli*. Chaudhary *et al.* (2014) found that among 49 *E. coli* isolates, 5 (10.2%) tested positive for *bla*_{OXA-51}, while among 74 *P. aeruginosa* isolates, the prevalence was 5.4%. El-Badawy *et al.* (2020) studied *bla*_{OXA-51} in carbapenems resistant *K. pneumoniae* isolates in Egypt and found that 10.5% of the isolates produced OXA-51. El-Hendawy *et al.* (2016) reported that 10.53% (2/19) of carbapenems resistant *K. pneumoniae* isolates from hospital specimens in Egypt carried *bla*_{OXA-51}. In a Sudanese study, 6 out of 60 *E. coli* isolates (10%) tested positive for *bla*_{OXA-51} (Khalid, 2023). Another study in Sudan revealed a *bla*_{OXA-51} rate of 27.8% (10/36) among carbapenems resistant clinical Gram-negative bacteria (Mohamed Elmutasim *et al.*, 2019). In Iran, a cross-sectional hospital-based

study reported that the frequency of *bla*_{OXA-51} in patients with urosepsis was 13%. The frequency of *bla*_{OXA-51} among isolates included *P. aeruginosa* at 25% (1/4), *K. pneumoniae* at 17% (1/6), *E. coli* at 8% (3/37), and *P. mirabilis* at 10% (1/10) (Ibrahim *et al.*, 2022). Another Iranian study reported that 3 (23.1%) out of 13 *E. coli* isolates and 3 (11.1%) out of 27 *K. pneumonia* isolates carried a gene encoding OXA-51 enzymes (Pourbaghi *et al.*, 2022). A study in an Iranian hospital found that among 141 bacterial isolates, exhibited carbapenem resistance. The *bla*_{OXA-51} was most prevalent in *E. coli* (15/81, 19%), followed by *A. baumannii* (7/15, 47%) and *K. pneumoniae* (2/16, 13%) (Setareh *et al.*, 2025). In contrast to our results, the study conducted by Cetinkol *et al.* (2016) did not observe *bla*_{OXA-51} in carbapenems resistant *K. pneumoniae* isolates. Additionally, research in India found that *bla*_{NDM} were present in 88 (91.66%) of *E. coli* isolates, while the *bla*_{OXA-48} was found in 8 (8.33%) isolates. Other genes, such as OXA-23 and OXA-51, were absent (Tiwari *et al.*, 2025). In Iraq, Al-Hasnawy *et al.* (2024) reported the detection of the *bla*_{OXA-51} gene in all 20 *A. baumannii* isolates, indicating a prevalence of 100%. The findings from these studies suggest the interspecific dissemination of the *bla*_{OXA-51} gene, which aligns with the current study's results. However, to the best of our knowledge, this is the first report of the occurrence of the *bla*_{OXA-51} gene in carbapenems resistant *E. coli* from Thi-Qar.

This study revealed that the primary mechanism of resistance to carbapenems in *E. coli* is the production of carbapenemases, as demonstrated by both phenotypic and genotypic methods. However, 6 out of 40 carbapenems resistant isolates (15%) were found to be incapable of harboring carbapenemase genes through molecular testing. This suggests the involvement of other resistance mechanisms or possibly the presence of other carbapenemase genes not included in this study. Further corroborating this finding, a recent study conducted in Iraq found that out of 300 *E. coli*

isolates, 11 (3.66%) were phenotypically resistant to carbapenems, but only 3 tested positive for carbapenemase genes (Mhawesh *et al.*, 2021). Similarly, Kuskucu *et al.* (2016) reported that only 7 of the 24 carbapenems resistant *E. coli* isolates (29.1%) were positive for carbapenemase genes. In another study, researchers examined a carbapenems resistant *E. coli* isolates from a patient with peritonitis and concluded that the resistance resulted from a combination of OmpC porins loss and AmpC (CTX-M) production (Lartigue *et al.*, 2007).

Worryingly, this study found the co-existence of genes for at least two carbapenemases in *E. coli* isolates. Specifically, 11 (61.11%) of the *bla*_{NDM-1} positive isolates also harbored the *bla*_{OXA}, while one isolate (5.56%) contained combinations of *bla*_{VIM} and *bla*_{NDM-1}, as well as *bla*_{NDM-1} with *bla*_{OXA-51}, and *bla*_{OXA} with *bla*_{OXA-51}. Additionally, two isolates (11.11%) were found to have *bla*_{VIM} with *bla*_{OXA} and *bla*_{OXA} with *bla*_{OXA-48} genes (Table 4-9). It is important to note that carbapenemase genes are part of complex transposons or integrons on conjugative plasmids, further facilitating their horizontal transfer. This mobile nature of carbapenemase genes likely contributes to the emergence of strains harboring two or sometimes even three resistance genes (Logan and Weinstein, 2017). However, whether these genes are carried on a single plasmid or located on separate plasmids remains unclear. It was previously described that the plasmids carrying the *bla*_{NDM} gene are diverse and can harbor numerous unrelated resistance genes, including *bla*_{OXA}, *bla*_{OXA-48}, and *bla*_{VIM}, AmpC, ESBLs, and genes for other classes of antimicrobials (Sonnevend *et al.*, 2015; Lutgring *et al.*, 2018; Lloyd *et al.*, 2018). Therefore, the diversity in plasmid content underscores the complex nature of resistance mechanisms and their evolution. The co-occurrence of genes for at least two classes of carbapenemases in *E. coli* has also been reported globally. In Pakistan, for instance, a study revealed the co-existence of *bla*_{NDM}/*bla*_{OXA-48} (15.4%,

16/104), *bla*_{NDM}/*bla*_{VIM} (2%, 2/104), and *bla*_{OXA-48}/*bla*_{VIM} (3.8%, 4/104) (Gondal *et al.*, 2022). In India, Kazi *et al.* (2014) revealed that 3.6% of the isolates possessed *bla*_{NDM} and *bla*_{VIM} genes. Moreover, Singh *et al.* (2023) found that *bla*_{OXA-48}-positive strains harbored various antibiotic resistance genes, including other carbapenemases (*bla*_{NDM}, *bla*_{IMP}, and *bla*_{VIM}) and ESBLs (*bla*_{TEM}, *bla*_{SHV}, and *bla*_{CTX-M}). Zanganeh Matin *et al.* (2021) reported the co-existence of *bla*_{OXA-48} and *bla*_{NDM} genes in four UPEC isolates. Studies from Iraq highlight similar issues, with findings from Baghdad documenting the coproduction of *bla*_{VIM} and *bla*_{OXA-48} in all carbapenems resistant *K. pneumoniae* isolates (Al-Ouqaili *et al.*, 2018). In another study in Baghdad, it was reported that 12 isolates (28.5%) harbored a combination of *bla*_{OXA-48} and *bla*_{VIM}. In contrast, one isolate (2.4%) co-harbored the *bla*_{OXA-48} and *bla*_{NDM} genes, and another isolate (2.4%) contained *bla*_{OXA-48}, *bla*_{VIM}, and *bla*_{NDM} genes (Hamed *et al.*, 2019). In Najaf province, carbapenems resistant *K. pneumoniae* isolates exhibited the co-existence of *bla*_{NDM} with *bla*_{VIM} (7.4%) and NDM with other oxacillinase genes, including *bla*_{OXA} (11.1%), *bla*_{OXA-48} (22.2%), and *bla*_{OXA-51} (37.1%). The presence of *bla*_{VIM} with *bla*_{OXA-51} genes was noted in 2 isolates (7.4%) (Mohanna and AL-Yasseen, 2024). Furthermore, studies from Mosul city indicate that isolates with multiple carbapenemase genes, such as *bla*_{KPC}/*bla*_{VIM} and *bla*_{KPC}/*bla*_{NDM}, were notably common (Al-Hasso, 2023).

5.8. Prevalence of Class 1 and Class 2 Integrins

In this study, class 1 integron was found to be predominant among the tested isolates, with 100% of isolates confirmed to have class 1 integron, while 13 isolates (32.50%) tested positive for class 2 integron (Table 4-12). Numerous investigations over the last decade have documented that the prevalence of class 1 integrons is generally higher than that of other classes, a finding that is consistent with the results of this study. Supporting studies have reported a 100% prevalence of class 1 integrons within MDR

Enterobacteriaceae, predominantly *E. coli* (Canal *et al.*, 2016; Firoozeh *et al.*, 2019; Vásquez-Ponce *et al.*, 2022). A study in China indicated a 100% prevalence of integron class 1 in *E. coli*, 100% in *P. aeruginosa*, 11% in *K. pneumoniae*, 18% in *A. baumannii*, and 33.3% in *E. cloacae* from clinical isolates (Xu *et al.*, 2009). Other studies have documented varying percentages for class 1 and 2 integrons within *E. coli* isolates. For example, a study in Egypt found class 1 integron prevalence at 98.33%, followed by class 2 at 8.33% (Abdel-Rhman *et al.*, 2020). In Iran, a study reported class 1 and class 2 integron prevalence rates of 86% and 8%, respectively (Ranjbaran *et al.*, 2013). Additionally, another Iranian study found that class 2 integrons were present in 14.1% (n=23) of 138 MDR *E. coli* isolates (Barzegar *et al.*, 2022). Moreover, in Iran, Razzaq *et al.* (2024) reported that the majority of detected integrons among *E. coli* isolates belonged to class 1 (62.2%), with class 2 observed in 18.9%. In Nepal, a study revealed that 92.3% (n=48/52) of the carbapenems resistant *E. coli* isolates carried the class 1 integron (Thapa *et al.*, 2024).

In Iraq, multiple studies have generally indicated a higher prevalence of these integron classes, although there are differences based on the source of infection and regional dissemination. In Babylon province, a study reported a class 1 integron prevalence of 99.1% (111 out of 112) among β -lactamase-producing *E. coli* isolates (Khulaif and Al-Charrakh, 2023). Another study in Najaf and Babylon hospitals found class 1 integrons were present in 98.3% of *E. coli* identified, while none of the isolates tested positive for class 2 integron (Al-Mamouri, 2019). A study by Alfarras and Al-Daraghi (2024) reported that 100% of *P. aeruginosa* isolates carried class 1 integron, compared to 10% that had class 2. Omar and Ibrahim (2023) indicated that among 15 *K. pneumoniae* isolates, 100% carried class 1 integron genes, while 45% (n=7) had class 2 genes. However, the prevalence rate in our study was slightly higher than that observed in a study by

Mohemmad *et al.* (2023), which found 89.4% of bacteria isolated from patients with bladder cancer in Thi-Qar hospitals carried class 1 integrons. A study from Najaf found that 82.7% of *E. coli* isolates had class 1 integrons, while the occurrence of class 2 integrons was 3.8% (Hadi *et al.*, 2020). A study conducted in Baquba city, Diyala province, showed that 16 (80%) of the extensively drug-resistant *E. coli* isolates in patients with diabetic foot ulcers were positive for the integron class 1, while class 2 integron were not found (Alsaadi *et al.*, 2021). Additionally, lower prevalence rates were reported in a study from Basrah province, which found that among 62 *E. coli* isolates from clinical and environmental sources, 32 (51.61%) contained class 1 integrons, and 5 (8.06%) harbored class 2 integrons (Othman and Abdulkhaliq, 2024). In Baghdad province, the presence of integrons in clinical pathogenic isolates was found in 74.4% of cases. Specifically, 55 (61.4%) of isolates harbored class 1 integrons, while 11 (13.3%) contained class 2 integrons (Nasif *et al.*, 2024). In Sulaimani city, a study involving 68 ESBL-producing Enterobacteriaceae found that the prevalence rate of class 1 integrons was 45 (66.2%), while class 2 integrons were present in 12 (17.6%) of the cases, predominantly in *E. coli*, which was the most common species harboring both classes (Abubaker and Anwar, 2023). Lastly, in Al-Diwaniya city, a study of 301 commensal *E. coli* isolates obtained from stools of healthy individuals revealed that 30 (10%) of these isolates contained class 2 integrons (Kazaal, 2018).

The high prevalence of antibiotic resistance patterns observed in this study may be attributed to integrons (Table 4-12). Bacterial strains that possess integrons are more likely to develop antibiotic resistance than those that do not a phenomena observed in *E. coli* (Souque *et al.*, 2021; Kubomura *et al.*, 2020). The incorporation of multiple cassettes into an integron is associated with resistance to various antibiotics. Additionally, carbapenem resistance is recognized as a key indicator of XDR and PDR Gram-negative

bacteria, as it often correlates with co-resistance to a wide range of other antimicrobial drugs (Tacconelli *et al.*, 2018). Consequently, integrons can confer resistance to commonly available antimicrobials, including β -lactams, fluoroquinolones, chloramphenicol, trimethoprim, and even carbapenems (Thapa *et al.*, 2024). These prevalence is higher than that reported in a study by Thapa *et al.* (2024), which found that among 48 integron class 1-positive *E. coli* isolates, only 3 (6.3%) showed XDR phenotypes, while 7 (14.6%) exhibited PDR phenotypes, with the remaining strains demonstrating MDR patterns. Another study conducted in Babylon province reported that the majority of 111 *E. coli* isolates carrying class 1 integrons exhibited MDR, with only 8 (7.1%) isolates demonstrating an XDR phenotype and 4 (3.6%) exhibiting PDR (Khulaif and Al-Charrakh, 2023).

In the current study, all carbapenems resistant *E. coli* carried class 1 integrons, and consequently, all carbapenemase genes found were associated with this integron class. Carbapenemase-encoding genes are predominantly located in mobile genetic elements such as bacterial plasmids, integrons, and transposons. They can easily spread among bacteria when the outer membrane is damaged and/or permeability increases, which explains the widespread dissemination of carbapenems resistant Enterobacteriaceae globally (Carattoli, 2013; Antunes *et al.*, 2014). The predominance of *bla*_{NDM-1} is not surprising, as it is particularly associated with class 1 integron gene cassettes and plasmids, which facilitate their mobilization (Lopez-Diaz *et al.*, 2022). Furthermore, the presence of the *bla*_{VIM}, though only found in 2 isolates (5%) that harbored both classes of integrons in our study, is noteworthy. Several studies have reported that the *bla*_{VIM} gene is carried as mobile gene cassettes inserted in class 1 integrons (Bolourchi *et al.*, 2021; Wei and Wang, 2013). While Oxacillinase-encoding genes are generally not associated with integrons and are typically encoded on the chromosome, some reports indicate their association with plasmids (Walther-Rasmussen

and Høiby, 2006). Interestingly, *bla*_{OXA} are usually found in non-Enterobacteriaceae bacteria. Their ability to rapidly mutate and broaden their spectrum of activity (Diene and Rolain, 2014) makes their occurrence among *E. coli* particularly noteworthy, even in this study. Thapa *et al.* (2024) reported that among the integron-positive population, the prevalence of the *bla*_{NDM}, *bla*_{OXA-23}, *bla*_{OXA-48}, and *bla*_{VIM}-encoding genes was 79.2% (38/48), 62.5% (30/48), 54.2% (26/48), and 6.2% (3/48), respectively. In Najaf province, a study showed that carbapenems resistant genes including *bla*_{OXA-48} and *bla*_{VIM} were identified in isolates carried class 1 integron (Hadi, 2015).

CONCLUSIONS

AND

RECOMMENDATIONS

Conclusions

The following conclusions can be drawn based on the findings:

1. Carbapenems resistant *E. coli* infections are demonstrably prevalent among patients admitted to hospitals in Thi-Qar.
2. These isolates exhibit high resistance levels to a wide range of antimicrobial agents.
3. The alarming rates of XDR and PDR highlighted in this study pose a significant therapeutic challenge for patient management.
4. Carbapenemase production was confirmed as the dominant mechanism of resistance among carbapenems resistant *E. coli* isolates. Most harbored at least one carbapenemase-encoding gene, primarily *bla*NDM-1, *bla*VIM, *bla*OXA-48, and notably, *bla*OXA-51. A high prevalence of the *bla*OXA β -lactamase gene was also observed among the isolates.
5. A high frequency of integrons, Class 1 and Class 2, was detected among the isolates, suggesting their potential role in mediating MDR.

Recommendations

1. Establishing and enforcing comprehensive antimicrobial stewardship programs in all healthcare facilities in Iraq, focusing on promoting evidence-based prescribing, reducing broad-spectrum agents, and restricting carbapenems use, to prevent the transmission of MDR/XDR/PDR isolates.
2. Developing systematic national surveillance programs utilizing molecular techniques to monitor the prevalence of key carbapenemase genes and integrons.
3. Conducting further studies to characterize non-carbapenemase mechanisms of carbapenems resistance in local isolates.
4. Performing sequencing of the variable regions of identified integrons to determine the specific cassette arrays of antibiotic resistance genes they carry.
5. Extending surveillance efforts to include other clinically significant Enterobacteriaceae species beyond *E. coli* to understand the full burden of carbapenems resistance.

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الخلاصة

تعد الكاربابينيمات من اقوى المضادات الحيوية التابعة لصنف البيتا-لاكتام، وتعتبر حاليًا من الأدوية المفضلة لعلاج أخطر أنواع العدوى التي تسببها البكتيريا متعددة المقاومة. في الوقت الحاضر تعد مقاومة المضادات الحيوية تحديا كبير في معالجة العدوى البكتيرية. وتشكل مقاومة الكاربابينيم في بكتريا *Escherichia coli* تهديداً متزايدا لنظام الرعاية الصحية بسبب محدودية الخيارات العلاجية المتاحة. هدفت هذه الدراسة لتحديد مدى انتشار وخصائص عزلات *E. coli* المقاومة للكاربابينيم المعزولة من مختلف العينات سريرية في مستشفيات ذي قار.

أُجريت هذه الدراسة المستعرضة على ٢٢٠٣ عينة سريرية تم جمعها عشوائيًا من مرضى الراقيدين او الذين راجعوا لمستشفيات رئيسيين في ذي قار خلال فترة عشر أشهر في عام ٢٠٢٤. وباستخدام الاختبارات المظهرية والكيموحيوية التقليدية تم تشخيص عزلات *E. coli* وتأكيدها بواسطة جهاز VITEK-2. كما تم تقييم الحساسية الدوائية لهذه العزلات باستخدام طريقة انتشار الاقراص (Discs Diffusion Method) ضد ثلاثة وعشرين مضادًا حيويًا عائدة الى إحدى عشرة صنف. كما تم التحري مظهرياً عن انتاج انزيمات البيتا-لاكتاميز واسعة الطيف (ESBLs)، وانزيمات البيتا-لاكتاميز من نوع (AmpC)، وانزيمات الكاربابينيم في العزلات باستخدام طرق انتشار الأقراص. كما تم التحري عن وجود جينات المقاومة للكاربابينيم والانتيكرون (النوع الاول والنوع الثاني) باستخدام تقنية تفاعل انزيم البلمرة المتسلسل (PCR). أرسلت نتائج هذا الاختبار الخاصة بجينات (bla_{NDM-1}) و (bla_{OXA}) من العزلات الموجبة لمعرفة التسلسل الجيني فيها.

أظهرت النتائج وجود ١٨٦ (١٥,٣٥%) عزلة من بكتيريا *E. coli* من اجمالي العينات السريرية المختلفة. وكانت نسبة المقاومة عالية لمعظم للمضادات الحيوية المدروسة وتراوحت هذه المقاومة من ١١,٢٩% ضد ايميبينيم (Imipenem) الى ١٠٠% ضد الامبيسلين (Ampicillin). تم تحديد مقاومة الكاربابينيم في ٤٠ (٢١,٥١%) عزلة، وقد لوحظت أعلى معدلات المقاومة في الدوريبينيم (Doripenem) (٨٠,٠٠%)، تليها ميروبينيم (Meropenem) (٥٥,٠٠%) وإيميبينيم (Imipenem) (٥٢,٥٠%) من بين العزلات المقاومة للكاربابينيم. أظهرت النتائج وجود ٢٧ (٦٧,٥٠%) عزلة ذات مقاومة دوائية واسعة النطاق (XDR)، بينما أظهرت ١٣ (٣٢,٥٠%) مقاومتها لجميع المضادات الحيوية (PDR). أظهرت جميع العزلات مؤشر مقاومة متعددة للمضادات الحيوية (MAR) أكبر من ٠,٢، مع وجود ١٢ عزلة (٣٠,٠٠%) أظهرت اقصى قيمة للمؤشر (١)، مما يثبت مقاومتها لجميع المضادات الحيوية المدروسة.

كشفت الاختبارات التأكيدية لإنتاج انزيمات ESBL أن ١٠ عزلات (٢٥,٠٠%) كانت إيجابية. أظهرت النتائج الأولية وجود (٧٠,٠٠%) من العزلات إيجابية لانزيمات AmpC البلازميدية بينما لم تظهر أي من العزلات نتيجة إيجابية لإنتاج انزيمات AmpC المحفزة

الكروموسومية. تم الاستدلال على إنتاج انزيمات الكاربابنيميز بواسطة اختبار التآزر المزدوج للأقراص (Double Discs Synergy Test) واختبار الأقراص المدمج (Combined Discs Test)، واقترحت النتائج إلى أن ١١ (٢٧,٥٠%) عزلة كانت منتجة لأنزيمات البيتا-لاكتاماز المعدنية (MBLs). بالإضافة إلى ذلك، تم تحديد ٢,٥% من العزلات على أنها منتجة لأنزيمات الكاربابنيميز من الصنف A أو OXA-48 باستخدام اختبار هودج المحور (Modified Hodge Test). أظهر اختبار التدفق المناعي الجانبي (Lateral Flow Immunoassay) أن ١١ (٢٧,٥٠%) عزلة كانت منتجة لأنزيم (NDM) وعزلة واحدة (٢,٥٠%) كانت منتجة لأنزيم (OXA-48).

من بين جينات الكاربابنيميز المختلفة التي تم دراستها احتوت ٣٤ (٨٥,٠٠%) عزلة على جين واحد على الأقل. وظهرت الدراسة إلى أن جين (bla_{NDM-1}) كان الأكثر انتشارًا ووجد في ٢٤ عزلة (٦٠,٠٠%)، يليه جين (bla_{OXA}) في ١٦ (٤٠,٠٠%) عزلة، وجين (bla_{OXA-48}) في ٦ (١٥,٠٠%) من العزلات، وجين (bla_{OXA-51}) في عزلتين (٥,٠٠%)، وجين (bla_{VIM}) في عزلتين (٥,٠٠%) أيضاً. كما وجد ظهور مشترك لهذه الجينات بين العزلات مع ملاحظة ست أنماط مختلفة. تم الكشف في هذه الدراسة عن انتشار النوع الأول من الانتيكرون في جميع العزلات بينما ظهر النوع الثاني في (٣٢,٥٠%) من العزلات المدروسة. وأكدت نتائج التسلسل الأحماض النووية لأربعة وعشرين عزلة تحتوي على جين (bla_{NDM-1}) أن ٢٢ منها كانت (bla_{NDM-1})، بينما أظهرت نتائج تسلسل الأحماض النووية لـ ١٦ عزلة التي تحتوي جين (bla_{OXA}) أن ١١ كانت (bla_{OXA-1}). في الختام، فإن عزلات *E. coli* المقاومة للكاربابنيم الحاملة لجينات الكاربابنيميز منتشرة حالياً على نطاق واسع في مستشفيات ذي قار، على حد علمنا. قد يزيد الظهور المشترك لجينات الكاربابنيميز مع الانتيكرون من خطر انتشار العزلات ذات المقاومة المتعددة في هذه المستشفيات. يعد التعرف السريع للعزلات المقاومة للكاربابنيم واستخدام تدابير مكافحة العدوى المناسبة أمراً ضرورياً لمنع المزيد من انتشار العدوى التي تسببها هذه البكتيريا في المستشفيات العراقية.



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الانتشار والتوصيف الجزيئي لبكتريا *Escherichia coli* المقاومة للكاربابانيم في محافظة ذي قار

أطروحة

مقدمة إلى مجلس كلية العلوم/جامعة القادسية وهي جزء من متطلبات نيل درجة دكتوراه فلسفة
في علم الاحياء المجهرية الطبية

من قبل

مسلم محسن خلف

بكالوريوس علوم الحياة (٢٠١٢)

ماجستير علوم الحياة (٢٠١٥)

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