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Occurrence and Distribution of *ampC* Beta Lactamase Genes Among Multidrug Resistant Clinical *Escherichia coli* Isolates in Babylon Province

A Thesis

Submitted to the Council of College of Medicine, University of Babylon, in a Partial Fulfillment of the Requirements for the Degree of Doctorate of Philosophy in Science/ Medical Microbiology

By

Mohammed Jasim Khulaif Al-Taee

B.Sc. Microbiology/ University of Babylon- 2006

M.Sc. Microbiology / University of Babylon-2014

Supervised by

Professor

Dr. Alaa Hani AL-Charrakh

2023 A. D 1444 A. H.

بسم الله الرحمن الرحيم

(وَيَسْأَلُونَكَ عَنِ الرُّوحِ ۖ قُلِ الرُّوحُ مِنْ أَمْرِ رَبِّي وَمَا أُوتِيتُم مِّنَ الْعِلْمِ إِلَّا قَلِيلًا)

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الإسراء ٨٥

Supervisor Certification

I certify that this thesis **(Occurrence and Distribution of *ampC* Beta Lactamase Genes Among Multidrug Resistant Clinical *Escherichia coli* Isolates in Babylon Province)** was prepared under my supervision at the Department of Medical Microbiology, College of Medicine, University of Babylon, in a partial fulfillment of the requirements for the degree of Doctorate of philosophy in Science/ Medical Microbiology

Professor

Dr. Alaa Hani Al-Charrakh

University of Babylon College of Medicine

/ / 2023

Recommendation of Head of Department of Microbiology

According to the available recommendations, I forward this thesis for debate by the examining committee

Professor

Dr. Hayam Khalis Al-Masoudi

Head of Department of Medical Microbiology

College of Medicine

University of Babylon

/ / 2023

Certification Committee

We certify that we read this thesis entitled **" Occurrence and Distribution of *ampC* Beta Lactamase Genes Among Multidrug Resistant Clinical *Escherichia coli* Isolates in Babylon Province "** and as examining committee, examined the student **"Mohammed Jasim Khulaif Al-Taee "** in its content, and in our opinion it meets standards of a thesis for degree of Doctorate of Philosophy in Medical Microbiology accepted with **"Excellent"**

degree.

Signature:
Name : Ihsan Edan A. Al-Saimary
Title: Professor
Address: College of Medicine,
University of Basrah
Date: / / 2023
(Chairman)

Signature
Name: Jawad Kadim T. Al-Khafaji
Title: Professor
Address: College of Medicine,
University of Babylon
Date: / / 2023
(Member)

Signature:
Name : Baha Hamdi Al-Amiedi
Title: Professor
Address: College of Dentistry,
University of Babylon
Date: / / 2023
(Member)

Signature
Name : Samah Ahmed AL- Jebory
Title: Professor
Address: College of Pharmacy,
University of Babylon
Date: / / 2023
(Member)

Signature:
Name : Zeena Hadi Obaid Alwan
Title: Professor
Address: College of Science,
University of Babylon
Date: / / 2023
(Member)

Signature
Name : Alaa Hani H. AL-Charrakh
Title: Professor
Address: College of Medicine,
University of Babylon
Date: / / 2023
(Member and Superviser)

Approved for the college committee of gradute studies.

Signature:
Name: Mohend Abbass Nori Al-Shalah
Title: Professor
Address: Dean of College of medicine/ Babylon University
Date: / / 2023

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Dedication

To....

My Father

My Mother

My Dear Wife

My Lovely Sons

Theker, Zahra, Ameer, and Abbas

I dedicate this work

Mohammed / 2023

Summary:

Escherichia coli is well known to be a universal commensal flora in humans as well as in several animal species, in the human gastrointestinal tract, it lacks virulence. whereas, when found outside of the intestinal tract, *E. coli* can cause several diseases.

Antimicrobial resistance (AMR) has emerged as one of the principal public health problems of the 21st century. Extended-spectrum β-lactamases (ESBLs) and AmpC enzymes have been detected in virtually all species of the family Enterobacteriaceae.

A total 1874 clinical isolates were collected from four main hospitals in Hilla city including Marjan Teaching Hospital, Al-Hilla General Teaching Hospital, Babil Teaching Hospital for Maternity and Children and Al-Imam Al-Sadiq Hospital during a period extended from February to June, 2022.

In the current study, *E. coli* were isolated from different clinical samples including urine, burn exudate, vaginal discharge, sputum, diabetic foot ulcer (DFU), stool, CSF, blood, and ear swab by culturing on MacConkey's agar and Eosin Methylene blue in addition to using several biochemical tests for bacterial identification.

The detection of the ability of all *E.coli* isolates for β-lactamase production was done using nitrocefin disk. The antibiotics susceptibility testing (AST) of β-lactamase-producing *E.coli* isolates was done by using Vitek 2 system and disc diffusion method and the results were interpreted according to CLSI, 2023 guidelines.

Polymerase chain reaction (PCR) was achieved for genetic detection of plasmid AmpC genes, ESBL genes, cabapenemase genes, plasmid-mediated quinolones resistance (PMQR) genes, aminoglycosides resistance genes, colistin-resistance

genes, and integron class 1. Finally, DNA sequencing was done for some *ampC* genes.

The results found that out of 1874 clinical samples, 231 isolates were belonged to *E.coli,* of which, 112 (48.5%) were found to be β-lactamase producers. The isolation rate of *E.coli* were mostly from stool 38(61.4%) followed by urine 118(18.4%). Regarding to patients' sex, *E.coli* from urine were mostly isolated from female patients 77(65.3%), while 19(63.3%) and 9(69.3%) of burn and DFU were mostly from males respectively.

For AST, *E.coli* were tested against 30 types of antibiotics belonging to 15 classes, 14 types were β-lactam and 16 types were non β-lactam. The results revealed all isolates were resistant to ampicillin, amoxicillin, amoxicillin/ clavulonate, and piperacillin 111(99.1%). Regarding to clinical samples *E.coli* isolated from urine were high resistant to cefazolin 53(94.6%), ceftriaxone 52(92.8%), aztreonem 42(75%), and nalidixic acid 50(89.3%). *E.coli* isolated from vaginal exudate were high resistant to cefazolin 17(100%), aztreonem17(100%), ciprofloxacin 17(100%), levofloxacin 17(100%), sparfloxacin 17(100 %), and nalidixic acid 17(100%). *E.coli* isolated from DFU were high resistant to cephazoline 7(100%), ceftriaxone 7(100%), aztreonem 7(100%), ciprofloxacin 6(85.7), levofloxacin 6(85.7%), sparfloxacin 6(85.7%), and norfloxacin 6(85.7%).

However, *E.coli* isolates were sensitive to meropenem 112(100%), tigycycline 112(100%), ertapenem 108(96.5%), impenem 107(95.5%), amikacin 103(92%), and isepamicin 110(98.2). All isolates were revealed MDR pattern of resistance. Out of 112 β-lactamase-producing *E.coli* 74(66%) isolates were ESBL producer, 4(3.5%) isolates were carbapeneme resistant, 37(33%) isolates were aminoglycosides resistant, 73(65.1%) isolates were quinolones resistant, and 27(24.1%) isolates were colistin resistant.

PCR results for ESBL genes revealed high prevalence of *bla*$_{CTX-M}$(100%), among ESBL producing *E.coli* isolates, followed by *bla*$_{SHV}$ (87.8%), *bla*$_{OXA}$ (85.1%) and *bla*$_{TEM}$ (79.7%).

The results of PCR for carbapenemase genes were *bla*$_{OXA}$ (100%) and *bla*$_{NDM}$ (75%), plasmid- mediated quinolone resistance (PMQR) genes *qnr A*(37.5%) and *qnr B* (32.2%), aminoglycosides gene *ACC(6)Ib-cr* were (100%), colistin resistant genes *mcr 2* (55.5) and *mcr 3* (22.2), finally *Int1*were detected in (99.1%).

Regarding to *pampC* genes, PCR results showed high prevalence of *bla*$_{CMY}$ (88.5%) followed by *bla*$_{CIT}$(57.7%) and *bla*$_{DHA}$(42.3%), *bla*$_{FOX}$, *bla*$_{MOX}$, *bla*$_{EBC}$, *bla*$_{ACC}$ genes were not detected in present study.

Out of 26 AmpC producing *E.coli* isolates, 1(3.85%) isolates carry 3 types of *pampC* genes, 19(73%) isolates carry 2 types of *pampC*, 5(19.3%) isolates carry 1 type and 1(3.85%) isolates do not carry any one from study genes.

DNA sequencing to *bla*$_{DHA}$ and *bla*$_{CIT}$ revealed that all *bla*$_{DHA}$ were compatible with NCBI database with 100% purity, *bla*$_{CIT}$ highly similar *bla*$_{CMY-42.}$

From this study, it can conclude that ESBL were more prevalence than AmpC in BPEC but AmpC were more antibiotics resistant when compared with ESBL. All *E.coli* isolates were resistant to ampicillin, amoxicillin, amoxicillin/ clavulonate, whereas susceptible for amikacin, impeneme, meropeneme, and tigycycline. High prevalence of ESBL genes, *pampC* genes such as *bla*$_{CMY}$ gene, *ACC(6)Ib-cr gene*, and *Int1* gene were detected among MDR *E.coli* isolates.

List of abbreviation

Abbreviation	Meaning
ACC	Ambler class-C
AmpC	AmpC enzyme
pampC	Plasmid-ampC gene
AMR	Anti- microbial resistance
AST	Antibiotic susceptibility test
BPEC	β- lactamase producing *E.coli*
*bla*gene	β- lactamase gene
CIT (enzyme)	*Citrobacter freundii*
CLSI	Clinical and Laboratory Standards Institute
CMY	Cephamycins
CRE	Carbapenemase resistance enterobacteriaceae
CTX-M	Cefotaximase
DAEC	Diffusely adherent *E.coli*
DHA	Dhahran Hospital in Saudi Arabia
DFU	Diabetic foot ulcer
EBC (enzyme)	Enterobacter cloacae
EHEC	Enterohaemorrhagic *E. coli*
EPEC	Enteropathogenic *E. coli*
EAEC	Enteroaggregasive *E. coli*
ETEC	Enterotoxogenic *E. coli*
EIEC	Enteroinvasive *E. coli*
E.M.B.	Eosin methylene blue
ESBL	Extended spectrum β-lactamases
EDR	Extensive durg resistance
ExPEC	Extra-intestinal pathogenic *E.coli*
FOX	Cefoxitin
GIT	Gastro- intestinal tract
InPEC	Intestinal pathogenic *E.coli*
Int1	Integron class 1 gene

LPS	lipopolysaccharide
MDR	Multi drug resistance
mcr	Mobile colistin resistance
MBL	Metallo beta lactamase
MOX	Moxalactam
MHA	Mueller Hinton agar
µg	Microgram
µl	Microliter
NA	Nutrient agar
NB	Nutrient broth
NMEC	newborn meningitis
PCR	Polymerase chain reaction
PDR	Pan-drug resistance
PBPs	Penicillin binding proteins
SHV	Sulfhydryl variable
SePEC	Septicaemia *E.coli*
TBE	Tris-Borate-EDTA
TEM	Temoniera
UPEC	Uropathogenic *E. coli*
UTI	Urinary infections tract

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Chapter One

Introduction and Literatures Review

1.1. Introduction

Escherichia coli is well known to be a universal commensal flora in humans as well as in several animal species, in the human gastrointestinal tract lacks virulence. However, when found outside of the intestinal tract, *E. coli* can cause several diseases (Jena *et al*. 2017). Antimicrobial resistance (AMR) has emerged as one of the principal public health problems of the 21st century (Prestinaci *et al.* 2015).

Beta-lactams (β-lactams) are among the most commonly prescribed antibiotics in Iraq as well as world-wide. The group is distinguished by a βlactam ring in their molecular structure. They include penicillins, cephalosporins, carbapenems, monobactams, and β-lactamase inhibitors. These antibiotics have activity against gram-positive and gram-negative bacteria (Pandey and Cascella, 2021).

AmpC β-lactamase production is one of the commonest causes of resistance to β-lactam antibiotics among Gram negative bacteria. AmpC β-lactamases are resistant to aminopenicillins, carboxypenicillins, ureidopenicillins, cephalosporins, broad as well as extended spectrum cephalosporins (cephamycin) and monobactams (aztreonam). AmpC β-lactamases are resistant to β-lactamase inhibitors like clavulanic acid. Clinical isolates of AmpC β-lactamase producing *E. coli* and their antimicrobial resistance have been described from different parts of the world (Ejaz *et al.,* 2014).

Extended-spectrum β-lactamases (ESBL) are enzymes that can hydrolyze third-generation cephalosporins (oxyimino-cephalosporins), such as ceftriaxone, cefpirome, and cefepime. They are also partially susceptible to cephamycin and carbapenem. The ESBL are usually inhibited by β-lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam (Thomson, 2010; Stefani *et al*., 2014).

Carbapenems possess broad spectrum antibacterial activity and have a unique structure that is defined by a carbapenem coupled to a β-lactam ring which confers

protection against most β lactamases such as metallo-β-lactamase (MBL) as well as extended spectrum β-lactamases. Consequently, carbapenems are considered one of the most reliable drugs for treating bacterial infections and the emergence and spread of resistance to these antibiotics constitute a major public health concern. Carbapenem-Resistant Enterobacteriaceae (CRE) is emerging as an important challenge in health-care settings and a growing concern worldwide (Korach-Rechtman, *et al.,* 2020).

Colistin is a cationic antimicrobial polypeptide, it exerts antibacterial activity via interacting with the lipid A moiety of lipopolysaccharide (LPS) and subsequently disrupting the outer membrane of Gram-negative bacteria. Despite the potential of colistin as a high-efficient antimicrobial agent, its massive use in clinical as a cationic antimicrobial polypeptide (Cai *et al*., 2015; Caniaux *et al*., 2016). Despite the potential of colistin as a high-efficient antimicrobial agent, its massive use in clinical herapeutics and as a feed additive has led to the development of colistin resistance in *E. coli*. The most common reported mechanisms for colistin resistance in *E. coli* have involved the plasmid borne mobile colistin resistance *(mcr)* gene, which was newly discovered in 2016 and denoted as *mcr-1*. Several amino acid variants have been described, such as *mcr-2* and *mcr-3* (Yin *et al.,* 2017).

Fluoroquinolones such as ciprofoxacin are effective broad-spectrum antibiotics used for the treatment of bacterial infections making them a recommended choice of therapy for enteric infections (Bruzzese *et al.,* 2018). Fluoroquinolone resistance is mediated by two mechanisms: chromosomal mutations in DNA gyrase and topoisomerase IV enzymes and plasmid-mediated quinolone resistance (PMQR). Mutations in fuoroquinolone binding sites during DNA replication mediate high-level fuoroquinolone resistance (Hopper and Jacoby 2015). Mechanisms of PMQR genes

include protection of DNA gyrase and topoisomerase IV from quinolone activity mediated by qnr genes: qnrA, qnrB, qnrC, qnrD, and qnrVC (Kariuki *et al*., 2023).

1.2. Aim of Study

The aim of this study is to determine the occurrence and molecular characterization of plasmid-mediated AmpC β-lactamase producing *E. coli* clinical isolates collected from hospitals in Babylon province.

1.2.1.Objectives of Study

1-Isolation and characterization of *E. coli* isolates from different clinical sources.

2-Investigatation of the ability of *E.coli* to β- lactamase production.

3-Investigation of the antibiotic susceptibility patterns of *E. coli* isolates to different antibiotics according to CLSI, 2023, guidelines.

4-Phenotypic detection of plasmid-mediated AmpC beta-lactamase by cefoxitin disk

5-Molecular characterization of the plasmid-mediated AmpC genes (*bla*$_{ACC}$, *bla*$_{CIT}$, *bla*$_{EBC}$, *bla*$_{FOX}$, *bla*$_{MOX}$ *bla*$_{CMY}$ and *bla*$_{DHA}$) among *E. coli* isolates from different clinical sources.

6-Detection the presence of common plasmid β-lactamases ESBL genes (*bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{OXA}$, *bla*$_{CTX-M}$), carbapenemase genes (*bla*$_{OXA}$, *bla*$_{NDM}$, *bla*$_{KPC}$, *bla*$_{VIM}$ and *bla*$_{IMP}$), PMQR genes (*qnr A and qnr B*), aminoglycosides resistance gene (*ACC(6) Ib-cr*), plasmid-borne colistin resistance genes (*mcr-2* and *mcr-3*), and class I integrons (*Int1*) gene among isolates.

7- DNA sequencing for some *amp C* genes

1.3.Literature Review

1.3.1. Feature of *E.coli*

E. coli is a mesophilic, facultative, Gram-negative bacteria with a rod shape that may colonize the gastrointestinal tracts of warm-blooded mammals. Its optimal growth temperature is around 37 °C., the microorganism moves by means of peritrichous flagella. Several strains have fimbriae and capsules (Leimbach *et al*., 2013).

The majority of *E. coli* strains are harmless and are found in the typical GIT flora. However, a few pathogenic *E. coli* have developed particular virulence characteristics that enable them to cause disorders of the gastrointestinal tract or other organs (Lee *et al*., 2022). Numerous pathogenic strains have the ability to produce extraintestinal and gastrointestinal illnesses in both healthy and immunosuppressed people (Gomes *et al*., 2016).

Diarrheal illnesses are a severe public health problem and a major cause of morbidity and mortality in infants and young children (Gomes *et al*., 2016) strain of *E.coli* are utilize their high levels of intrinsic and acquired resistance mechanisms to counter most antibiotics. The emergence of antibiotic-resistant bacteria is a major cause of treatment failure in infected patients especially newborns (Wu *et al*., 2021). *E. coli* is one of the most common Gram-negative microorganism causing bacteremia. Patients with multidrug-resistant (MDR) *E. coli* bacteremia had a much lower incidence of right empirical antibiotic therapy than patients with non-MDR *E. coli* bacteremia . Multidrug-resistant (MDR) *E. coli* and especially extended spectrum βlactamase (ESBL) -producing *E. coli* are much concern because of their increased frequency and their resistance to a wide range of β-lactams and to other groups of antimicrobial factors (Peralta *et al*., 2007).

1.3.2. *E. coli* Classification

According to the virulence factors and clinical signs which *E.coli* can cause , pathogenic *E.coli* have been widely classified into 2 major categories : the extra-intestinal pathogenic *E.coli* (ExPEC) and the intestinal pathogenic *E.coli* (InPEC) (Riley, 2014).

The majority of extra-intestinal infections associated with *E.coli* are caused by commensal strains that become pathogenic by adaption stratagems or the possession of virulence determinants(Extraintestinal Pathogenic *E. coli* (ExPEC)) and later reach a specific sterile body site (Saez-Lopez *et al*., 2016).

Extra-intestinal Pathogenic *E. coli* (ExPEC) is responsible for infections outside the digestive tract which include newborn meningitis (NMEC) , uropathogenic *E.coli* (UPEC), and Septicaemia *E.coli* (SePEC) (Allocati *et al*., 2013).

By contrast , The InPEC are principally responsible for diarrheic infections and are the major cause of mortality specifically in children . (Rehman *et al*., 2017).

According to their virulence factors, *E.coli* can be classified into 6 groups as follows :

1-Enterohemorrhagic *E. coli* (EHEC) / Shiga toxin-producing *E.coli* (STEC) associated with hemorrhagic colitis and hemolytic uremic syndrome in human .

2- Enteropathogenic *E. coli* (EPEC) which cause diarrhea in children .

3- Enterotoxigenic *E. coli* (ETEC) which are associated with traveler`s diarrhea .

4- Enteroaggregative *E. coli* (EAEC) associated with persistent diarrhea in children.

5- Enteroinvasive *E. coli* (EIEC) which are involved in invasive intestinal infection ; watery diarrhea and dysentery inhuman .

6- Diffusely adherent *E. coli* (DAEC) which bind to the small bowel in a diffusive adhesion pattern (Croxen *et al*.,2013; Andersen *et al*., 2015; Bozal *et al*., 2016).

It is realized that *E. coli* can be divided phylogenetically into 4 groups A , B1 , B2 and D, these groups are appointed according to 3 common genes used in phylogeny there are *yjaA* , *chuA* and *TspE4C2* . Many studies have indicated the relationship between phylogeny and pathogenicity of *E.coli* , a great deal can be acquired by determining the phylogroup of anonymous *E.coli* strains (Tenaillon *et al*., 2010; Barzan *et al*., 2016).

1.3.3.Pathogenicity of *E.coli*

1.3.3.1.Virulence factors

Virulence factors (VF) related to the pathogenicity of *E.coli* are numerous and have a wide range of activities, from those related to bacteria colonization to those related to virulence, including adhesins, toxins, iron acquisition factors, lipopolysaccharides, polysaccharide capsules, and invasins, which are usually encoded on pathogenicity islands (PAIs), plasmids and other mobile genetic elements (sarowska *et al*. 2019). Generally the important virulence factors of Escherichia coli can broadly be divided into two groups: bacterial cell surface and secreted virulence factor. Bacterial cell surface virulence factors most commonly include fimbriae like mainly type 1 fimbriae and P fimbriae. These fimbriae help in adhesion to host cell surface, tissue invasion (which is important in pathogenesis of UPEC causing UTIs), biofilm formation and cytokine induction. Bacterial cell surface virulence factor also include flagellum, capsular lipopolysaccharide and outer membrane proteins (Shah *et al*., 2019).

Haemolysin and siderophores are secreted virulence factor. (Emody *etal*., 2003). These virulence factors are important in enabling the bacteria to colonize the urinary

tract and persist despite the effectively functioning host defense mechanism (Vagarali *et al*., 2008).

There are six pathotypes than can produce intestinal disease in humans, *E. coli* strains are classified into these pathotypes depending on the presence/ absence of several combinations of virulence genes (Cabal *et al*, 2016). In EPEC, the virulence machinery is based on the carriage of eae, tir, and other proteins required for causing attachment and effacement (A/E) lesions and they are encoded on a chromosomal pathogenicity island known as the locus for enterocyte effacement (Kaper *et al*., 2004).

The activation of biofilm formation is mediated by Quorum-sensing encoded by the suppressor of the division inhibitor (*SdiA*) gene. However, for biofilm formation, bacteria demand structures to support these characteristic curli fimbriae, type-I fimbriae, and the cellulose encoded in EPEC by *csgA*, *fimA*, and *bcsA* genes, respectively . (Ruano-Gallego., 2021)

The most prominent and effective virulence factor of ETEC is the secretion of enterotoxins. Two types of enterotoxins, heat-stable toxins (STs) and heat-labile toxins (LTs), are secreted by ETEC and activate cyclic nucleotide production, contributing to intestinal net water, salt, and fluid loss, causing secretory diarrhea in humans and animals. LTs, STs, or a combination of both toxins might be expressed by ETEC strains. (Pakbin *et al*., 2021)

1.3.4. Epidemiology of *E.coli*

Escherichia coli are gram-negative bacteria within the family Enterobacteriaceae that can harmlessly colonize the human gut or cause intestinal or extraintestinal infections, including severe invasive disease such as bacteremia and sepsis. *E. coli* is the most common cause of bacteremia in high-income countries, exceeding other

leading bacteremia-causing pathogens such as *Staphylococcus aureus* and *Streptococcus pneumoniae*, and is a leading cause of meningitis in neonates (Bonten *et al*., 2021).

E. coli colonizes majority of neonates within a few hours of birth, most likely during delivery. *E. coli* has dual nature. Most strains are inhibited with their host without incident, while others are greatly pathogenic. The divergence in the capability of strains to incident disease resides in specific gene encoding virulence factors, the gaining of which mostly dependent on horizontal transfer. *E. coli* can incident disease at several sites even outside the gastrointestinal tract, including the urinary tract, meninges, biliary tract, peritoneum, lungs, skin and soft tissues (Kessler *et al*., 2015).

Escherichia coli O157:H7 was first recognized as a pathogen in 1982 during an outbreak investigation of hemorrhagic colitis. *E. coli* O157 infection can lead to hemolytic uremic syndrome (HUS), characterized by hemolytic anemia, thrombocytopenia, and renal injury (Rangel *et al*., 2005).

Accurate detection of AmpC is not only critical for proper health management of patients suffering from Enterobacteriaceae infections but also useful for epidemiological analyses of the geographical distribution of AmpC genes (joji *et al*, 2021).

1.3.5. Antimicrobial Resistance of *E.coli*

Antibiotic resistance mechanisms reported in Gram-negative bacteria are causing a worldwide health problem. Antimicrobial resistance (AMR) is one of the leading causes of death (estimated five million associated deaths annually) globally. In recent years, AMR has made therapeutic drugs ineffective against bacterial infections (Murray *et al*, 2022).

E. coli is intrinsically susceptible to almost all clinically relevant antimicrobial agents, but this bacterial species has a great capacity to accumulate resistance genes, mostly through horizontal gene transfer. The most problematic mechanisms in *E. coli* correspond to the acquisition of genes coding for extended-spectrum β-lactamases (conferring resistance to broad-spectrum cephalosporins), carbapenemases (conferring resistance to carbapenems), 16S rRNA methylases (conferring pan-resistance to aminoglycosides), plasmid-mediated quinolone resistance (PMQR) genes (conferring resistance to [fluoro]quinolones), and *mcr* genes (conferring resistance to polymyxins) (Poirel *et al* ., 2018).

1.3.5.1.Intrinsic Resistance

The intrinsic antibiotic resistance of a bacterial species refers to innate ability to diminish the efficacy of a specific antibiotic through inherent structural or functional characteristics (Blair *et al*., 2015).

E.coli produces the AmpC enzyme , but in this case its production is at a low level and is not inducible because there is no ampR regulatory gene in this species . However, even this low level of the enzyme can contribute to the intrinsic resistance of *E. coli*, as shown earlier by the comparison of β-lactam susceptibility in strains producing this enzyme at different levels (mazzariol *et al*., 2000).

1.3.5.1.1. Outer Membrane

In addition to their cytoplasmic (inner) membrane, Gram-negative cells have an outer membrane (OM) that poses a significant permeability barrier. The Gram-negative outer membrane is a unique asymmetrical bilayer composed of an inner leaflet of phospholipid and an outer leaflet of lipopolysaccharide (LPS). The outer membrane barrier, in conjunction with active efflux, is effective at protecting these pathogens from many antibiotics (Shen *et al*., 2019).

Membrane-associated antibiotic resistance is a key mechanism in Gram-negative bacteria that efficiently controls the intracellular concentration of various drugs . Two complementary processes, membrane impermeability and the expression of efflux pumps, limit the concentration of deleterious compounds inside bacteria by impairing the entry and expelling the internal molecules . Consequently, they play a key role in protecting the bacterial cells against aggressive chemicals, such as antibiotics, disinfectants, conservatives, detergents, and others (Davin-Regli., 2021).

In fact, the outer membrane acts as the first-line of defense against the penetration of multiple toxic compounds, including several antimicrobial agents. Hydrophilic molecules such as β-lactams, tetracyclines and some fluoroquinolones are particularly affected by changes in permeability of the outer membrane since they often use water-filled diffusion channels known as porins to cross this barrier (Pages *et al*., 2008)

The prime example of the efficiency of this natural barrier is the fact that vancomycin, a glycopeptide antibiotic, is not active against gram-negative organisms due to the lack of penetration through the outer membrane. Several types of porins have been described, and they can be classified according to their structure (trimeric vs. monomeric), their selectivity and the regulation of their expression. Among the best-characterized porins, the three major proteins produced by *E. coli* (known as OmpF, OmpC and Pho). Alterations of porins could be achieved by 3 general processes i) a shift in the type of porins expressed, ii) a change in the level of porin expression, and iii) impairment of the porin function. (Munita *and* Arias, 2016)

1.3.5.1.2. Efflux Systems

Multidrug efflux mechanisms in bacteria contribute significantly to intrinsic and acquired resistance to antimicrobial agents. Genome analysis have confirmed the

broad distribution of these systems in Gram-negative as well as in Gram-positive bacteria. Among resistance mechanisms, the multidrug efflux system or pump deserves special attention mechanisms associated with modifications of membrane permeation processes, such as decreasing passive uptake (influx) or increasing active efflux of antibiotics, are now being reported as key contributors of the bacterial MDR phenotype (Nikaido *et al*., 2012).

Regarding the drug transporters, multidrug efflux pumps encoded on the bacterial genomes belong to the ABC, MFS, SMR, MATE, PACE, and RND (super) families. Except for the ABC transporters, which use the ATP as an energy source to transport the drugs across the membrane, the other described multidrug efflux pumps are H+(or Na+)/drug antiporters (Kornelse and Kumer, 2021). These transporters carry drugs from the cytoplasm or the inner leaflet of the inner membrane to the periplasm or to external medium with tripartite systems that comprise an inner membrane transporter (ABC, MFS, or RND), a periplasmic adaptor protein (PAP, previously known as the MFP for Membrane Fusion Protein), and an outer membrane channel (OMF, Outer Membrane Factor) (Alav *et al.*, 2021). The RND family contributes to the major membrane-associated mechanisms of efflux in Enterobacteriaceae and the more relevant complex described in clinical isolate belong to AcrAB-TolC system (ferrand *et al*., 2020) .

These systems may be substrate-specific (for a particular antibiotic such as tet determinants for tetracycline and mef genes for macrolides in pneumococci) or with broad substrate specificity, which are usually found in MDR bacteria (Munita and Arias, 2016).

1.3.5.1.3. AmpC β-lactamase Expression

In Gram-negative bacteria, AmpC beta-lactamase production is chromosome or plasmid mediated. Chromosomal ampC genes are expressed constitutively at a low level,enteric organisms with the potential for high-level AmpC β-lactamase production by mutation, the development of resistance upon therapy is a concern. Some Enterobacteriaceae, such as Enterobacter spp., Citrobacter spp., and Serratia spp., carry an inducible ampC gene. In these cases, the gene is strongly induced by β-lactams, such as cefoxitin and imipenem, with expression mediated by the regulator AmpR. Mutations in the repressor gene ampD may lead to overproduction of AmpC beta-lactamases (Jacoby *et al*., 2009). The regulation of chromosomal ampC expression in *E. coli* differs considerably from that in other Enterobacteriaceae. *E. coli* lacks ampR, and thus ampC expression is not inducible. In *E. coli*, ampC is expressed constitutively at a low level (Kong *et al*.,2005). Various mutations in the ampC promoter/attenuator region of *E. coli* have been identified that result in constitutive overexpression (Tracz *et al*., 2007). In addition to chromosomal ampC, *E. coli* may contain plasmids carrying ampC (pampC), transferred via horizontal gene transfer and derived from the chromosomal ampC genes of other Enterobacteriaceae spp. Plasmid-based ampC genes are expressed constitutively in most cases (Jacoby *et al*., 2009).

However, some plasmid-carried *ampC* genes, such as the $bla_{\text{DHA-1}}$ gene, are inducible by β-lactams, with expression regulated similarly to that of inducible chromosomal *ampC* genes. All plasmid-carried *ampC* genes are considered to be of significant clinical relevance. AmpC overproduction in addition to porin mutations of the outer membrane can reduce susceptibility to carbapenems, in particular in plasmid-mediated AmpC producers. AmpC beta-lactamases can confer resistance to amino-penicillins, cephalosporins, oxyimino-cephalosporins (e.g.,ceftriaxone,

cefotaxime, and ceftazidime), cephamycins (e.g.,cefoxitin and cefotetan), and monobactams. Cloxacillin and 3-aminophenylboronic acid inhibit AmpC beta-lactamases , while AmpC beta-lactamase activity is not affected by the ESBL inhibitor clavulanic acid (Peter-Getzlaff *et al*., 2011).

1.3.5.2. Acquired Resistance.

The acquired, or extrinsic, and continuously increasing resistance of *E. coli* to antibiotics is already considered a major public health problem around the world. In 2018, more than half of the *E. coli* isolates reported to the European Centre for Disease Prevention and Control were resistant to at least one antimicrobial group under surveillance, and combined resistance to several antimicrobial groups was frequent ,in the United States in 2017, the national prevalence of extended spectrum β-lactamases (ESBL)-producing *E. coli* strains isolated from urinary tract infections (UTI) was 15.7%, whereas levofloxacin and trimethoprim-sulfamethoxazole-resistant rates were ≥ 24% among all isolates (Galindo-Mendez.,2020).

the development of acquired resistance in *E.coli* can result from the acquisition of external genes responsible for resistance through horizontal gene transfer and chromosomal gene mutation. Acquired resistance resulting from mutations impacts resistance against several classes of antibiotics, including β-lactamase, fluoroquinolones and aminoglycoside (Munita and Arias , 2016).

However, the rate at which *E. coli* strains are becoming resistant to the vast majority of antibiotics is increasing worldwide. In addition, Enterobacteriaceae harbor gene(s) conferring resistance to almost all antibiotics and plasmids harboring these resistance determinants can be transferred between bacteria, even between species, such that the acquisition of resistance to new antibiotics may only be a matter of time (Lee *et al*., 2018).

A subset of bacterial cells derived from a susceptible population develop mutations in genes that affect the activity of the drug, resulting in preserved cell survival in the presence of the antimicrobial molecule. Once a resistant mutant emerges, the antibiotic eliminates the susceptible population and the resistant bacteria predominate. In many instances, mutational changes leading to resistance are costly to cell homeostasis (i.e., decreased fitness) (Munita and Arias, 2016)

Mutations are one of the common means by which bacteria acquire resistance to antibiotics(Cho and misra., 2021). Mutational changes are able to cause reduced antibiotic uptake, modifications of antibiotic targets, and overexpression of efflux pumps and antibiotic-inactivating enzymes; all of which allow bacteria to survive in the presence of antimicrobial molecules (Munita and Arias, 2016).

1.3.6. β-lactam Antibiotic

β-lactam is a generic name for all β-lactam antibiotics that contain a β- lactam ring, a heteroatomic ring structure, consisting of three carbon atoms and one nitrogen atom. The principal classification of β-lactams is based upon the structure (Wilke *et al.*, 2005).

These antibiotics have been isolated from numerous sources but principally from bacteria and fungi, the history of β-lactam antibiotics began when Alexander Fleming descripted antibacterial activity of a substance produced by penicillium mold and gave it the name penicillin in 1929. However, the phenomenon of antibiotic was already well known at this time (Laursen *etal.*, 2004)

Penicillins are a group of either natural or semi-synthetic β -lactam antibiotics that are characterized by the presence of a β-lactam ring and a thiazolidine ring; this structure is defined as 6-amino-penicillanic acid and its presence is essential for the activity of these antimicrobial agents. Hydrolysis of the C=O union by the activity of

β-lactamases gives rise to a bacteriologically inactive molecule. The differences of activity among the members of this group are due to the lateral chain found in the position 6 of the 6-aminopenicillanic acid (Samaha-Kfoury and Araj, 2003).

Cephalosporins are structurally similar to the penicillins; however, the members of this group present a dihydrothiazine ring instead of the thiazolidine ring which is present in the penicillins. As for the penicillins, the introduction of additional side chains will give rise to different cephalosporins with a different level of antimicrobial activity (Livermore and Williams, 1996).

Carbapenems are also structurally very similar to the penicillins, but the sulphur atom in the position 1 of the structure has been replaced with a metal group. The additional side-chains and its special disposition confer an important affinity towards the penicillin binding proteins (PBPs) and make this group of antibiotics resistant to a great part of β-lactamases (Queenan and Bush, 2007; Jacoby and Bush, 2009).

Monobactams are monocyclic compounds derived from the 3-amino monobactamic acid. Their major characteristic is the presence of the β-lactam ring alone and not fused to another ring (Livermore and Williams, 1996; Samaha-Kfoury and Araj, 2003).

The clinical use of β-lactamases inhibitors is exclusively based on clavulanic acid, tazobactam and sulbactam. The clavulanic acid has a structure similar to the penicillins but the sulphur atom has been substituted for an oxygen atom which increases the affinity of these molecules for the β -lactamases. On the other hand, the sulbactam and the tazobactam have an oxidation of the sulphur present in the β-lactam ring (Jacoby and Bush, 2009).

1.3.6.1. Mode of β-Lactam Antibiotics Action

Cell wall synthesis in bacteria is dependent on the PBPs enzymes transpeptidases, carboxypeptidases and transglycosylases. These enzymes complete the final stages of cross-linking the peptidoglycan polymers in the wall. β -lactams interfere during this stage directly inhibiting one or several of the enzymes (Perry and Jstaley, 1997).

The β-lactam antibiotics are analogues of the terminal amino acid (Dalanyl-D-alanine) residues on the precursor N-acetylmuramic acid and Nacetylglucose amine peptide subunits of the peptidoglycan layer. The nucleus of the -lactam molecule irreversibly binds to the Serine 403 residue of the PBP active site. This prevents the transpeptidation of the peptidoglycan layer, and hence disrupts the synthesis of the cell wall (Bradford, 2001).

The inhibition leads to bacteriolysis caused by autolysins activation of the cells autolytic system. This happens when precursors of the cell wall accumulate and the wall is unable to resist osmotic pressure (Perry and Jstaley, 1997; Ryan and Ray, 2004).

1.3.6.2. Mechanisms of β-Lactam Antibiotics Resistance

There are several mechanisms of resistance to β-lactam antibiotics and they are generally due to point mutations on the chromosome or to the acquisition of mobile elements such as plasmids or transposons. The resistance to -lactam antibiotics can be due to the expression of a single mechanism of resistance or to the additive effect of several mechanisms, resistance to β-lactam antibiotics in bacteria could be due to four mechanisms (Lawley *et al*., 2004).

1.3.6.2.1. Resistance by Increased Efflux Pump

Genes encoding efflux pumps may be on the chromosome or on transmissible elements, such as plasmids, and are present in antibiotic susceptible and antibiotic-resistant bacteria, efflux systems are now recognized as an important contributor to antimicrobial resistance, and are more commonly found in Gram-negative bacteria, with resistance mediated by increased expression of the efflux pump protein or a mutation in the protein increasing the efficiency of export (Piddock, 2006).

1.3.6.2.2. Resistance by Decreased Antibiotics Uptake

Before a β-lactam reaches bacterial PBP targets which are on the outer surface of the cell membrane it must diffuse across the outer membrane of the cell, by using the pores that are formed by porins, and then cross the periplasm. The porins, which represent one family of outer membrane proteins (Omps), form channels to permit diffusion of small hydrophilic solutes through the outer membrane (Kumar *et al*., 2005).

1.3.6.2.3. Resistance by Alteration of the Target Site

Resistance caused by alterations in PBPs can occur by acquisition of an increased target PBP number and reduced affinity of this target. PBPs with reduced affinity are an important mechanism of resistance to β-lactamse specially in Gram-positive when β-lactamases are absent (Livermore, 2002).

1.3.6.2.4.Resistance by Enzymatic Inactivation

Enzymes, like β-lactamses, are the most important single cause of resistance to β-lactams. Over 700 unique enzymes have been identified and they are either chromosomally or plasmid mediated. These enzymes make biologically inactive products of the antibiotic by efficient hydrolysis of the amide bond in the β-lactam

ring (Livermore, 2003). β-Lactams are the most common antibiotics prescribed to treat infections caused by Gram-negative rods . The production of β-lactamases is the most common antimicrobial resistance mechanism against b-lactams in Gram-negative bacilli (Abdalhamid *et al*, 2017). β-lactamases were initially noted in *Staphylococcus aureus* and are now common in Gram-negative bacteria such as *E. coli, K. pneumoniae* and *Proteus mirabilis*, but have also been found in other Enterobacteriaceae (Ali Shah *et al*., 2004)

1.3.6.3. AmpC β-lactamase.

AmpC enzymes typically have molecular masses of 34 to 40 kDa and isoelectric points of 8.0, although the isoelectric points of plasmid-mediated FOX enzymes are lower. The enzymes are located in the bacterial periplasm, with the exception of the AmpC β-lactamase of *Psychrobacter immobilis*, which is secreted mainly into the external medium (Jacoby, 2009). AmpC β-lactamases confer resistance to β-lactam/ β-lactamase inhibitor combinations, narrow as well as broad-spectrum cephalosporins, and aztreonam (Joji *et al*, 2021). The first bacterial enzyme reported to destroy penicillin was the AmpC β-lactamase of *Escherichia coli*, although it had not been so named in 1940. Plasmid-mediated AmpC -lactamases have been found worldwide but are less common than extended-spectrum β-lactamases (ESBLs), and in *E. coli*, they appear to be less often a cause of cefoxitin resistance than an increased production of chromosomal AmpC -lactamase (Jacoby, 2009).

There are two main types of class C β-lactamases (AmpC) in Enterobacteriaceae species: chromosomal and plasmid derived (Philippon *et al*, 2002). The former is often generated by chromosomal deregulation, whereas the latter comes from gene transfer from plasmids. Subtypes of plasmid-derived β-lactamases have been named according to their resistance to cefoxitin (FOX), cephamycins (CMY), and moxalactam (MOX) or latamoxef (LAT). In addition, these β-lactamases could be

classified by the species origin such as CIT (*Citrobacter freundii*) and EBC (*Enterobacter cloacae*); their genetic features such as Ambler class C (ACC) and AmpC type (ACT); their discovery sites such as the Dhahran Hospital in Saudi Arabia (DHA) and Miriam Hospital in Providence (MIR-1); or the name of the subject in which the species was discovered (BIL-1, patient Bilal). Among these, ACC, FOX, MOX, DHA, CMY, CIT, and EBC genotypes are most commonly reported (Shanthi *et al*, 2012 and Joji *et al*, 2021)

The dissemination of *bla*CMY-2 was mainly due to the spread of IncI1-γ and IncK plasmids.(Borjesson *et al*., 2016).

In Europe and the United States, the prevalence of AmpC genes and antibiotic resistance due to AmpC enzymes have been reported. Similarly, the AmpC-producing Enterobacteriaceae isolates have also been documented in Saudi Arabia.(Abdalhamid *et al*, 2017 and Ibrahim *et al*, 2019). Furthermore, many studies have reported an alarming rise of ESBL and AmpC co-producer strains, causing significant challenges for the management of infections as these Enterobacteriaceae bacteria are multidrug-resistant organism.(Mohamudha *et al*., 2012 and Rizi *et al*, 2020).

1.3.6.4. Extended-Spectrum β-lactamase (ESBL)

Extended-spectrum beta-lactamase (ESBL)-producing bacteria are one of the more recent bacterial evolutions observed in the ongoing antibiotic-resistant pandemic. Since the first detection of these organisms, an increasing prevalence of ESBL-producing bacteria has been described worldwide (Ajuga *et al*., 2021).

ESBLs belong mostly to class A of the Ambler classification and group 2 according to the updated functional classification of β-lactamases by Bush and Jacoby. Class A ESBL enzymes are the most common sources of acquired resistance

to broad-spectrum cephalosporins in *E. coli*. TEM- and SHV-ESBLs were among the first described ESBLs in the 1980s, and they were predominant until 2000. Since then, CTX-M-ESBLs emerged and have been predominantly identified in commensal and pathogenic ESBL-producing *E. coli* isolates of human and animal origin around the world (poirel *et al*., 2018). The ESBLs themselves are generally of different types. The less commonly reported ESBLs include the OXA type, PER type and GES type . Those belonging to the SHV, TEM and CTX-M families have, however, been described as the most commonly encountered among clinical isolates (Ouchar Mahamat *et al*. 2019)

ESBL-producing bacteria are typically resistant to penicillins, first-and second-generation cephaloporins as well as the third generation oxyimino cephalosporins (cefotaxime, ceftazidime, ceftriaxone) and monobactams (aztreonam), retaining susceptibility only to cephamycins, fourth-generation cephalosporins (cefepime, cefpirome) and carbapenems, ESBLs are inhibited by β-lactamase inhibitors such as clavulanic acid and tazobactam, which confer an extended spectrum of activity (Poole, 2004).

1.3.6.4.1. CTX-M β –lactamases

In 1986, a non-TEM and a non-SHV ESBL cephalosporinase was discovered in a cefotaxime-resistant *E. coli.* A few years later in 1989, similar cefotaxime-resistant strains were found in Germany, France and Argentina (Bauernfeind *et al*., 1990). This cefotaxime resistance was due to enzymes named cefotaximases (CTX-M), and these CTX-M showed a much higher degree of activity to cefotaxime than to ceftazidime (Chia *et al*., 2005). The CTX-M represent plasmid acquisition of β-lactamase genes that can normally be found on the chromosome of *Kluyvera spp* (Humeniuk *et al*., 2002).

1.3.6.4.2. TEM β -Lactamases

The TEM-1 (named after a Greek patient Temoneira) was first reported in 1965 from an *E. coli* strain and represents today one of the most prevalent β-lactamase in Enterobacteriaceae (Dowling *et al*., 2017). Isolates harbouring TEM-1 β-lactamases are resistant to ampicillin. Although TEM-type -lactamases are most often found in *E. coli* and *K. pneumoniae*, they are also found with increasing frequency in other Gram-negative species (Bradford, 2001). The TEM-1 β-lactamase encoded by the bla_{TEM-1} gene is present on Tn2 and Tn3 transposons (Partridge and Hall, 2005)

1.3.6.4.3. SHV β –Lactamases

The SHV enzymes, named after the "sulfhydryl variable" active site are commonly associated with *K. pneumoniae* (Shah *et al*, 2004). SHV-1 was first described in 1972 as Pit-2, while SHV-2 was detected in Germany in the beginning of the 1980s (Liakopoulos *et al.*, 2016). The SHV β-lactamases have a similar structure to TEM, with which they share 68% of their amino acids (Shah *et al*, 2004). Both chromosomal, $bla_{\text{SHV-1}}$ and $bla_{\text{SHV-11}}$, and plasmid-mediated bla_{SHV} genes are found (Ford and Avison, 2004). The amino acid changes at positions 238 and 240 according to the Ambler classification are present in the SHV variant with ESBL activity (Rasheed *et al*., 2000)

1.3.6.4.4. OXA β -Lactamases

Class D β-lactamases, also known as oxacillinases or OXA-type β-lactamases (OXAs), are active-serine-site enzymes like Ambler class A and class C βlactamases, differing from class A and C enzymes in amino acid structure, which can confer resistance to penicillins, cephalosporins and, in some cases, carbapenems (Evans and Amyes, 2014). The OXA enzymes may be chromosomal or plasmid-mediated and therefore some OXA variants may be transferred between pathogenic species. OXA

enzymes are generally with widely differing sensitivities to inhibitors (Stojanoski *et al*., 2015).

Class D functionally described as penicillinases capable of hydrolyzing βlactamases were hydrolyzing oxacillin and cloxacillin, this definition is no longer accurate, as recently described enzymes are inadequately inactivated by cloxacillin and oxacillin, and sometimes even save these substrates. However, all OXA significantly hydrolyzes aminopenicillins and carboxypenicillins (Gupta *et al.,* 2020).

1.3.6.5. Carbapenems Antibiotic

Carbapenem antibiotics (including imipenem, meropenem, ertapenem and doripenem), with a broad spectrum of antibacterial activity, play an extremely important role in the field of anti-infective treatment for severe infections. They are stable against most chromosomal broad spectrum β-lactamases and cephalosporinases found in Gram-negative bacteria (Li *et al*., 2020). Carbapenems are β-lactam antibiotics possessing a β-lactam ring and a five-membered ring which differs from that of penicillin in being unsaturated and having a carbon atom rather than sulfur. This unique molecular structure confers remarkable stability against the majority of β-lactamases, including extended spectrum β-lactamases (ESBLs). In 1976, thienamycin, a naturally derived product of *Streptomyces cattleya*, was the first discovered carbapenem (Elshamy and Aboshanab, 2020)

Thienamycin's instability in water limited its clinical use (Lee and Bradley, 2019). However, this instability was overcome by the semisynthetic production of its N-formimidoyl derivative, called imipenem (Foye *et al*., 2013). Imipenem is degraded by a renal tubular dipeptidase enzyme, dehydropeptidase I. For this reason, imipenem is co-administered with cilastatin, a competitive antagonist, which inhibits

imipenem's renal degradation . Cilastatin also protects the kidneys from the toxic effects caused by higher doses of imipenem (Zhu *et al*., 2020).

1.3.6.5.1. Mechanisms of Carbapeneme Action

Bacterial cell walls are complex structures composed of a peptidoglycan polymer. The last transpeptidation step in the synthesis of peptidoglycan is enabled by transpeptidase enzymes, which are penicillin-binding proteins (PBPs). The structure of carbapenems (and other β-lactams) is closely related to acylated D-alanyl-D-alanine – the terminal amino acid residues of the peptidoglycan. This structural similarity allows carbapenems to bind irreversibly to the active site of the PBPs, leading to the inhibition of transpeptidation of the peptidoglycan layer via crosslinking, this in turn disrupts the cell wall synthesis (Fisher *et al*., 2006).

1.3.6.5.2. Molecular Classification of Carbapenemases

β-lactamases have been classified based on the molecular structure classification of Ambler and the functional classification of Bush-Jacobi-Medeiros. In the Ambler classification, β-lactamases are grouped into four classes A, B, C, and D according to motifs composed of primary sequences constituting the protein molecules. β-lactamases of classes A, C, and D use a serine as an enzyme active center, whereas β-lactamases of class B use the metal zinc (Sawa *et al*., 2020).

1.3.6.5.2.1. Ambler Class A Carbapenemases

A number of carbapenemases belonging to Ambler class A have been identified with broad hydrolytic profile for all β-lactams except cephamycins. As such, they mediate serine-directed hydrolysis of aminopenicillins, ureidopenicillins, first-generation and second-generation cephalosporins, aztreonam, and carbapenems (Hammoudi *et al*., 2014). Bacteria expressing these enzymes are characterized by

reduced susceptibility to imipenem, with minimum inhibitory concentration MICs ranging from mildly elevated to fully resistant. These carbapenemases, therefore, may go unrecognized following routine susceptibility testing (Queenan and Bush, 2007). They are inhibited by clavulanate, tazobactam and boronic acid derivatives (Yigit *et al.*, 2001). Class A carbapenemases are plasmid-encoded like IMI (imipenemase), KPC (*Klebsiella pneumoniae* carbapenemase), and some varieties of GES (Guiana extended spectrum). The plasmid-encoded enzymes are often associated with mobile elements responsible for their genetic transfer (Queenan and Bush, 2007).

1.3.6.5.2.2. Ambler Class B Carbapenemases (Metallo-β-Lactamases)

Unlike the serine-dependent carbapenemases belonging to classes A and D, class B carbapenemases are MBLs that require a heavy metal like zinc for catalysis. MBLs have a broad substrate spectrum and can catalyze hydrolysis of virtually all β-lactam antibiotics including carbapenems with the exception of monobactams, such as aztreonam (Jeon *et al.*, 2015). Because MBLs are metallo enzymes, they are resistant to the commercially available β-lactamase inhibitors but are susceptible to inhibition by metal ion chelators like ethylene diamine tetra acetic acid (EDTA).The most common MBL families include the IMP, VIM, NDM, GIM, and SIM enzymes, which are genetically located within a variety of integrons, where they have been incorporated as gene cassettes. When these integrons become associated with plasmids or transposons, transfer between bacteria is readily facilitated (Yoon & Jeong, 2021).

The first transferrable imipenem resistance through MBLs was reported from *P. aeruginosasa* in 1991 in Japan, was designated IMP-1, and its gene was located on a conjugative plasmid conferring resistance to β-lactams, gentamicin, and sulfonamide. This plasmid was transferable by conjugation to *P. aeruginosa* but not

to *E. coli* (Walsh *et al*., 2005).The second dominant group of MBLs is the VIM category, so named due to initial discovery of VIM-1 in a *P. aeruginosa* isolate from Verona, Italy, in 1997. Similar to bla_{IMP}, bla_{VIM} was also carried on a gene cassette inserted into a class 1 integrons and when cloned into *E. coli*, resulted in a significant decrease in susceptibility to a broad array of β-lactams (ampicillin, carbenicillin, piperacillin, mezlocillin, cefotaxime, cefoxitin, ceftazidime, cefoperazone, cefepime, and carbapenems) (Lauretti *et al*., 1999). The bla_{VIM} genes are typically embedded in class 1 integrons, which can be incorporated in either plasmids or chromosomes. Plasmids carrying bla_{VIM} genes in *Enterobacteriaceae* belong, most frequently, to the IncA/C or IncN group (Carattoli, 2009).

1.3.6.5.2.3. Ambler Class D Carbapenemases (Oxacillinases)

These β-lactamases can hydrolyze cloxacillin and oxacillin at rate of more than 50 percent compared to benzylpencillin and is therefore known as OXA enzymes, carbenicillin is easily hydrolyzed, OXA related enzymes form the second largest class of β-lactamases (Zhanel *et al*., 2007). These β-lactamases are encoded plasmids and are commonly found in *P. aeruginosa* and *Enterobacteriaceae* (Walther-Rasmussen and Hoiby, 2006). These enzymes are serine-β-lactamases that have been poorly inhibited by EDTA or clavulanic acid. These carbapenemases are of the type OXA enzyme and have weak carbapenem activity. Enzymes are found primarily in non-fermenting organisms such as *Acinetobacter baumannii*, *P. aeruginosa* and rarely in *Enterobacteriaceae* isolates in most countries, including the United Kingdom and the United State (Moquet *et al*., 2011; Poirel *et al*,. 2011).

The OXA β-lactamase with carbapenemase activity was first identified by Paton *et al*. (1993), and the enzyme isolated from the MDR *A. baumannii* was isolated in 1985 from patient in Scotland , Edinburgh. OXA-48-type producers are among the most difficult carbapenemase, producers to be identified due to their point mutant ESBL

analogues, therefore, their true occurrence rates are difficult to estimate Over the years, 102 unique OXA sequences have been identified, of which 9 are ESBLs and at least 37 are considered to be carbapenemases (Queenan and Bush, 2007; Nordmann *et al.*,2011). OXA- 50 has been observed in several *P. aeruginosa* strains as chromosome enzymes, which are not communicated all the time and may not cause an obvious resistance to carbapenem (Queenan and Bush, 2007). OXA-48 is primarily found in *K. pneumoniae*, that is labeled from UK, India, China, and Turkey (Costa *et al.*, 2003; Cuzon *et al.*, 2010).

1.3.6.5.3. Resistance to Carbapenems Antibiotics.

Resistance to carbapenems may be attributed to three major mechanisms: porin-mediated resistance to reduce uptake of carbapenems, efflux pumps, which pump the carbapenem outside the cells and enzyme-mediated resistance which is mediated via the acquisition of carbapenemase genes. The reduced uptake or increased efflux of antibiotics are usually associated with an overexpression of β-lactamases possessing weak affinities for carbapenems (Elshamy and Aboshanab, 2020)**.**

1.3.7. Flouroquinolone Antibiotics.

Quinolones are a family of synthetic antimicrobial agents with a broad antibacterial activity commonly used as a suitable therapy in patients with UTI. This family has been classified into four generations based on their antimicrobial activity. The most well-known quinolone antibiotics are nalidixic acid, ciprofloxacin, and levofloxacin as members of the first, second, and third generations, respectively, Quinolones prevent bacterial DNA synthesis through inhibiting DNA gyrase and topoisomerase IV enzymes leading to cell death. (Malekzadegan *et al*., 2019)

1.3.7.1. Resistance to Flouroquinolone Antibiotics.

Increased use of antibiotics has been recognized as a major factor driving E. coli resistance over the last decades . Fluoroquinolone resistance is easily established by point mutations in the DNA gyrase and via plasmid-mediated transfer, and various studies have indicated that the use of fluoroquinolone is most likely closely associated with the increase in fluoroquinolone resistance in *E. coli* . Moreover, a few published studies reported that lowering the use of fluoroquinolone decreased the resistance proportion.(Terahara and Nishiura., 2019).

Quinolone resistance is traditionally mediated by chromosomal mutations in bacterial topoisomerase genes, genes regulating expression of efflux pumps, or both (Chen *et al*, 2007). Quinolone resistance is caused by various mechanisms, particularly plasmid mediated quinolone resistance (PMQR) which contains the pentapeptide repeat family Qnr proteins (QnrA, QnrB, QnrS, QnrC, and QnrD). These proteins confer quinolone resistance by physically protecting DNA gyrase and topoisomerase IV from quinolone acts. (Malekzadegan *et al*., 2019).

1.3.8. Aminoglycoside Antibiotics.

Aminoglycosides are potent, broad-spectrum antibiotics that act through inhibition of protein synthesis. The class has been a cornerstone of antibacterial chemotherapy since streptomycin was first isolated from *Streptomyces griseouss* and introduced into clinical use in 1944 (Krause *et al.*, 2016). The aminoglycosides that have been, or are still, important in medical practice are amikacin, gentamicin, isepamicin, kanamycin, neomycin, netilmicin, paromomycin, sisomicin, streptomycin and dihydrostreptomycin, and tobramycin (Yokoyama *et al.*, 2023)

1.3.8.1. Mechanism of Action

The mechanism of aminoglycosides action on bacteria are a multistep process beginning at the plasma membrane, followed by internalization and by effects on various intracellular processes. Binding of the drugs to the 16S ribosomal RNA leads to inhibition of protein synthesis. This inhibition is specifically directed against bacteria since mammalian ribosomal RNA has a different structure. Whether this step causes the bactericidal effects remains to be established (Beringer and Winter, 2011). Aminoglycoside entry into bacterial cells is comprised of three distinct stages, the first of which increases permeability of the bacterial membrane, whereas the second and third are energy-dependent (Krause *et al*., 2016).

1.3.8.2. Resistance Mechanism

About the aminoglycosides resistance there are four mechanisms of resistance to aminoglycosides in bacterial human pathogens. These including (I) reduced uptake or decreased cell permeability, this mechanism are likely chromosomally mediated and results in cross-reactivity to all aminoglycosides, (II) active efflux (III) deactivation of aminoglycosides by aminoglycoside-modifying enzymes (AMEs) and (IV) altered ribosome binding sites by mutations at the site of aminoglycoside attachment may interfere with ribosomal binding (Zweier, 2014). In 2003, the fiveth mechanism of resistance had been emerged represented by ribosomal methylation 16S rRNA A site (Yokoyama *et al*., 2003).

1.3.8.2.1.Aminoglycoside-modifying Enzymes (AMEs)

AMEs are a large family of enzymes consisting of three subclasses categorized according to the type of chemical modification (Krause *et al*., 2016). They impart on their aminoglycoside substrates: N-acetyltransferases (AACs), that acetylate an amino group using acetyl-coenzyme A, O-nucleotidyltransferases (ANTs), that

transfer an adenyl group from ATP to a hydroxyl group of the antibiotic; and O-phosphotransferases (APHs), which phosphorylate a hydroxyl group also employing ATP (Garneau-Tsodikova and Labby, 2016).

1.3.8.2.2.Aminoglycoside N-Acetyltransferases (AACs)

The family of enzymes is the largest within the AMEs, with 48 sequences identified so far, and they all weigh around 20-25 kDa and comprise a wide range of positions susceptible to modification at the 1 [AAC(1)], 3 [AAC(3)], 2′ [AAC(2′)], or 6′ [AAC(6′)] positions (Zárate *et al*., 2018). AACs are found both in Gram-positive and Gram-negative bacteria and are able to reduce the affinity of the antibiotic for the receptor 16S rRNA by enzymes catalyze the acetylation of -NH_2 groups in the acceptor molecule using acetyl coenzyme A as donor substrate, in the case of AACs the acceptor is an aminoglycoside antibiotic (Gillings *et al*., 2017). Genes encoding these enzymes are widespread in plasmids, transposons, and integrons (Vetting *et al*., 2004). There are nine recognized subclasses of AAC(3) enzymes described to date, all of them in Gram-negatives (Lalitha Aishwarya *et al*., 2020). AAC(3) enzymes were the first to be described to confer resistance to gentamicin, kanamycin, fortimicin, and tobramycin and can acetylate either N/O groups of the aminoglycoside (Wolf *et al*., 1998).

The subclass AAC(3)-I includes five enzymes that confer resistance to gentamicin, sisomicin, and fortimicin (astromicin) and are present in a large number of *Enterobacteriaceae* and other Gram-negative clinical isolates (Wilson and Hall, 2010).

Aminoglycoside N-acetyltransferases (6′) enzymes are the most prevalence in clinical strains, conferring resistant to amikacin, gentamicin, kanamycin, neomycin, dibekacin, sisomicin, isepamicin and tobramycin and have an ordered kinetic

mechanism (Vetting *et al.*, 2004). There are two main subclasses of AAC(6′) enzymes that differ in their activity against amikacin and gentamicin C1. While, AAC(6′)-I shows high activity against amikacin and gentamicin C1a and C2 but very low towards gentamicin C1. They are present in Gram-negatives as well as Gram-positives, the genes have been found in plasmids and chromosomes, and are often part of mobile genetic elements, some of them are with unusual structures (Tolmasky, 2007 b).

The structural gene of the AAC(3) and AAC(6′) is generally found on transposable elements (Gillings *et al.*, 2017). The AAC(6′)-I enzymes can be divided into three sub-families, basedupon their structural and kinetic properties. The AAC(6′)-Ie and AAC(6′)-Ib enzymes are classified together in sub-family C, whose members are all monomeric enzymes. The AAC(6′’)-Ig, AAC(6′)-Ih and AAC(6′)-Iy enzymes are in sub-family A and are domain-swapped dimers. The AAC(6′)-Ii enzyme is currently the sole member of sub-family B, and although this enzyme is also dimeric (Stogios *et al*., 2016). Aminoglycoside N-acetyltransferases (6′)-II enzymes actively mediate acetylation of all three forms of gentamicin but not amikacin (Tolmasky, 2007 b). The AAC(6')-Ib-cr enzyme is a variant of AAC(6′)-Ib conferring resistance to tobramycin, amikacin and kanamycin. This is a bifunctional variant able to acetylate quinolones with a piperazinyl substituent (e.g. ciprofloxacin and norfloxacin). Other quinolones without this substitution (i.e. levofloxacin) are not affected by this enzyme (Mamouri, 2015). The *aac(6')-Ib-cr* gene is the most prevalent plasmid mediated quinolones resistance (PMQR) mechanism detected in both quinolone- susceptible and -resistant clinical isolates. Moreover, this gene is usually found in a cassette as part of integrons in multi-resistance plasmids, which may contain other PMQR genes (Tao *et al*., 2020).

1.3.8.2.3.Aminoglycoside O-Nucleotidyltransferases (ANTs)

Aminoglycoside O-nucleotidyltransferases are the smallest family of AMEs, mediate inactivation of aminoglycosides by catalyzing the transfer of an AMP group from the donor substrate ATP to hydroxyl group in the aminoglycoside molecule. There are five classes of ANTs that catalyze adenylylation at the 6 [ANT(6)], 9 [ANT(9)], 4′ [ANT(4′)], 2″ [ANT(2″)], and 3″ [ANT(3″)] positions, of which only ANT(4′) includes two subclasses, I and II. The genes coding for ANT enzymes are found in plasmids, transposons, and chromosomes (Cox *et al*., 2015). ANTs(2″) class consists only of ANT(2″)-Ia, an enzyme that is widely distributed as a gene cassette in class 1 and 2 integrons (Ramirez *et al*., 2005) and mediates resistance to gentamicin, tobramycin, dibekacin, sisomicin, and kanamycin. Therefore, it is commonly encoded by plasmids and transposons (Cox *et al*., 2015). The ANT(3″), are the most commonly found ANT enzymes, they specify resistance to spectinomycin and streptomycin and the coding genes are most commonly named aadA (Chen *et al*., 2015).

1.3.8.2.4.Aminoglycoside O-Phosphotransferases (APHs)

Aminoglycoside O-phosphotransferases are the second most abundant family of AMEs, that use ATP as the second substrate and are able to phosphorylate specific hydroxyl groups of the antibiotics. There are seven classes of APH enzymes, APH(3'), APH(2"), APH(3"), APH(4), APH(7"), APH(6) and APH(9) (Vakulenko and Mobashery, 2003). APH(3'), the largest class of aminoglycoside phosphotransferases includes enzymes that modify the hydroxyl groups of antibiotics at the 3' position. Seven different types of APH(3'), APH(3')-I to APH(3')VII, have been identified among Gram-negative and Gram-positive bacteria and also aminoglycoside producing microorganisms (Ramirez and Tolmasky, 2010). APH(3')-I produces resistance to kanamycin, neomycin, lividomycin, paromomycin, and

ribostamycin. The gene for the first APH(3')-I enzyme *aph(3')-Ia* was discovered on transposon Tn 903 in *E. coli*. Subsequently, it was identified on plasmids and transposons in many Gram-negative bacteria (Shaw *et al.*, 1993). APH(3')-II is rarely found in clinical isolates despite the fact that it confers a similar spectrum of resistance and the gene for APH(3')-I and subclass includes three isozymes that specify resistance to kanamycin, neomycin, butirosin, paromomycin, and ribostamycin (Wright and Thompson, 1999). The gene for APH(3')-IIb has been identified in the chromosome of *P. aeruginosa* (Winsor *et al.*, 2005), and the subclass, *aph(3')-IIc*, was distinct in *Stenotrophomonas maltophilia* (Okazaki and Avison, 2007). APH(3')-III produces resistance to kanamycin, neomycin, lividomyci, paromomycin, butirosin, and ribostamycin. The genes for the APH(3′)-III were originally isolated from *S. aureus* and *S. faecalis* (Kim and Mobashery, 2005). The genes for APH(3')-IV and -V are detected only in antibiotic- producing microorganisms (Vakulenko and Mobashery, 2003).

1.3.8.2.5. 16 S rRNA Methylation

This mechanism involved methylation step occurs via a methylase enzyme at specific nucleotides in 16S rRNA, a site causing restriction in binding between antibiotics to its target due to loss the affinity of binding, thus producing elevated resistance to the clinically important aminoglycosides such as amikacin, tobramycin and gentamicin (Wachino and Arakawa, 2012). This process is mediated by posttranscriptional methylation of two sites of methylation within 16S rRNA that lead to different aminoglycoside-resistance phenotypes have been identified. One group of 16S rRNA methylases, such as that produced by the istamycin producer *Streptomyces tenjimariensis*, methylates residue A1408 confers resistance to kanamycin and apramycin but not gentamicin. Another group of 16S rRNA methylases, exemplified by those produced by gentamicin-producer *Micromonospora*

purpurea, methylates residue G1405 confers resistance to kanamycin and gentamicin but not apramycin. As shown in both of these residues are located within the A-site–decoding region of 16S rRNA, where aminoglycosides are known to bind and interfere with accurate translation through blocking translocation of peptidyl-tRNA from the A-site to the peptidyl-tRNA site (Kotra *et al*., 2000). Ribosomal RNA methyltransferase was first described in a *P. aeruginosa* isolate from Japan in 2003 (Vakulenko and Mobashery, 2003). This isolate contained a plasmid-encoded *rmt*, named *rmtA* for ribosomal methyltransferase. Consequently, numerous additional plasmid-borne RMTs, have appeared in isolates that show high-level resistance to multiple aminoglycosides (Wachino and Arakawa, 2012). At present, ten 16S rRNA methylase genes have been identified, which are capable of conferring high levels of resistance to most clinically important aminoglycosides, including amikacin, gentamicin, kanamycin, and tobramycin (Moura *et al.*, 2017; Braun *et al*., 2018).

1.3.9. Nitrofurantoin

Nitrofurantoin, is a synthetic , broad-spectrum activity against the major uropathogens (i.e., *Escherichia coli* , *Staphylococcus aureus* , *S. epidermidis* , *Klebsiella pneumonia* , and *Enterobacter spp.*) (Cunha, 2013). There is no clinically significant resistance development has occurred. Nitrofurantoin susceptibility in bacteria correlates with the presence of bacterial nitroreductases which convert nitrofurantoin to highly reactive electrophilic intermediates. These intermediates were shown to attack bacterial ribosomal proteins non-specifically, causing complete inhibition of protein synthesis. Resistance to this antibiotic occurred due to mutations in the nitroreductase genes nfsA and nfsB, lead to loss of antibiotics action. (Pereira *et al.*, 2023)

1.3.10. Polymyxins

Polymyxins have historically been viewed as a last-resort antibiotic treatment choice for infections with carbapenemase-producing Enterobacteriaceae . Colistin (polymyxin E) and other polymyxins are cationic polypeptides that interact with the bacterial outer membrane . In the outer membrane , the initial linkage of colistin with the membrane of bacteria occurs by electrostatic interactions between colistin which regarded as a cationic polypeptide and lipopolysaccharide (LPS) molecules as an anion , that leading to the disorder of the membrane . Colistin is making to displace calcium (Ca+2) and magnesium (Mg+2) , which stabilize the lipopolysaccharide molecules from its negative charge, that lead to a local disorder of the outer membrane ,which caused a high permeability of the cell envelope and infiltration of cell contents and thereby cell lysis (Newton-Foot *et al.* , 2017).

However , increasing the use of colistin has resulted in the emergence of colistin resistance in Gram-negative pathogens universally and the rates of resistance are constantly expanding . The most common mechanism of colistin resistance is , in general , thought to be related to the chromosomally-encoded PmrA/PmrB and PhoP/PhoQ twocomponent regulatory systems . Changes in these regulatory systems produce modification of the lipopolysaccharide (LPS) molecules target by adding positively charged [4-amino-4-deoxy-L-arabinos (L-Ara4N)] and phosphoethanolamine molecules , which reduces the negative charge of the outer membrane and thus decrease the intensity of its interaction with colistin (Eiamphungporn *et al.* , 2018).

1.3.10.1. Resistance to Colistin Antibiotics

The polymyxin group includes five different chemical compounds (polymyxins A, B, C, D, and E) ; only two polymyxins are used clinically: polymyxin B and colistin,

also called polymyxin E . For clinical use, two forms of colistin are available: a prodrug, colistin methanesulfonate sodium (CMS), for parenteral use, and colistin sulfate (CS) for oral, inhalator, or topical use (Andrade *et al*., 2020)

Colistin is a polymyxin antibiotic of last resort for the treatment of infections caused by multi-drug-resistant Gram-negative bacteria. By targeting lipopolysaccharide (LPS), the antibiotic disrupts both the outer and cytoplasmic membranes, leading to bacterial death and lysis. Colistin resistance in *E. coli* occurs via mutations in the chromosome or the acquisition of mobilized colistin-resistance (*mcr*) genes. Both these colistin-resistance mechanisms result in chemical modifications to the LPS, with positively charged moieties added at the cytoplasmic membrane before the LPS is transported to the outer membrane.(Humphrey *et al*., 2021).

Resistance to first- and second-line antibiotics frequently necessitates the use of drugs of last resort such as the polymyxins, colistin and polymyxin B, which were used to treat 28% of infections caused by carbapenem-resistant Enterobacteriaceae in the USA in the 12 months to January 2019 (Clancy *et al*.,2019).

The drug was initially widely prescribed, but its use quickly dwindled due to its lack of efficacy and frequent side effects, such as nephrotoxicity and neurotoxicity . Despite these limitations, colistin is considered by the WHO to be a 'highest priority critically important antimicrobial for human medicine' because of its ability to treat infections caused by bacteria that are otherwise resistant to antibiotic treatment. mcr-1-mediated LPS modification protects the cytoplasmic but not the outer membrane from damage caused by colistin, enabling bacterial survival. (Humphrey *et al*., 2021).

1.3.11.Fosfomycin

Fosfomycin is a broad-spectrum antibiotic that was discovered in 1969, low international use since its discovery has resulted in retained susceptibility to a large number of organisms, and this antibiotic provides significant promise against MDR organisms in children, due to its comprehensive spectrum of activity and encouraging safety profile. There is increasing global interest regarding fosfomycin's potential for the treatment of infections caused by many MDR organisms, particularly due to its synergistic effects when combined with aminoglycosides and carbapenems (Souli *et al*. 2011). Fosfomycin is a phosphoenolpyruvate analogue and orally available as Fosfomycin-Trometamol. It has been used extensively for uncomplicated cystitis in women, has a good safety profile (Ten Doesschate *et al*. 2018).

Mechanisms of fosfomycin resistance is induced via one of three mechanisms: (i) reducing the production of functional transporters (impacting the GlpT or UhpT transport systems); (ii) reduced affinity to MurA, the target enzyme; or (iii) production of fosfomycin modifying enzymes. The enzymes responsible for modifying fosfomycin can be chromosomally encoded or encoded on transferable plasmids, as occurs in *E. coli* (Willaims, 2020).

1.3.12. Class 1 integrons

These elements are natural cloning and expression systems that incorporate ORFs by site-specific recombination and convert them to functional genes due to the presence of a promoter sequence. It is now well established that these mobile elements constitute the major vectors of antibiotic multi resistance in Gram-negative and, to a lesser extent, in Gram positive bacteria. Five different classes of mobile integrons have been defined to date, based on the sequence of the encoded integrases. It is known that three (classes 1, 2 and 3) of these classes have an important role in

the dissemination of antimicrobial resistance genes. These classes are associated to multi resistant phenotypes. (Pagano *et al*., 2016)

Antibiotic resistance genes can be carried on plasmids, transposons, integrons and prophages, and bacteria can acquire these genes via horizontal gene transfer from the same or different bacterial species (Breidenstein *et al.*, 2011). Integrons are genetic elements that insert mobile gene cassettes into a specific genetic site via site-specific recombination (Marquez *et al*, 2008)**.**

Chapter Two
Materials and Methods

2.1. Materials

2.1.1.Apparatuses and Equipment

The instruments and equipment with manufacturing companies and origin of countries used in present study are listed in the (Table 2-1).

Table (2-1): The instruments and equipment

Type of equipment	Manufacturer (Origin)
Autoclave	Hirayama (Japan)
Bench centrifuge	Eppendorf (Germany)
Biological cabinet class I	Nuaire, Airegrand (USA)
Compound light microscope	Olympus (Japan)
Conventional PCR system	Gene Amp. (Singapore)
Deep freezer	GFL (Germany)
Electrophoresis unit	Labner (Taiwan)
Electric balance	Memmert (Germany)
Forceps	Himedia (India)
Gel documentation system	Biometra (Germany)
Incubator	Ginder (Germany)
Loops	Himedia (India)
Micropipette set (1-1000 µl)	Eppendorf (Germany)
Mini spine centrifuge	Eppendorf (Germany)
Nano drop biophotometer	Eppendorf (Germany)
Petri dish (15 and 9 cm)	China
pH-meter	LKB (Sweden)
Refrigerator	Ishtar (Iraq)
Spectrophotometer	Eppendorf (Germany)
UV- illuminator	Eppendorf (Germany)
VITEK2 compact system	Biomerieux (France)
Vortex mixer	Eppendorf (Germany)
Water distillater	GFL (Germany)
Water bath	GFL (Germany)
UV illuminator	Eppendorf (Germany)

2.1.2.Biological and Chemical Materials

The biological and chemical materials that used in this study are showed in the (Table 2-2).

Table (2-2): Biological and chemical materials used in the study

Materials	**Manufacturing company (Origin)**
Agarose	Promega (USA)
Ethanol (96%)	BDH (UK)
Free nuclease water	Promega (USA)
Glycerol ($C_3H_8O_3$)	Fluka (Switzerland)
Gram stain	Himedia (India)
H_2O_2 (3%)	Himedia (India)
Kovac's reagent	Himedia (India)
Methyl red	Himedia (India)
McFarland standard tubes (0.5)	Biomerieux (France)
α-Naphthanol ($C_{10}H_8O$)	BDH (UK)
N.N.N.N tetra methyl *p*-phenyl diamine-dihydrochloride	BDH (UK)
Phenol red	BDH (UK)
Ethidium bromide	Intron (USA)
Tries-borate-EDTA buffer (TBE buffer)	Promega (USA)
Primer Pairs	Macrogen (Korea)
DNA ladder	Promega (USA)
Nitrocefin	HARDY diagnostic (USA)

2.1.3.Culture Media

The culture media that are used in study showed in Table (2-3).

Table (2-3): Culture media that used in the study

Type of medium	**Manufacturing company (Origin)**
Brain heart infusion broth	Himedia (India)
Blood agar base	Himedia (India)
Kligler iron agar	Himedia (India)
MacConkey agar	Himedia (India)
Muller-Hinton agar	Himedia (India)
Methyl red-Vogas-Proskawer broth	Oxoid (UK)
Nutrient broth	Himedia (India)
Nutrient agar	Himedia (India)
Peptone water	Himedia (India)
Simmons citrate agar	Mast Diagnostic (UK)
Eosine methylene blue (E.M.B.)	Himedia (India)

2.1.4. Antimicrobial Agents

In current study used two listed of antibiotics, including list of antibiotics in VITEK 2, AST GN76 kit and list antibiotics disc. In AST GN76 kit there are 21 types of antibiotics that shown in the (Table 2-4)

Table (2-4) List of antibiotics in GN76 kit

No.	antibiotics	Class	Subclass
1	ESBL	Cephems	-
2	Amoxicillin	Pencillins	Aminopencillin
3	Ampicillin	Pencillins	Aminopencillin
4	Piperacillin /tazobactam	Beta-Lactam combination agent	-
5	Cefazolin	Cephems	Firest generation
6	Cefoxitin	Cephems	Second generation
7	Ceftazidim	Cephems	Thired generation
8	Ceftriaxone	Cephems	Thired generation
9	Cefepime	Cephems	Fourth generation
10	Ertapenem	Penems	Carbapenems
11	Imipenem	Penems	Carbapenems
12	Meropenem	Penems	Carbapenems
13	Amikacin	Aminoglycosides	-
14	Gentamicin	Aminoglycosides	-
15	Isepamicin	Aminoglycosides	-
16	Ciprofloxacin	Quinolone	Fluoroquinolone
17	Levofloxacin	Quinolone	Fluoroquinolone
18	Sparfloxacin	Quinolone	Fluoroquinolone
19	Nitrofurantoin	Nitrofurans	-
20	Tigyecycline	glycycline	-
21	Trimethoprim /sulfamethazole	Folate pathway antagonists	-

Ten types of antibiotic disks, belonging to nine classes applied in this study are showed in the (Table 2-5).

Table (2-5): Types of antimicrobial agents, provided from Himedia (India)

No.	Class	Antibiotic	Symbol	Concentration (μg)
1	Macrolid	Azithromycin	AZM	15
2	Tetracyclines	Doxycycline	DO	10
3	Pencillin	Piperacillin	PRL	100
4	B-lactam combination agents	Amoxicillin-clavulonate	AMC	30
5	Monobactams	Aztreonam	ATM	30
6	Lipopeptides	Colistin	CS	10
7	Quinolone	Nalidixic acid	NA	30
8	Quinolone	Norfloxacin	NOR	30
9	Phenicole	chloroamphenicole	C	30
10	Fosfomycins	Fosfomycin	FOS	200

2.1.5.Kits

Types of kits that used in this study are listed in (Table 2-6).

Table (2-6): Kinds of kits used in the study

Type of kit	Origin
Plasmid DNA Extraction	Favorgen (Taiwan)
GN 76 AST VITEK-2	Biomerieux (France)

2.1.6.Polymerase Chain Reaction (PCR) Materials

2.1.6.1.Master Mix

The master mix used in the study is showed in Table (2-7).

Table (2 -7): Master mix tag green used in the study

Type	**Description**	**Origin**
Master mix tag green	2X Green Taq reaction buffer pH 8.5, 400 µM dATP, 400 µM dGTP, 400 µM dCTP, 400 µM dTTP, and 3mM $MgCl_2$.	Promega (USA)

2.1.6.2. Molecular DNA Ladder

The molecular DNA ladder used in the study is shown in Table (2-8).

Table (2-8): Molecular weight DNA marker used in the study

DNA marker	Description	Origin
100 bp ladder with loading dye	100-1500 base pairs (bp). The ladder contains of 11double strand DNA fragment ladder with size of 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500 (bp). The 500 bp present at triple the intensity of other fragments and serve as a reference. All other fragments appear with equal intensity on gel.	Promega (USA)

2.1.6.3. The Primers

primers that used in the study were prepared by Macrogen company in Korea , all primers showed in (Table 2-9).

Table (2-9): The primers used in this study

Primer	Gene name	Oligo sequence (5-3)	Product size(bp)	Reference
FOX	*bla*$_{FOX}$	F: AACATGGGGTATCAGGGAGATG R: CAAAGCGCGTAACCGGATTGG	190	Perez-perez and Hnson, 2002
CIT	*bla*$_{CIT}$	F: TGGCCAGAACTGACAGGCAAA R: TTTCTCCTGAACGTGGCTGGC	462	
DHA	*bla*$_{DHA}$	F: AACTTTCACAGGTGTGCTGGGT R: CCGTACGCATACTGGCTTTGC	405	
EBC	*bla*$_{EBC}$	F: TCGGTAAAGCCGATGTTGCGG R: CTTCCACTGCGGCTGCCAGTT	302	
ACC	*bla*$_{ACC}$	F: AACAGCCTCAGCAGCCGGTTA R: TTCGCCGCAATCATCCCTAGC	346	
MOX	*bla*$_{MOX}$	F: GCTGCTCAAGGAGCACAGGAT R: CACATTGACATAGGTGTG GTG C	520	
CMY	*bla*$_{CMY}$	F: GACAGCCTCTTTCTCCACA R: TGGAACGAAGGCTACGTA	1000	Kozak *et al.* (2009)
SHV	*bla*$_{SHV}$	F:CTT TAC TCG CCT TTATCG R:TCCCGCAGATAAATCACCA	827	Chanawong *et al.,(*2000)
TEM	*bla*$_{TEM}$	F:TCAACATTTTCGTGTCGCCC R:AACTACGATACGGGAGGGCT	766	Murugan *et al.*(2018)
OXA	*bla*$_{OXA}$	F: ATATCT CTACTGTTGCATCTCC R: AAACCCTCTAAACCATCC	619	Colom (2003)
CTX-M	*Bla*$_{CTX-M}$	ATGTGCAGYACCAGTAA ACCGCRATATCRTTGGT	512	Amel *et al.* (2016)
KPC	*bla*$_{KPC}$	F:CGTCTAGTTCTGCTGTCTTG R:CTTGTCATCCTTGTTAGGCG	798	Francesco *et al.(*2021)
NDM	*bla*$_{NDM}$	F:GGTTTGGCGATCTGGTTTTC R:CGGAATGGCTCATCACGATC	621	Francesco *et al.*(2021)
IMP	*bla*$_{IMP}$	F: 5-GAAGGCGTTTATGTTCATAC-3 R: 5 - GTAAGTTTCAAGAGTGATGC-3	587	Fallah *et al* .(2014)
VIM	*bla*$_{VIM}$	F: 5- GATGGTGTTTGGTCGCATA-3 R: 5-CGAATGCGCAGCACCAG-3	390	Fallah *et al.*(2014)
Acc(6)Ib	*acc(6) Ib-cr*	F :TTGCGATGCTCTATGAGTGGCTA R :CTCGAATGCCTGGCGTGTTT	482	Park *et al.* (2006)
mcr-2	*mcr-2*	F :CAAGTGTGTTGGTCGCAGTT R :TCTAGCCCGACAAGCATACC	715	Rebelo *et al..* (2018)
mcr-3	*mcr-3*	F :AAATAAAAATTGTTCCGCTTATG R :AATGGAGATCCCCGTTTTT	929	
Int 1	*Int1*	F: CAGTGG ACATAAGCCTGTTC R: CCCGAGGCATAGACTGTA	160	Ranjbar and Farahani,(2019)
qnr A	*qnr A*	F:ATTTCTCACGCCAGGATTTG R:GATCGGCAAAGGTTAGGTCA	516	Wang *et al.* (2008)
qnr B	*qnr B*	F:GATCGTGAAAGCCAGAAAGG R:ACGATGCCTGGTAGTTGTCC	469	

2.2.Methods

2.2.1. Preparation of Reagents and Solutions

2.2.1.1. McFarland standard solution

The 0.5 McFarland's standard (1.5×10^8 CFU/ml) was prepared by adding 0.5 ml of 1.175% barium chloride [BaCl2.2H2O: H2O (w/v, 1.175g: 98.825 ml)] to 1% sulfuric acid [H2SO4: H2O (v/v, 1 ml: 99 ml)] in order to obtain a barium sulfate precipitate. The solution was used to visually compare the turbidity of a suspension bacteria with the turbidity of the 0.5 McFarland's standard. The McFarland's standard tubes were sealed with parafilm to prevent evaporation and stored in the dark for up to 6 months at room temperature. The accuracy of a prepared 0.5 McFarland's standard was checked by using a spectrophotometer. The optical density was measured at 625 nm that should be between 0.08 and 0.1 (CLSI, 2023).

2.2.1.2. Preparation of TBE Buffer (Tris-Borate-EDTA)

Tris-Borate-EDTA(TBE) running buffer is the most commonly used buffer for DNA gel electrophoresis. TBE was prepared and stored as a 10× stock solution. The 10× working solution was prepared by dissolving 108 g of Tris base, 55 g of boric acid, and 40 ml of 0.5 M EDTA in 1000 ml of D.W. However, final concentration of 1× TBE solution was prepared by adding 100 ml of 10× TBE buffer to 900 ml of sterile D.W. (Sambrook and Russel, 2001).

2.2.1.3.Oxidase Reagent

One percent of oxidase reagent was prepared by dissolving 0.1 gm of N.N.N.N tetra methyl *p*-phenyl diamine-dihydrochloride into 10 ml of sterile DW, mixed well and then let stand for 15 minutes. This reagent was prepared freshly in dark bottle daily and the unused portion was discarded. The reagent is used to determine if an

organism possesses the cytochrome oxidase enzyme (Frobes *et al*, 2007)

2.2.1.4.Catalase Reagent

It was prepared in a dark bottle by using a 3% concentration from hydrogen peroxide (MacFaddin, 2000).

2.2.1.5. Methyl Red (MR) Reagent

Methyl red solution (0.02%) was prepared by dissolving 0.1 gm of methyl red in 300 ml of ethanol (96%). Then, sufficient DW was added to make 500 ml and stored at 4˚C in brown bottle. This reagent is an indicator solution used to indicate the pH of the broth culture in the methyl red test (Frobes *et al*, 2007).

2.2.1.6. Vogas-Proskauer (VP) Reagent

It consists of two solutions:

Barritt's solution A (5% w/v): It is prepared by dissolving 5 gm of α-naphthanol in 100 ml of 96% ethyl alcohol.

Barritt's solution B (40% w/v): It is prepared by dissolving 40 gm of potassium hydroxide pellets in 100 ml of DW.

The VP reagent when used with MR-VP test can differentiate bacteria based on their ability to produce the end product, acetylmethylcarbinol (acetoin), from glucose fermentation (Frobes *et al*, 2007).

2.2.2.Culture Media Preparation

Growth media used in the present study were prepared following manufacturer's protocol. The media were sterilized by the autoclave at 121°C for 15 minutes (min).

2.2.2.1. MacConkey's Agar Medium

MacConkey (MA) agar is a selective and differential culture medium for bacteria designed to selectively isolate gram-negative (G-ve) bacteria enteric rods and differentiate them based on lactose fermentation. The MA was prepared by adding 49.5 of MA to 1000 ml D.W a selective and differentiating culture media, MA exclusively supports the growth of G-ve bacteria (Jung and Hoilat, 2022).

2.2.2.2. Eosin Methylene Blue Agar

Eosin methylene blue (EMB) is used for the identification and differentiation of G-ve enteric bacteria from clinical and nonclinical specimens. The EMB was prepared by dissolving 36 g in 1000 ml D.W.(Murray *et al.*, 2020)

2.2.2.3. Brain Heart Infusion Broth

Brain heart infusion broth (BHIB) is a highly nutritious general purpose growth medium recommended for the preservation (MacFaddin, 2000). The BHI Agar was prepared by dissolving 37 g in 1000 ml D.W.

2.2.2.4. Mueller Hinton Agar

Mueller Hinton agar (MHA) is used to determine the susceptibility of microorganisms to antimicrobial agents by either the disk diffusion or well diffusion methods (MacFaddin, 2000). The MHA was prepared by dissolving 38 g in 1000 ml D.W.

2.2.2.5. Nutrient Agar

Nutrient agar (NA) is a general purpose medium which can be used for cultivation of non-highly nutritious bacteria (MacFaddin, 2000). The NA was prepared by dissolving 28 g in 1000 ml.

2.2.2.6. Nutrient Broth

Nutrient broth (NB) is a general purpose medium which supports the growth of bacteria that are not very nutritionally demanding (McFadden, 2000). The NB was prepared by dissolving 25 g in 1000 ml D.W.

2.2.2.7. Peptone Water Medium

This medium was prepared according to the method suggested by the manufacturing company. It was used for the demonstration of the bacterial ability to decompose the amino acid tryptophan to indole. The positive result was formation of red ring at the top of broth (MacFaddin, 2000).

2.2.2.8. Simmon's Citrate Agar

This medium was prepared according to the method suggested by the manufacturing company. It was used for the demonstration of the bacterial ability to use citrate as sole source of energy. The positive result was conversion of color from green to blue (Forbes *et al*., 2007).

2.2.2.9. Kligler iron agar medium

This medium was used to detect the bacteria ability to produce (H2S), fermented sugars with producing acid and gas (Forbes *et al*., 2007).

2.2.2.10. Motility Medium

It was prepared by dissolving 0.5 gm of agar-agar in 100 ml of brain-heart infusion broth, then the contents was dispensed into test tube and autoclaved (MacFaddin, 2000).

2.2.3. Sample Collection

A cross-sectional study was performed during 5 months from (February to june 2022), clinical samples were collected from four main hospitals in Babylon province, that included Marjan teaching hospital, Al-Hilla General Teaching Hospital, Babil teaching hospital for maternity and children and Al-Imam Al-Sadiq Hospital. The clinical samples included urine samples from patients suspected with UTIs, burn exudate, vaginal discharge, sputum, diabetic foot ulcer, stool, CSF, blood, ear swab. The study included both sexes and all ages.

Specimen collection and handling including container types, patients preparation, and transportation to laboratory are recorded in (Table 2-10) according to Bailey and Scotts (2011).

2.2.4.Cultivation and Identification of *E.coli*

E.coli isolates were detected by colonial morphology on Eosin Methylene Blue (EMB) and MacConkey's agar after incubation at 37°C as well as microscopical, biochemical and physiological tests, including gram stain technique, oxidase, catalase, IMVC tests, KIA and motility test. The presumptive *E. coli* isolates were stored in the Brain Heart Infusion Broth with 15% glycerol at - 20°C for further analysis (LeBouguenec *et al.*, 1992).

Table (2-10) Collection, transport, and processing of specimens in current study

specimen	container	Patient preparation	Special instructions	Transportation to laboratory	Primary plasting media	Direct examination
Ear (inner)	Sterile, scew- top container or swab	Clean ear canal with mild soap solution	Aspirate material behind drum with syringe if ear drum intact, used swab to collect material from ruptured ear drum	Immediately R/T	BA and Mac	Gram stain
stool	Clean container		Routin culture	Within 24 hrs/ 4C	BA and Mac	
vagina	Swab moistened with Amies media	Remove exudate	Swab secretions and mucous membrane of vagina	Within 24 hrs/ RT	BA and Mac	Gram stain
Sputum	Sterile, scew- top container	Sputum: have patient brush teeth and then rinse or gargle with water before collection	Sputum: have patient collect from deep cough	Within 24 hrs/ RT	BA and Mac	Gram stain
CSF	Sterile screw-cap tubes	Disinfect before aspirating specimen	Consider rapid test e.g. (gram stain)	Immediately R/T	BA and Mac	Gram stain
Blood	Blood culture media	Disinfect venipuncture site with 70 alcohol and disinfectant such as detadine	Draw blood at time of febrile episode: draw 2 set from right and left arm	Within 2 hrs/RT	Blood culture bottle	
DFU or Burn	Aerobic swab moistened with Amies media	Wipe area with sterile saline or 70% alcohol	Swab along leading edge of wound	Within 24 hrs/RT	BA and Mac	Gram stain
Urine	Sterile screw-cap container	Clean area with soap and water, then rinse with water		Within 2 hrs/ 4C	BA and Mac	Gram stain

RT , room temperature; **BA**, blood agar: **Mac**, Maconkey agar; hrs, hours

2.2.5. Biochemical Tests

The following standard biochemical tests were used for identification of *E.coli* isolated from clinical samples as described by MacFaddin (2000).

2.2.5.1.Indol Production Test

The test was performed by aseptically inoculating the tubes containing 4 ml of pepton water broth by taking the growth from 18 to 24 hours culture and incubated 24 hours at 37°C. Indole production was detected following the overnight incubation by adding 0.5 ml of Kovac's reagent to the broth culture, which reacts with the indole showing a cherry red colored ring. This test demonstrates the ability of certain bacteria to decompose the amino acid tryptophan to indole, which accumulates in the broth.

2.2.5.2.Methyl Red Test

The Methyl red-Vogas-Proskawer broth (5 ml) was inoculated with 18-24 hours pure culture of the test organism and incubated aerobically at 37°C for 24 hours. Then, 5 or 6 drops of methyl red reagent were added to broth. The positive result gives red color change in the broth medium immediately. This test used to identifies bacterial ability to produce sufficient stable acid end products during the fermentation of glucose.

2.2.5.3.Vogas-Proskauer Test

The Methyl red-Vogas-Proskawer broth (5 ml) was inoculated with 18-24 hours pure culture of the test organism and incubated aerobically at 37°C for 24 hours. At the end of this time, 6 drops of 5% α-naphthanol were added and mix well to aerate. Then, 2 drops of 40% potassium hydroxide were added. The tube was shaking gently and allowed to remain undisturbed for 10 to 15 minutes. The positive result gives a

pink-red color at the surface within 15 minutes. The test is used to determine if an organism produces acetylmethyl carbinol from glucose fermentation.

2.2.5.4. Simmon's Citrate Test

The test is performed by picking a well-isolated colony from an 18-24 hours culture with a sterile inoculating needle. The citrate agar tubes are inoculated by streaking the surface of the slant first and finally stabbing the butt. The tubes are then incubated aerobically at 37°C. The test tubes should be examined daily for 4 days before discarding the result as a negative. A positive citrate test is seen as bright blue. The Simmon's citrate test is used to differentiate organisms on the basis of their ability to use citrate as a sole source of energy.

2.2.5.5.Kligler Iron Agar (KIA) Test

The Kligler iron agar test was performed by using a straight wire to pick the test organism; 18-24 hours culture growing on solid media. Then first stabbing the butt within 3-5mm from the bottom and streaking along the slope in a zigzag pattern. This was incubated afterwards at 37°C for 24 hours. The tubes were read for acid production of the slant/butt, gas, and hydrogen sulfide reactions. This test is used for differentiation of Gram negative bacteria on the basis of dextrose and lactose fermentation, gas production and hydrogen sulfide (H_2S) production.

2.2.5.6.Oxidase Test

The filter paper was saturated with oxidase reagent that was placed in a petri dish. Then, a portion of a single, well isolated colony from a 24-hours-old culture was removed from the agar plate with a wooden stick and rubbed onto filter paper impregnated with the reagent. Development of a blue color at the site of inoculation within 10 seconds indicated a positive reaction. This test is used to determine the presence of the enzyme cytochrome oxidase in a given isolate.

2.2.5.7. Catalase Test

A clean, dry glass slide is placed inside a petri dish. A small amount of organism is collected from a well-isolated 18 hours colony with a sterile wooden stick and placed onto the slide. Then, a drop of 3% H_2O_2 was placed onto the organism on the slide by using a dropper. A positive result is the rapid evolution of bubble.

2.2.5.8. Motility Test

A semisolid agar medium was prepared in test tube. A straight needle was touched to colony of a young (18 hours) culture growing on agar medium. The needle stabbed once down the center of the tube to about half the depth of the medium, incubate at 37°C and examined daily for up to 7 days. Motile bacteria typically give a diffuse zone of cloudy growth flaring out from the line of inoculation.

2.2.6. Preservation and Retrieval of isolates

Each isolate was sub-cultured onto nutrient agar to assess purity. To store the isolates for the short-term, overnight growth were sub-cultured on nutrient agar slant and incubated at 37°C for 18 hours. After incubation, the slants stored at 4°C for weekly or monthly.

For long-term storage, 4-5 colonies of the isolate from an overnight pure growth were inoculated in 5 ml of nutrient broth and incubated at 37°C. After 4-5 hours of incubation when the growth is in mid-log phase, 0.5 ml of broth were took and inoculated screw capped tubes containing nutrient broth with 15% glycerol and the tubes were vortex to make the suspension homogenous. They were then kept frozen at -20°C until used. When *E.coli* isolates were required for further testing, they were retrieved from frozen storage and the content was thawed rapidly by placing in lukewarm water for 1 to 3 hours, then cultured on blood agar and incubated at 37°C for 24 hours (Mahon *et al*., 2007) .

2.2.7.Detection of the Ability of *E.coli* to β- lactamase Production.

Number of required nitrocefin discs were placed into sterile empty petri dish and these discs were moisted with one drop of sterile D.W. (not over moisten), then the disc was hold by sterile forceps and wiped a cross a young colony on the plate. The development of a red color in the area of the disc indicated positive result.

2.2.8.Antibiotic Susceptibility Test (AST)

In vitro antibiotics susceptibility of 112 *E. coli* isolates was tested via Vitek 2 compact system (Biomerieux France) and disk diffusion method according to Clinical and Laboratory standards Institute instructions (CLSI, 2023). The bacterial isolates were cultured on a MacCkonkey's agar and then sub cultured in brain heart infusion broth for 18 h at 37ºC. The turbidity was adjusted in 0.85% sterile normal saline solution to 0.5 McFarland's standard (10^8 CFU/mL) and then cultured on Mueller Hinton agar with a cotton swab. Antibiotic disks were put onto MHA by using a sterile forceps, then, pressed down to ensure complete contact with the agar. The plates were incubated for 18 h at 37°C and the antibacterial activity was expressed as mean of the inhibition zone diameter in millimeters (mm). The zone size was measured the next day.

2.2.9.Determination of MDR, XDR and PDR Patterns of *E.coli* Isolates

Defining of MDR, XDR and PDR in *E.coli* isolates were done according to standardized international document (Magiorakos *et al*., 2012), isolates of *E.coli*, which have shown non-susceptibility to at least one agent in three or more antimicrobial categories known as MDR, while isolates exhibit non-susceptibility to at least one agent in all but two or less antimicrobial categories considered as XDR, and isolates reveal non-susceptible to all antimicrobial agents listed distinguish as PDR.

2.2.10.Detection of AmpC, ESBL, Carbapenemase-Producing *E.coli* Isolates

Depending on the results of antibiotics sensitivity that accomplished by vitek 2 system, cefoxitin resistance detected as Amp C- producing isolates, ESBL positive (as item in GN76 kit) detected ESBL-producing isolates and the resistance to carbapenems detected as carbapenemase-producing isolates.

2.2.11.Extraction of Plasmid DNA

The plasmid DNA extraction was done according to manufacturing origin company protocol (Favorgen, Taiwan) using chemical methods. In this study, plasmid DNA was extracted from the isolates cultured in nutrient medium using the plasmid DNA Purification Kit. Bacterial isolates were cultivated in LB medium at 37ºC for 24 hours, and two ml broth culture was precipitated by centrifugation (13000 rpm for 10 minutes). The cell suspension was centrifuged at 6000 rpm for 5 minutes and the supernatant was removed. The deposit cells were treated by solution of the kits according the procedure of Gram negative bacteria. After completion of DNA extraction, the concentration of the DNA was measured by nanodrops.

2.2.12.Genetic Study of *E.coli*

2.2.12.1. Preparation of the Reaction Mixture

After preparing all of the DNA template , Forward primer and Reverse primer and Master mix as follows :

1. The Taq G2 green master mix was kept at room temperature. Vortex the master mix, then spin it briefly in a micro centrifuge to collect the material at the bottom of the tube.

2. Prepare the reaction mix as followed in (Table 2-11) for Monoplex PCR reaction mixture and (Table 2-12) for multiplex PCR reaction mixture

Table (2-11): Monoplex PCR reaction mixture

PCR reaction mixture	Promega protocol (final volume 25 µl)
Master mix 2X	12.5 µl
Primer forward (10µM)	2.0 µl
Primer reverse (10µM) DNA	2.0µl
template	5.0 µl
PCR grade water	3.5 µl

Table (2-12): Multiplex PCR reaction mixture

PCR reaction mixture	Promega protocol (final volume 50 µl)
Master mix 2X	25 µl
Primer forward (10µM)	1.5 µl * 6
Primer reverse (10µM) DNA	1.5 µl * 6
template	5.0 µl
PCR grade water	2.0 µl

3-If using the thermal cycler without a heated lid, overlay the reaction mix with 1-3 drop (Q-solution) of mineral oil to prevent evaporation during thermal cycling. Centrifuge the reaction in a micro centrifuge for 5 seconds.

4. Place the reaction in a thermal cycler that has been preheated to 95 C. preform PCR using your standard parameters.

2.2.12.2. PCR Thermo Cycling Conditions

In this study, the system conditions for the conventional PCR thermal cycler were optimized before PCR mixing tubes were within for efficient amplification of the DNA and priming sets of interest. Three steps formed the PCR condition, the first step is called Initiation denaturation which performed at 95 C°. The second called Annealing which the temperature of this step vary from one gene to another as shown in (Table 2-13). Finally, the last step called final extension that are performed at 72 C°.

Table (2-13): Thermo cycling conditions for detection genes in the study

genes	Temperature (°C) / Time					Cycle No.
	Initial denaturation	Cycling condition			Final extension	
		denaturation	annealing	extension		
***bla*FOX**	94/3 min	94/30 sec	64/30 sec	72/1 min	72/7 min	25
***bla*MOX**						
***bla*CIT**						
***bla*EBC**						
***bla*ACC**						
***bla*DHA**						
***bla*KPC**	94/3 min	95/1 min	55/31 sec	73/1 min	72/5 min	30
***bla*NDM**						
mcr 2	94/15 min	94/30 sec	58/90 sec	72/60 sec	72/10 min	25
mcr 3						
***bla*IMP**	94/5 min	94/60 sec	54/60 sec	72/60 sec	72/5 min	36
***Bla*VIM**						
***bla*OXA**	95/5 min	94/30 sec	54/30 sec	72/60 sec	72/10 min	32
***bla*SHV**	94/3 min	95/15 sec	52/30 sec	72/90 sec	72/5 min	30
***Bla*TEM**	94/5 min	94/30 sec	56/30 sec	72/90 sec	72/ 90 sec	35
***Bla*CTX-M**	94/5 min	94/30 sec	56/30 sec	72/90 sec	72/7 min	35
qnr A	94/5 min	94/45 sec	53/45 sec	72/60 sec	72/7 min	32
qnr B						
bla_{CMY}	94/ 15 min	94/1 min	55/1 min	72/ 1 min	72/10 min	30
ACC(6) Ib-cr	94/5 min	94/45 sec	55/45 sec	72/45 sec	72/7 min	30
Int1	95/1 min	94/30 sec	/40 sec	72/90 sec	72/5 min	35

2.2.12.3. Agarose Gel Preparation

The Agarose gel preparation according to Bartlett and Stirling (1998) as the following:

Agarose gel in this study was prepared by adding 1.5 gm of agarose powder to100 ml of TBE buffer previously prepared (90 ml DW were added to 10 ml TBE buffer l0X). The combination was placed in boiling water bath until it converts clear, and then was allowed to cool to 50°C, and ethidium bromide stain at concentration 0.5 mg/ml was added. The agarose poured kindly in equilibrated gel tray earlier was set with 2 combs fixed in the end and in the middle, and the 2 ends of gel tray were sealed. The agarose was permitted solidifying for 30 minutes at room temperature. The combs and the seal were detached gently from the tray. The gel plate was fixed in electrophoreses and powered by a TBE buffer conducted at 65 volts for 2 hours. Finally, the outcome was recognized by utilizing the gel documentation system.

2.2.13. Sequencing of *pampC* Genes

PCR products of 12 specimens of *E. coli* were stored at -20 C^{o}, and then the nucleotide sequences of *pampC* genes (sanger sequencing) carried out by sending the specimens and primer to Macrogen Company in Korea.

2.2.14. Statistically Analysis

Statistical Package for Social Sciences-version 20 (SPSS v20) was used for data input and analysis. Continuous variables presented as mean and discrete variables presented as numbers and percentages. *P*.value is asymptotic and two sided. Findings with *P*. value less than 0.05 was considered significant (Wayne, 2011).

2.2.15. Ethical Approval

The present research was reviewed and approved by the committee of University of Babylon, College of Medicine along with Al-Hilla Hospitals . The approval form is available in its original version from the corresponding author on request.

Chapter Three
Results and Discussion

3.1. Samples Sources

During a five months period (from February to june 2022) of a cross-sectional study, a total of 1874 clinical samples were collected from four main hospitals in Babylon province, that included Marjan Teaching hospital, Al-Hilla General Teaching Hospital, Babil Teaching Hospital for Maternity and Children, and Al-Imam Al-Sadiq Hospital. The clinical samples included urine samples from patients suspected with UTIs, burn exudate, vaginal discharge, sputum, diabetic foot ulcer, stool, CSF, blood, ear swab. The essential information included both genders and all ages except stool specimens were collected from children less than 5 years.

The distribution of clinical samples according to hospitals as the following, Al-Imam Al-Sadiq Hospital and Marjan Teaching Hospital were 615(32.8%) and 590(31.5%) respectively, followed Babil Teaching Hospital for Maternity and Children 501(26.7%) and Al-Hilla General Teaching Hospital 168(9.0%) (Table 3-1)

Table (3-1) Distribution of samples according to the clinical status and sources

Sample types	Al-Imam Al-Sadiq Hospital	Marjan Teaching Hospital	Babil Teaching Hospital for Maternity and Children	Al-Hilla General Teaching Hospital	Total No.(%)
Urine	130	227	173	112	642(34.3)
Burn exudate	416	0	2	4	422(22.5)
Vaginal discharge	13	0	238	2	253(13.5)
Sputum samples	35	181	22	2	240(12.8)
Diabetic foot ulcer	4	101	0	13	118(6.3)
Stool samples	4	11	45	2	62(3.3)
C.S.F.	3	43	7	4	57(3)
Blood samples	6	27	12	8	53(2.8)
Ear swabs	4	0	2	21	27(1.5)
Total (%)	615 (32.8)	590 (31.5)	501(26.7)	168(9.0)	1874

Most types of clinical samples were urine 642(34.3%), burn exudate 422(22.5%) followed by vaginal discharge 253(13.5%), sputum samples 240(12.8%) (Figure 3-1)

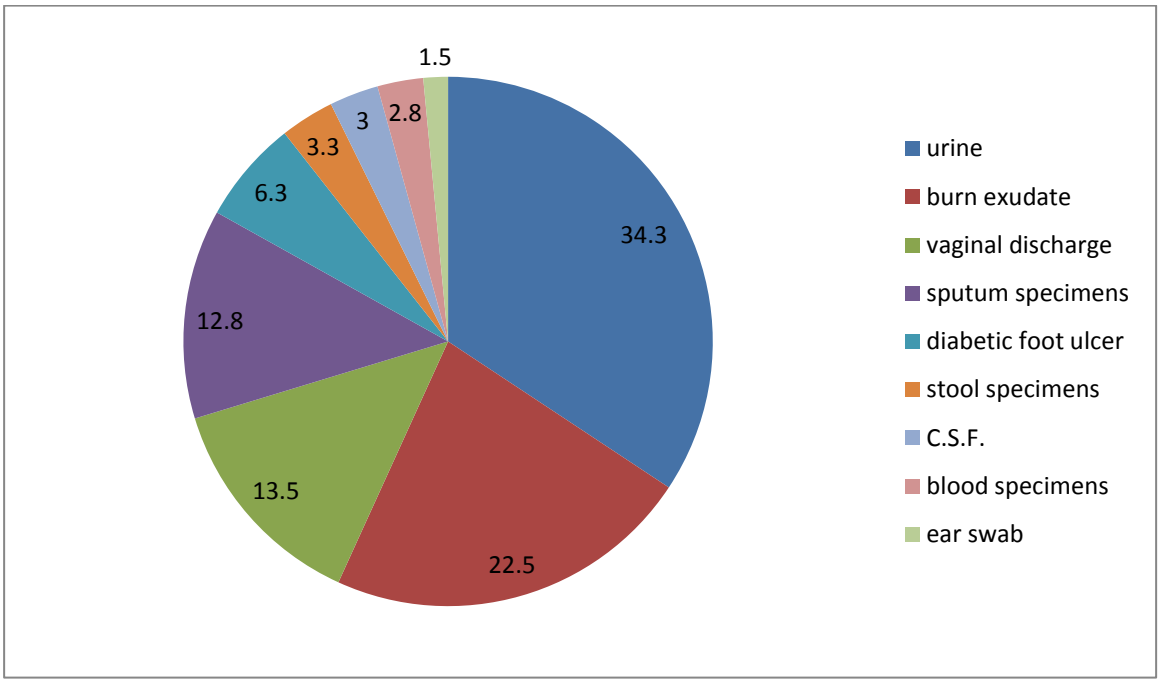

Figure (3-1) Types of various clinical samples

Urinary tract infection (UTI) is common between population in most developing countries, UTI is the second most common type of infection in the body and the most common cause of UTI is Gram-negative bacteria that belong to the family Enterobacteriaceae. Members of this family include *Escherichia coli*, Klebsiella, Enterobacter, and Proteus. (Shayan and Bokaeian, 2015).

3.2. Patients' Sex

Regarding to sex, urine samples were collected mostly from females (61.8%), whereas burns, sputum and diabetics foot ulcer (DFU) were mostly collected from males than females (Table 3-2).

The predominance of UTIs in females in this study was compatible with several authors due to the short urethra and close approximation of the urethra to the vagina and anus.

Table (3-2) Distribution of different samples regarding to patients' sex

Sample types	Total	Male(%)	Female(%)
Urine	642	245(38.2%)	397(61.8%)
Burn exudate	422	288(68.2)	134(31.8%)
Vaginal discharge	253	(0.00)	253(100%)
Sputum samples	240	147(61.3%)	93(38.7%)
Diabetic foot ulcer	118	82(79.8%)	36(20.2%)
Stool samples	62	27(43.5%)	35(56.5%)
C.S.F	57	25(43.8%)	32(56.2%)
Blood samples	53	31(58.5%)	22(41.5%)
Ear swabs	27	16(59.3%)	11(40.7%)

Gu *et al*. (2022) found that (57%) of patients with UTI were females and (43%) males. John *et al*. (2016) reported women are usually more susceptible to this infection compared to the men.

Some of the risk factors responsible for this high prevalence is due to menopause, poor personal hygiene, pregnancy and the close anatomical relationship of the female urethra and the anus (John *et al.,* 2016)

Galal *et al.* (2021) reported that diabetic foot ulcer were more in males (52.2%) than in females (47.8%). In contrast with results in present study, Navarro-Peternella *et al*. (2016) who reported 58.6% of diabetic foot ulcer patients were females.

Regarding to burns, Obed *et al.*, (2023) found that (68%) of patients were males, this result compatible with our study.

3.3. Patients' Age

Regarding to age, most urine samples were collected from patients with ages between 36 to 65 years, (20.7%) of samples were collected from patients with age group 36-45 years, (19.0%) of clinical samples from patients with age group 46-55 years followed by (27.1%) of samples from patients with age group 56-65 years (Table 3-3), concluded most urine samples were collected from elderly patients.

Regarding to vaginal discharge, (48.3%) of samples collected from females with age group 26-35 years. Burn exudate (28%) of patients with age group 46-55 years, (48.4%) of diabetic foot ulcer patients with age group 56-65 years (Table 3-3).

Matthews *et al.* (2011) reported Urinary tract infections (UTIs) are common in the elderly population,

Regarding to burns, Obed *et al*. (2022) found that the largest age group effected were 45–64 year, that forming (34%) of patients, this a result similar the result in our study.

Regarding to diabetic foot ulcer (DFU), most clinical isolates (48.4%) were from patients at age group (56-65) years, Ghanassia *et al.* (2008) reported the prevalence of DFU mostly in elderly, Lu *et al.* (2020) found that DFU related with patients > 80 years.

Vaginal discharge samples, were mostly collected from patients at age group (26-35) years, Fonseca *et al.* (2013) found that vaginosis mostly from younger women.

Table (3-3) Distribution of different samples regarding to age groups of patients

samples	1day-5 years No.(%)	6-15 years No.(%)	16-25 years No.(%)	26-35 Years No.(%)	36-45 years No.(%)	46-55 years No.(%)	56-65 years No.(%)	66-75 years No.(%)	total
Urine	35(5.5)	21(3.3)	56(8.7)	64(10.0)	133(20.7)	122(19.0)	174(27.1)	37(5.7)	642
Burn exudate	2(0.5)	66(15.6)	43(10.2)	89(21)	83(19.7)	118(28)	20(4.7)	1(0.3)	422
Vaginal discharge	(0.00)	(0.00)	66(26)	122(48.3)	51(20.2)	8(3.2)	5(1.9)	1(0.4)	253
Sputum samples	(0.00)	1(0.4)	11(4.6)	33(13.7)	63(26.2)	76(31.7)	42(17.5)	14(5.9)	240
Diabetic foot ulcer	(0.00)	1(0.8)	(0.00)	2(1.7)	2(1.7%)	37(31.3)	57(48.4)	19(16.1)	118
Stool samples	62(100)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	62
C.S.F.	(0.00)	2(3.5)	1(1.8)	21(36.9)	7(12.3)	10(17.5)	8(14)	8(14)	57
Blood samples	2(3.8)	3(5.7)	(0.00)	7(13.2)	18(34)	19(35.8)	(0.00)	4(7.5)	53
Ear swabs	(0.00)	2(7.4)	7(25.9)	2(7.4)	1(3.7)	8(29.7)	(0.00)	7(25.9)	27

3.4. Isolation and Identification of *E.coli*

The clinical samples are collected from hospitalized patients, by using sterile trasporter media, each specimen was immediately inoculated into MaCconkey, EMB agar and blood agar, incubation occurred at 37°C for 18 hours. Laboratory diagnosis procedure depending on the steps recommended by (MacFaddin, 2000).

Escherichia coli isolates when cultured on EMB agar produced a green metallic sheen colonies due to the fact that under acidic conditions the dyes produce a dark purple complex which is usually associated with a green metallic sheen. This metallic green sheen is an indicator of lactose and/or sucrose fermentation ability typical of fecal coliforms (Lal and Cheeptham, 2007).

3.5. Prevalence of *E.coli* isolates Among Clinical Samples

Out of 1874 clinical samples, 231 (12.3%) were identified as *E.coli* isolates, other bacterial isolates were 808 (43.1%) and 835 (44.6%) were negative culture (Table 3-4), Mustafai *et al.* (2023) found in cross sectional study, the prevalence of *E.coli* was (9.1%), this result relatively similar prevalence of *E. coli* in current study.

High prevalence of *E.coli* were isolated from stool samples (61.4%), followed by urine (18.4%), diabetic foot ulcer (11%), vaginal discharge (10.7%), burn exudate (7.1%).

A 62 stool samples, *E.coli* isolated from 38 samples (61.4%), showed high prevalence of *E.coli* in stool when compared with other clinical samples. This results consistent with findings of Adinortey *et al*. (2017) found that out of 167 stool samples, *E.coli* isolated from 114 (69%) samples.

Table (3-4) Distribution of *E.coli* and other bacterial isolates according to clinical samples.

Sample types	No. of clinical samples	No.(%) of bacterial isolates	No.(%) of E.coli isolates	No.(%) of other bacterial isolates	No.(%) of negative cultures
Urine	**642**	**163(25.4)**	**118(18.4%)**	**45(7)**	**479(74.6)**
Burn exudate	**422**	**352(83.4)**	**30(7.1%)**	**322(76.3)**	**70(16.6)**
Vaginal discharge	**253**	**124(49)**	**27(10.7%)**	**97(38.3)**	**129(51)**
Sputum samples	**240**	**204(85)**	**2(0.8)**	**202(84.2)**	**36(15)**
Diabeti foot ulcer	**118**	**98(83)**	**13(11)**	**85(72)**	**20(17)**
Stool samples	**62**	**61(98.4)**	**38(61.4)**	**23(37)**	**1(1.6)**
C.S.F.	**57**	**4(7)**	**(0.00)**	**4(7)**	**53(93)**
Blood samples	**53**	**9(17)**	**(0.00)**	**9(17)**	**44(83)**
Ear swabs	**27**	**24(88.9)**	**3(11.1)**	**21(77.8)**	**3(11.1)**
Total	**1874**	**1039(55.4)**	**231(12.3)**	**808(43.1)**	**835(44.6)**

Regarding to urine, John *et al.* (2016) reported The predominant organisms responsible for UTI were mostly the Enterobacteriaceae especially *E. coli* which are the cause of 80–85% of urinary tract infections. This result was high when compared with current study. Yadav *et al*. (2015) found that the prevalence of *E.coli* in urine samples were 67/450(14.9%), this result relatively similar the result in current study. The frequency of clinical isolates of *E. coli* in urine samples was higher in females than in males (Assafi *et al.*2015; Naqid *et al.*2020), and most urine samples were from females.

In Iraq/ wasit province, a study on diabetic foot ulcer (DFU) by Jassim *et al*. (2022) reported 13.2% of DFU were *E.coli*, in Athiopia, Atlaw *et al.* (2022) reported that (16.5%) of isolates were *E.coli*. These results consistent with our study.

Regarding to vaginal discharge samples, a study in Najaf city by Al-kraety *et al*. (2022) reported that (43.4%) of isolates were *E.coli*, the percentage of *E.coli* isolates were more than in our study.

In Ghana (Adinortey *et al*., 2017) found that the prevalence of *E.coli* in vaginal swab (8%) this result compatible with result in our study. Several authers found Enterobacteriaceae were isolated from patients with aerobic vaginitis symptoms in many studies .This might be due to the poor some women's hygiene and pass of gut flora into genital tract causing vaginal (Hayat *et al.* 2015; Kumar and Singh 2015).

3.6. Distribution of *E.coli* Isolates According to Sex

Regarding to association of *E.coli* to sex, (60.6%) of different isolates were from females, statistical analysis was significant *p*- value (0.001), 65.3% of urine origin *E.coli* isolates from females, whereas, *E.coli* isolated from diabetic foot ulcer and burn exudate mostly collected from males more than females, (69.3%) from diabetic foot ulcer, (63.3%) from burns (Table 3-5). The statistical analysis revealed all *p*-values were significant.

Table (3-5) Distribution of *E.coli* isolates according to sex

Sample types	Total *E.coli* isolate	Male (%)	Female (%)	*P* - value
Urine	**118**	**41(34.7)**	**77(65.3)**	**< 0.001**
Burn exudate	**30**	**19(63.3)**	**11(36.7)**	**< 0.001**
Vaginal discharge	**27**	**(0.00)**	**27(100)**	**< 0.001**
Sputum samples	**2**	**2(100)**	**(0.00)**	**< 0.001**
Diabetic foot ulcer	**13**	**9(69.3)**	**4(30.7)**	**0.001**
Stool samples	**38**	**18(47.4)**	**20(52.6)**	**0.018**
Ear swabs	**3**	**2(66.7)**	**1(33.3)**	**< 0.001**
Total	**231**	**91(39.4)**	**140(60.6)**	**0.011**
High significant (P < 0.001), Significant (P value < 0.05), Non-significant (P value > 0.05)				

Regarding to high frequency of *E.coli* in urine, A study by Gu *et al*., (2022) revealed that *E.coli* high prevalence in females (53.2%) of isolates, Lin *et al*., (2022) found (75%) *E.coli* isolates from females, whereas (25%) of *E.coli* isolates from males. John *et al.* (2016) reported the female urethra appears to be particularly prone to colonization because of its proximity to the anus, some organism particularly

uropathogenic *E. coli* (UPEC) which is present within bowel flora can infect the urinary tract by expressing some specific virulence factors that permit adherence and colonization of the lower urinary tract causing urinary tract infections

Bruun *et al*. (2013) reported that there was an association between diabetic foot ulcer and male as well as peripheral neuropathy, peripheral arterial disease, retinopathy and myocardial infarction, whereas Dinh and Veves (2008) found that no differences between male and female in age (59 ± 12) years.

A study by Obed *et al*. (2022) revealed 68% of the *E.coli* isolated from burns patients were males, this result compatible with present study, In contrast with present study, Dissanaike *et al*. (2017) found there were significant increase in the risk of burn injury in women, Yakupu *et al*. (2022) found the burn almost evenly split between men and women.

3.7. Detection of β-Lactamase Producing *E.coli* (BPEC)

Nitrocefin is chromogenic cephalosporin, when changing from yellow to red indicated a positive result, out of 231 *E.coli* isolates 112(48.5%) were β- lactamase producer, high percentage of *E.coli* isolates from vaginal discharge (63%), diabetic foot ulcer (53.8%), burn exudate (53.3%), urine (47.5%). (Table 3-6).

Nitrocefin used as substrate for the rapid and quantitative detection of β-lactamase-producing *Escherichia coli* (Chantemesse *et al.*, 2017). High prevalence of β-lactamase producer compatible with resistance to β- lactam antibiotics in present study, each *E.coli* isolate positive to nitrocefin was resistant to one or more β-lactam antibiotics and several studies depended on ampicillin and amoxicillin resistant instead of nitrocefin.

Table (3-6) Distribution of β- lactamase producing *E.coli* (BPEC) according to clinical isolates

Sample types	Total *E.coli* isolate	*E.coli* produce beta lactamase (nitrocefin positive)
Urine	118	56(47.5%)
Burn exudate	30	16(53.3%)
Vaginal discharge	27	17(63%)
Sputum samples	2	(0.00)
Diabetic foot ulcer	13	7(53.8%)
Stool samples	38	15(39.5%)
Ear swabs	3	1(33.3%)
Total	231	112(48.5%)

3.8. Antibiotics Susceptibility of β-Lactamase - producing *E coli*

3.8.1. Bacterial Susceptibility to β-Lactam Antibiotics

β-lactam antibiotics are one of the most important groups of antimicrobial agents administered worldwide, covering as much as 50% of all prescribed drugs. This is because they are highly effective and their efficacy, broad spectra, and low toxicity (Samaha-Kfoury and Araj, 2003), Beta-lactam antimicrobials have been used extensively in human healthcare settings and animal husbandry to treat bacterial infections (Mahazu *et al*. 2022).

The results of present study revealed that all *E.coli* isolates (No.= 112) were highly resistant to ampicillin (100%), amoxicillin (100%), piperacillin (99.1%), amoxicillin-clavulonate (100%), (Figure 3-2).

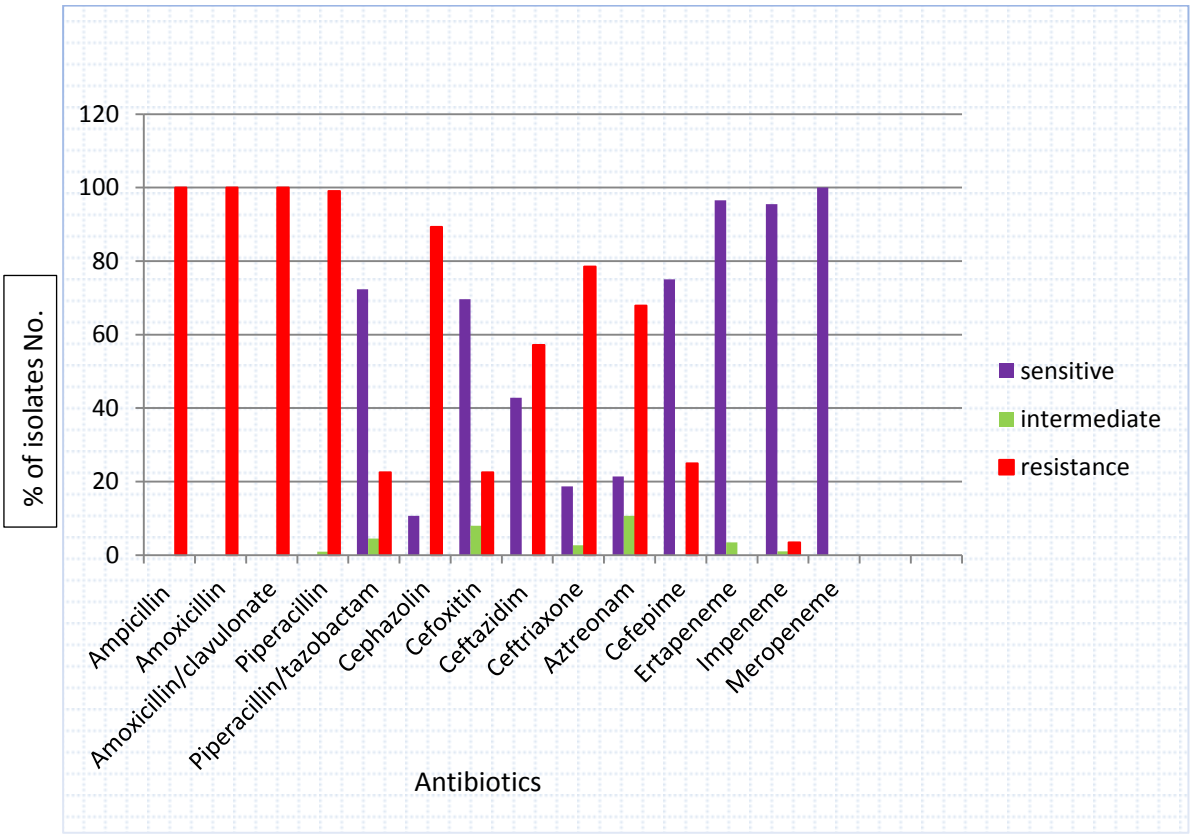

Figure (3-2) Antibiotic susceptibility to β–lactam drugs of β- lactamase producing *E.coli* isolate (No.=112)

This result is most known by several textbooks regarding ability of *E.coli* clinical isolates to resistance penicillins (ampicillin, amoxicillin and piperacillin) by means of mechanisms that includes inactivation of these agents by the production of β-lactamase, decreased penetration of the antibiotics to the target site, alteration of target site PBPs and efflux pumps (Mayers 2009; Drlica and Perlin 2010).

Allami *et al*. (2022) in southern Iraq, recorded resistance to piperacillin and amoxicillin/ clavulonic acid were 92% and 88% respectively, Hasan and Ibrahim (2022), in Duhok city, Kurdistan region/ Iraq, found that resistance to penicillin including penicillin and amoxicillin were 85%, Ghaffoori1 and Suleiman (2022) in Anbar city found resistance to ampicillin (94%), Bahramian *et al*. (2021) in Iran found that resistance to ampicillin were (100%) Jain *et al*. (2021) found (98%) of

E.coli resistant to amoxicillin, these results consistent with our findings in present study, Mustafai *et al*. (2023) were reported resistance to amoxicillin-clavulonate (97.1%), Said *et al* .(2021) in Saudi arabia reported resistance to ampicillin (75.5%), most studies were consistent with current studies. Atif *et al.* (2023) in Baghdad city/ Iraq were noted most β-lactamase producers had multiple plasmids, where's single plasmid profile predominant in non-β-lactamase-producing isolates. The presence of common plasmid among the isolates increases the distribution of resistant plasmid in the community.

Regarding resistance of various origin *E.coli* to cephalosporins agents, cephazolin (89.3%), ceftriaxone (78.5%), aztreonem (67.9%) and cefoxitin (23.2%) (Figure 3-2) (Table 3-7).

Table (3-7) β- lactam antibiotics resistance profiles of *E.coli* isolated from various clinical sources.

B- lactam antibiotics	**Number of Isolates Recovered from Clinical Sources (Percent (%) of Resistance)**						
	Urine (N=56)	**Burn exudate (N=16)**	**Stool (N=15)**	**Vaginal discharge (N=17)**	**DiabetiC foot ulcer (N=7)**	**Ear swab (N=1)**	**Total (N =112)**
Ampicillin	**56(100)**	**16(100)**	**15(100)**	**17(100)**	**7(100)**	**1(100)**	**112(100)**
Amoxicillin	**56(100)**	**16(100)**	**15(100)**	**17(100)**	**7(100)**	**1(100)**	**112(100)**
Piperacillin/tazobactam	**16(28.5)**	**1(6.2)**	**2(13.3)**	**1(5.9)**	**5(71.4)**	**(0.00)**	**25(22.5)**
Cephazolin	**53(94.6)**	**9(60)**	**17(100)**	**7(100)**	**7(100)**	**1(100)**	**94(89.3)**
Cefoxitin	**14(25)**	**2(12.5)**	**4(26.7)**	**1(5.9)**	**5(71.4)**	**(0.00)**	**26(23.2)**
Ceftazidim	**38(67.8)**	**5(31.2)**	**5(33.3)**	**10(58.8)**	**6(85.7)**	**(0.00)**	**64(57.2)**
Ceftriaxone	**52(92.8)**	**11(68.7)**	**4(26.7)**	**13(76.5)**	**7(100)**	**1(100)**	**88(78.5)**
Cefepime	**24(42.8)**	**(0.00)**	**(0.00)**	**2(11.8)**	**2(28.6)**	**(0.00)**	**28(25)**
Etrapeneme	**(0.00)**	**(0.00)**	**(0.00)**	**(0.00)**	**(0.00)**	**(0.00)**	**0(0)**
Impenem	**2(3.5)**	**(0.00)**	**(0.00)**	**1(5.9)**	**1(14.3)**	**(0.00)**	**4**
Meropeneme	**(0.00)**	**(0.00)**	**(0.00)**	**(0.00)**	**(0.00)**	**(0.00)**	**(0.00)**
Piperacillin	**56(100)**	**16(100)**	**15(100)**	**17(100)**	**7(100)**	**1(100)**	**112(100)**
Amoxicillin-clavulonate	**56(100)**	**16(100)**	**15(100)**	**17(100)**	**7(100)**	**1(100)**	**112(100)**
Aztreonem	**42(75)**	**5(31.2)**	**5(33.3)**	**17(100)**	**7(100)**	**(0.00)**	**76(67.9)**
***P* - value**	**0.0381**	**0.0011**	**0.0165**	**0.0087**	**0.043**	**1**	
High significant (P < 0.001), Significant (P value < 0.05), Non-significant (P value > 0.05)							

Naqid *et al.* (2020) in Duhok city/ Kurdistan Region of Iraq, found that antibiotics resistance for *E.coli* isolated from various clinical samples revealed resistant to ceftriaxone (63.9%), this result consistent with current study, whereas cephazolin (38.6%). Ghaffoori and Suleiman (2022) found that resistance to ceftriaxone (58%), cephazolin (64.1%), ceftazidim (67.1%), these results were lower than results in present study. The resistance to cephalosporins agents may be due to having AmpC and/ or ESBL genes.

Cefoxitin is antibiotics used for treatment of *E.coli* and other infections including anaerobic bacteria and for detection AmpC β- lactamase producer bacteria According to (CLSI, 2022). The result of cefoxitin resistance (23.2%) were consistent with other studies (Sadeghi- deylamdeh and Jafari-sales 2021; Sultan *et al.* 2013; Mansouri *et al*.2014)

Regarding to carbapenems resistance, the results revealed that *E.coli* isolates showed high susceptibility to carbapenems, no resistance to ertapenem and meropenem but slightly resistant to impenem (3.5%) (Figure 3-2), Liu and Liu (2016) explained *E.coli* resistance to carbapenem antibiotics including meropenem, ertapenem and impenem because high stability of this drugs against β- lactamase and reported there is no resistance to impenem (0%).

Several authors revealed that carbapeneme active agains *E.coli*. Carbapeneme especially meropeneme it has excellent bactericidal activity against gram positive, gram negative, and anaerobic bacteria. Carbapenemase is especially usefull in treating infections caused by gram–negative rods that produce ESBL. Carbapenemase are often" drugs of last resort " bacteria resistance to multiple antibiotics (Drlica and Perlin 2010; Levinson *et al.*, 2018)

Atif *et al.* (2023) in Baghdad city/ Iraq found that high suceptibility to impeneme (100%) and meropeneme (99%), Naqid *et al.*(2020) in Duhok city, Kurdistan

Region/ Iraq antibiotics resistance for ertapenem and impenem were (2.2%) and (3.9%) respectively, these results compatible with present results, Halaji *et al.* (2020) revealed *E.coli* resistant to impenem and meropenem (0%), Medugu *et al.* (2022) found that resistance to meropenem (7.5%), Mustafai *et al.* (2023) found that resistance to meropenem and impenem were (31.4%), Said *et al.* (2021) in Saudi Arabia reported resistance to meropenem and etrapenem were 30.1% and 37.1% respectively, these results differ from present results because differences in geographic regions.

Distribution of β–lactamase producing *E.coli* according to clinical origin, results revealed that all *E.coli* isolates recovered from different clinical samples were highly resistance to penicillins (Figure 3-2).

Regarding to clinical samples the statistical analysis were significant for all isolates, *P*- value < 0.05.

Regarding to urine isolates, the results revealed that *E.coli* showed high rate of resistance to cephazolin (94.6%), ceftazidim (67.8%), ceftriaxone (92.8%) and aztreonem (75%) (Table 3-7). A study in Baghdad city on (UTIs) recorded the resistance to ceftazidim (58%), ceftriaxone (79%) and recorded no resistance to impeneme, (Khadhim 2021), these results relatively similar the present results, Hussein (2017), in Duhok city/ Iarq , found that *E.coli* isolated from UTIs resistance to ceftazidim (64.4%), aztreonem (66.3%), impenem (2.1%) and cefepime (64.8%) these result consistent with present, in Iran a study by Sadeghi *et al.* (2021), found that *E.coli* resistance to ceftazidim (55.1%), meropenem (0.8%) and impenem (4.2%), these results consistent with our study but different in resistance to ceftriaxone (56.3%), whereas in present study (92.8%).

E.coli isolated from diabetic foot ulcer (DFU) resistance to cephazolin (100%), aztreonem (100%) and ceftriaxone (100%) (Table 3-7), a study in Kufa city/Iraq by

Wahid *et al.* (2022) found that resistance to aztreonam (79%) and ceftriaxone (95%), the resistance to aztreonem is high as in present study, Atlaw *et al.* (2022) reported resistance to cephazolin (33.3), aztreonem (28.8%) and ceftriaxone (52%), the result in this study were low resistance when compared with present study, SHI *et al.* (2023) in china reported moderated resistance to several antibiotics in contrast with our study shown high resistance (100%) to cefazolin, ceftriaxone and ztreonem. Alm *et al.* (2015) concluded that small PBP3 changes will affect the susceptibility to a broad range of β-lactams (aztreonem),

E.coli isolated from vaginal discharge were resistant to cephazolin (100%), aztreonem (100%), ceftriaxone (76.5%), as mention in (Table 3-7). A study by ALkratey *et al.(*2022) in Najaf province/Iraq reported vaginal *E.coli* resistance to ampicillin, ceftriaxone, and cephazolin. Atia (2021) in Libya how reported *E.coli* isolated from vagina resistance to ceftriaxone (76.5%) but sensitive to more antibiotics, whereas in present study all vaginal E.coli resistance to cephazoline and aztreonem.

E.coli that isolated from burns were resistant to ceftriaxone (68.7%), (Table 3-7) this result similar the result in a study in Isfahan/ Iran, the ceftriaxone resistance for *E.coli* isolated from burns (70%) (Haghighifar and Dolatabadi, 2020).

3.8.2. Bacterial Susceptibility to Non-β-Lactam Antibiotics

In present study, *E.coli* showed different degree of resistance to non- β-lactam antibiotics. The resistance to aminoglycosides revealed that *E.coli* were resistant to isepamicin (1.8%), amikacin (0%) and gentamicin (33%) (Figure 3-3), (Table 3-8).

Aminoglycosides are amongst the most important compounds used to treat serious nosocomial infections caused by aerobic, Gram-negative bacteria. They are

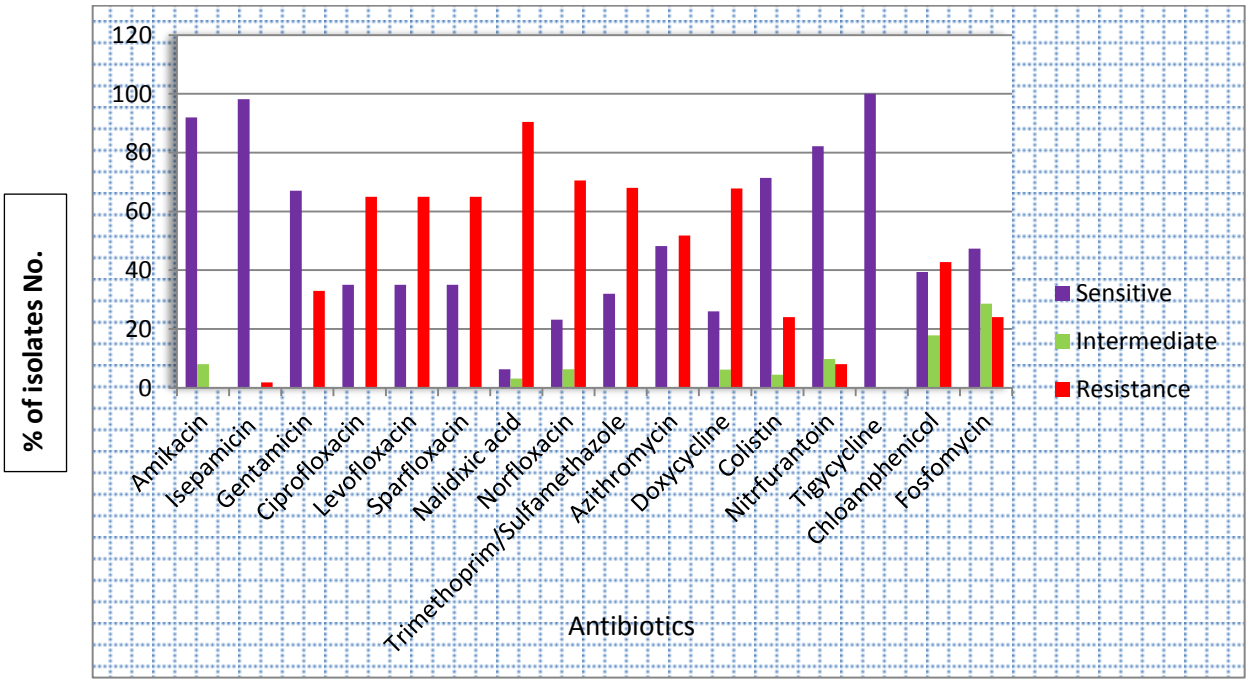

Figure (3-3) Antibiotics susceptibility to non β –lactam drug of *E.coli* isolate (No.=112)

pseudo-polysaccharides containing amino sugars and can therefore be considered polycationic species for the purpose of understanding their biological interactions. Since they are highly positively charged at physiological pH values, they show high binding affinity for nucleic acids, especially for certain portions of the prokaryotic ribosomal RNA (rRNA) (Mayers 2009).

Ghaffoori and Suleiman (2022) reported that resistant to amikacin (2%), similar result in present study. Jain *et al*. (2021) noted resistance to gentamicin (39%), relatively similar the results in current study.

Regarding to quinolone antibiotics *E. coli* resistant to ciprofloxacin (65%), levofloxacin (65%), sparfloxacin (65%), norfloxacin (70.5%) and nalidixic acid (90.5%).

Aiber *et al*.(2022) in mosul city/ Iraq found that resistance to ciprofloxacin and levofloxacin were 68.2% and 66.7% respectively, Ghaffoori and Suleiman (2022)

Table (3-8) Non B- lactam antibiotics resistance profiles of *E. coli* isolated from various clinical sources.

Non B- lactam antibiotics	Number of Isolates Recovered from Clinical Sources (Percent (%) of Resistance)						
	Urine (No. = 56)	Burn exudate (No.=16)	Stool (No.=15)	Vaginal discharge (No.=17)	Diabetic foot ulcer (No.=7)	Ear swab (No.=1)	Total
Amikacin	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
Isepamicin	1(0.9)	1(0.9)	(0.00)	(0.00)	(0.00)	(0.00)	2(1.8)
Gentamicin	19(33.9)	5(31.2)	3(20)	10(58.8)	(0.00)	(0.00)	37(33.4)
Ciprofloxacin	32(57.1)	9(56.2)	8(53.3)	17(100)	6(85.7)	1(100)	73(65.2)
Levofloxacin	32(57.1)	9(56.2)	8(53.3)	17(100)	6(85.7)	1(100)	73(65.2)
Sparfloxacin	32(57.1)	9(56.2)	8(53.3)	17(100)	6(85.7)	1(100)	73(65.2)
Nalidixic acid	50(89.3)	15(93.7)	13(86.7)	17(100)	6(85.7)	(0.00)	101(90.5)
Norfloxacin	38(67.8)	12(56.2)	10(66.7)	12(70.6)	6(85.7)	(0.00)	78(70.5)
Nitrofurntion	(0.00)	2(12.5)	3(20)	(0.00)	4(57.1)	(0.00)	9(8)
Trimethoprime/sulfamethazole	31(55.3)	10(62.2)	12(80)	16(94.1)	6(85.7)	1(100)	76(68)
Azithromycin	30(53.5)	5(31.2)	5(33.3)	14(82.4)	4(57.1)	(0.00)	58(51.8)
Doxycycline	45(80.3)	10(62.2)	7(46.7)	12(70.6)	2(28.6)	(0.00)	76(68)
Colistin	9(16.0)	6(37.5)	3(20)	5(29.4)	4(57.2)	(0.00)	27(24.1)
Chloroamphenicol	22(39.3)	9(75)	6(40)	6(35.3)	5(71.4)	(0.00)	48(42.8)
Fosfomycin	21(37.5)	3(18.7)	3(20)	(0.00)	(0.00)	(0.00)	27(24.1)
Tigycycline	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
***P* - value**	0.0359	0.0145	0.0035	0.0292	0.0149	1	-
High significant (P < 0.001), Significant (P value < 0.05), Non-significant (P value > 0.05)							

found that resistant to ciprofloxacin (59%), a study in Baghdad city Kadhim, M. (2022) reported *E.coli* resistant to ciprofloxacin (80%), these results were consistent with present study. Jain *et al*.(2021) in Bangladish reported resistance to ciprofloxacin (34%) and norfloxacin (39%) these results lower than findings in present study.

E.coli isolates resistant to nitrofurntoin and trimethoprime/ sulfamethazole were (8%) and (68%) respectively (Table 3-8), Ghaffoori and Suleiman (2022) found that resistance to nitrofurntoin (2.9%) slightly lower result in present study and found that resistance to trimethoprime/ sulfamethazole (89.5%), the result more than result in current study.

Resistance to doxycycline (67.8%) and fosfomycin (24.1%) (Table 3-8), a study in athiopia reported *E.coli* resistance to doxycycline was 81.2% (Assefa *et al*., 2022), this result consistent with our study. Sardar *et al*. (2017) found that all *E.coli* sensitive to fosfomycin. Batra *et al*. (2020) concluded that the level of antibiotic resistance in the community is increasing rapidly with the constant use and misuse of antibiotics, and reported fosfomycin resistance were 3.7%, 10.8%, 17.5% and 34.2% for 2015, 2016, 2017 and 2018.

In Gram-negative bacteria, fosfomycin resistance can result from reduced permeability, amino acid mutations in the active site of the MurA target involved in peptidoglycan biosynthesis, and the production of fosfomycin-inactivating enzymes (*fos* genes). Acquired resistance to fosfomycin in *E. coli* may be due to metalloenzymes of the FosA type that catalyze the conjugation of glutathione to fosfomycin, thereby inactivating this antibiotic (Nordmann, 2018).

Regarding to tigycycline, all *E.coli* isolates (No.= 112) were highly susceptibility to this antibiotic (Table 3-8), Chaudhary *et al*. (2023) reported different origin *E.coli* were sensitive to tigycycline (100%), Peterson (2008) reported the enterobacteriaceae sensitivity to tigycycline were in Asia (99.4%), Europe (97.3%) and North America (96.2%). This results were compatible with result in our study, Pournaras *et al*. (2016) were observed tigecycline appears to retain low non-susceptibility rates of <10% among Enterobacteriaceae worldwide in most large-scale surveillance studies

Urine *E.coli* high resistance to nalidixic acid (89.3%), doxycycline (80.3%) norfloxacin (67.8%) and moderate resistance (57.1%) for ciprofloxacin, levofloxacin and sparfloxacin (Table 3-8) Bahramin *et al*. (2021) in Iran found that *E.coli* resistance to ciprofloxacin (61.7 %) this result consistent with our study, whereas resistance to levofloxacin (38.3%) this result lower than result in our study, Halaji *et*

al. (2021) reported resistant to nalidixic acid, norfloxacin and ciprofloxacin were (78.3 %), (56.5%) and (54.3%) respectively, these result consistent with our study. Mohanty *et al*. (2022) in India reported *E.coli* resistant to ciprofloxacin (57.6%) and nalidixic acid (90.4%) relatively similar results in present study.

E.coli isolates from stool were resistant to nalidixic acid (86.7%) and trimethoprime/ sulfamethazole (80%), Ghaffoori and Suleiman (2022) found that *E.coli* resistance to trimethoprime/ sulfamethazole (89.5%), Park *et al*. (2022) in South Korea reported *E.coli* resistant to nalidixic acid (44%) and trimethoprime/ sulfamethazole (34.7%) the results lower than in present study may due to drug abuse in Iraq.

E.coli isolated from vaginal discharge and diabetic foot ulcer high resistance to different types of antibiotics. Vaginal isolates as well as resistance to 6 types of β-lactam antibiotics (100%) (Table 3-7), were resistant to ciprofloxacin, levofloxacin, sparfloxacin and nalidixic acid (100%), trimethoprime/ sulfamethazole (94.1%), azithromycin (82.4%) and (70.6%) for each doxycycline and norfloxacin. Raheem and Said (2023) found resistant to nalidixic acid (100%), ciprofloxacin (93.3%), trimethoprime/ sulfamethazole (93.3%), this results similar present study.

E.coli isolates from diabetic foot ulcer (DFU) were resistant to ciprofloxacin, levofloxacin, sparfloxacin, trimethoprime/ sulfamethazole, nalidixic acid, norfloxacin the resistance for all these antibiotics were (85.7%), as well as 7 types of β- lactam antibiotics were resistant (100%). A study in Kufa city by Wahid *et al*. (2022) found that resistance to ciprofloxacin (89.4%), levofloxacin (79%), norfloxacin (79%) and trimethoprime/ sulfamethazole (68.4%) these results consistent with present study.

3.9. Prevalence of AmpC- Producing *E.coli* Isolates and Associated with clinical samples.

Primary screening for detection of AmpC β- lactamase *E.coli* isolates depended on cefoxitin resistance (CLSI, 2023) in present study from 112 β-lactamase *E.coli* isolates, 26 (23.2%) isolates were cefoxitin resistant (Table 3-9).

Table (3-9) Antibiotic resistance pattern of β- lactamase–producing *E.coli* and associated with clinical samples.

Sample types (beta lactamase producing *E.coli*)	Tot. No.	No. (%) of AmpC producing *E.coli* isolates	No. (%) of ESBL producing *E.coli* isolates	No. (%) of Carbapenemase producing *E.coli* isolates	No. (%) of aminoglycosides resistance *E.coli* isolates	No. (%) of quinolone resistance *E.coli* isolates	No. (%) of colistin resistance *E.coli* isolates	*P* -value
urine	56	14(25)	44(78.6%)	2(3.6%)	19(34)	32(57)	9(16)	0.0029
Burn exudate	16	2(12.5)	10(62.5%)	(0.00)	5(31.2)	9(56.2)	6(37.5)	0.0082
Vaginal discharge	17	1(5.9)	14(82.4%)	1(5.9%)	10(58.8)	17(100)	5(29.4)	0.0036
Diabetic foot ulcer	7	5(71.4)	2(28.6%)	1(14.3%)	(0.00)	6(85.7)	4(57.2)	0.048
Stool samples	15	4(26.6)	3(20%)	(0.00)	3(20)	8(53.3)	3(20)	0.0345
Ear swabs	1	(0.00)	1(100%)	(0.00)	(0.00)	1(100)	(0.00)	1
Total	112	26(23.2%)	74(66%)	4(3.6%)	37(33%)	73(65.1%)	27(24.1%)	
High significant (P < 0.001), Significant (P value < 0.05), Non-significant (P value > 0.05)								

A study in Iran by Sadeghi-deylamdeh and Jafari-sales (2021) found that the *E.coli* isolated from different clinical sources generated AmpC (40.5%), Soltan-Dallal *et al*. (2013) in Tehran a research on clinical specimens including urine, stool, blood and wounds, out of 128 *E.coli* isolates, 13 isolates (10.2%) were AmpC generators. In a study conducted in Kerman by Mansouri *et al*. (2014) on clinical specimens including urine, blood, and body fluids, (39.3%) were generators for AmpC. In conclusion despite the heterogeneity in reported rates, results in present study were consistent with the average reported in these studies. Coolen *et al*. (2019)

reported that variance in prevalence is likely to be influenced by diagnostic strategies used in these studies, and there are also regional differences in prevalence.

Regarding to associated β- lactamase producing *E.coli* isolates (BPEC) to clinical samples, showed that diabetic foot ulcer (DFU) were high prevalenc in AmpC producing *E.coli* isolates (Table 3-9). Ranjini and Rangasamy (2015) found that the AmpC producing *E.coli* were high prevalence in DFU patients.

3.9.1. Comparison between AmpC and non- AmpC β-Lactamase producing *E.coli* (BPEC) isolates for resistant to several antibiotics.

Comparison between AmpC and non- AmpC β- lactamase producing *E.coli* isolates is necessary for discover the significant of AmpC isolates.

In present study out 26 isolates AmpC- producer, only 5 isolates were ESBL, on other hand, only 5 isolates out of ESBL producing isolates AmpC-producing isolates, when comparison between AmpC and non- AmpC β- lactamase producing isolates, relatively similar comparison between ESBL and AmpC.

the statistical analysis in (Table 3-10) revealed *p*-value were high significant for some antibiotics belong to AmpC producing isolates that included ceftazidim, cefepime, impeneme, colistin, ciprofloxacin, levofloxacin, sparfloxacin and chloroamphenicole, low significant for ceftriaxone, cefoxitin, aztreonem, norfloxacin, nalidixic acid, nitrofurantoin, Trimethoprim/ sulfamethoxazole, fosfomycin, doxycycline and azithromycin.

Peter-Getzlaff *et al*. (2011) AmpC beta-lactamases can confer resistant to aminopenicillins, cephalosporins, oxyimino-cephalosporins (e.g., ceftriaxone, cefotaxime, and ceftazidime), cephamycins (e.g., cefoxitin and cefotetan), and monobactams

Table (3-10) Frequency of AmpC and non- AmpC β- lactamase producing *E.coli* isolates resistant to several antibiotics

No.	Class	Antibiotics	Number and percentage of *E.coli* isolates resistant to several antibiotics						p-value
			Total *E.coli* No.=112		Non-AmpC producing *E.coli* No.=86		AmpC producing *E.coli* No.=26		
			No.	%	No.	%	No.	%	
1	B-lactam/penicillins	Ampicillin	112	100	86	100	26	100	1
2	B-lactam/penicillins	Piperacillin	111	99.1	85	98.8	26	100	0.84
3	B-lactam/penicillins	Amoxicillin	112	100	86	100	26	100	1
4	B-lactam/ B-lactam combination	Pipereacillin/tazobactam	25	22.3	18	20.9	7	26.9	0.084
5	B-lactam/ B-lactamase inhibitor	Amoxicillin/clavulonate	112	100	86	100	26	100	1
6	B-lactam/cephems	Cefazolin	112	100	86	100	26	100	1
7	B-lactam/cephems	Ceftazidim	64	57.1	38	44.2	26	100	< 0.001
8	B-lactam/cephems	Ceftriaxone	88	78.6	62	72	26	100	0.032
9	B-lactam/cephems	Cefepime	28	25	15	17.5	13	50	< 0.001
10	B-lactam/cephems	Cefoxitin	26	23.2	21	24.4	5	19.2	0.012
11	B-lactam/carbapenems	Ertapenem	0	0	0	0	0	0	-
12	B-lactam/carbapenems	Imipenem	4	3.6	0	0	4	15.4	< 0.001
13	B-lactam/carbapenems	Meropenem	0	0	0	0	0	0	-
14	B-lactam/monobactams	Aztreonam	76	67.9	56	65.1	20	76.9	0.037
15	Aminoglycosides	Amikacin	0	0	0	0	0	0	-
16	Aminoglycosides	Gentamicin	37	33	28	32.5	9	34.6	0.13
17	Aminoglycosides	Isepamicin	1	0.9	1	2.6	0		1
18	Lipopeptides	Colistin	27	24.1	9	10.5	18	69.2	< 0.001
19	Quinolones and fluoroquinolones	Ciprofloxacin	73	65.2	50	44.6	23	88.5	< 0.001
20	Quinolones and fluoroquinolones	Levofloxacin	73	66.2	50	44.6	23	88.5	< 0.001
21	Quinolones and fluoroquinolones	Sparfloxacin	73	65.2	50	44.6	23	88.5	< 0.001
22	Quinolones and fluoroquinolones	Norfloxacin	70	62.5	48	55.8	22	84.6	0.043
23	Quinolones and fluoroquinolones	Nalidixic acid	90	80.4	66	76.5	24	92.3	0.036
24	Nitrofurans	Nitrofurntoin	9	8.0	3	3.5	6	23	0.013
25	Folate pathway antagonists	Trimethoprim/sulfamethoxazole	76	67.9	56	65.1	20	76.9	0.024
26	Phenicols	Chloramphenicole	48	42.9	31	36	17	65.4	< 0.001
27	Fosfomycins	Fosfomycin	27	24.1	17	19.8	10	38.5	0.013
28	Tetracyclines	Doxycycline	76	67.9	57	66.3	19	73	0.039
29	Macrolides	Azithromycin	58	51.8	34	39.5	24	92.3	0.012
30	glycylclines	Tigycycline	0	0.00	0	0.00	0	0.00	-
High significant (P < 0.001), Significant (P value < 0.05), Non-significant (P value > 0.05)									

Estaleva *et al*. (2021) found that AmpC β-lactamases confer resistant to a wide range of β-lactam drugs as well as classical β-lactamase inhibitors like clavulanic acid and tazobactam to which ESBLs are sensitive.

poirel *et al*. (2018) found that *E. coli* of animal origin often also show resistances to other mostly older antimicrobial agents, including tetracyclines, phenicols, sulfonamides, trimethoprim, and fosfomycin. Plasmids, especially multi-resistance plasmids, but also other mobile genetic elements, such as transposons and gene cassettes in class 1 and class 2 integrons, seen to play a major role in the dissemination of resistance genes.

3.9.2. MDR Among β-Lactamase and AmpC β- Lactamase Producing *E.coli* Isolates

All BPEC *E.coli* isolates appeared multi-drug resistance (MDR), whereas XDR and Pan drug-resistant pattern were not detected among the β- lactamase producing *E.coli* isolates in present study, Wahid *et al*.(2022) in Kufa city / Iraq found that *E.coli* isolated from diabetic foot ulcer were MDR (100%), in Bangladish Jain *et al*. (2021) found that 98% of *E.coli* isolates were MDR, a study in athiopia by Dejene *et al*. (2022) reported 100% of *E.coli* isolates were MDR. The increase in multidrug-resistant (MDR) UPEC isolates is primarily due to antibiotic resistance, which is common, leading to a significant rise in MDR-UPEC isolates (Mohsin *et al*., 2022).

In present study major AmpC β- lactamase producing isolates were resistant to 9 classes (42%) (Table 3-11), whereas high resistant percentage of non-AmpC β- lactamase producing *E.coli* isolates were (25.6%) for 7 classes followed by 8 classes (22%), (Table 3-12). Totally β- lactamase producing *E.coli* were high percentage (22.3%) related with 7 classes (Table 3-13).

Regarding to resistance of AmpC β- lactamase producing *E.coli* isolates to antibiotics numbers, high percentage were (23.1%) for 22 types of antibiotics followed by (15.4%) for 23 types (Table 3-14), whereas in non-AmpC β- lactamase producing *E.coli* isolates high percentage were (11.6%) followed by (10.5%) for 15 and 14, 17 types respectively (Table 3-14). From these information, the AmpC β-lactamase producing *E.coli* isolates were more resistance than non- AmpC β-lactamase producing *E.coli* isolates.

Table (3-11) Frequency of different AmpC producing *E.coli* isolates (MDR) to antibiotics resistant classes

Sample Types	MDR Number of Classes							
	4 Classes	5 Classes	7 Classes	8 Classes	9 Classes	10 Classes	11 Classes	Total
Urine	0	2 (14.3%)	0	4 (28.5%)	4 (28.5%)	3 (21.4%)	1 (7.2%)	14
Stool	1 (25%)	0	2 (50%)	0	1 (25%)	0	0	4
Vaginal discharge	0	0	0	0	0	1 (100%)	0	1
Burn exudate	0	0	0	1 (50%)	1 (50%)	0	0	2
Diabetic foot ulcer	0	0	0	0	5 (100%)	0	0	5
Total	1(3.8%)	2(7.7%)	2(7.7%)	5(19.2%)	11(42.5%)	4(15.3%)	1(3.8%)	26

Table (3-12) frequency of different non-AmpC producing *E.coli* isolates (MDR) to antibiotics resistant classes

Sample Types	MDR Number of Classes									
	3 Classes	4 Classes	5 Classes	6 Classes	7 Classes	8 Classes	9 Classes	10 Classes	11 Classes	Total
Urine	0	1 (1.2%)	4 (9.5%)	12 (28.5%)	8 (19%)	9 (21.4%)	6 (14.2%)	1 (2.4%)	1 (2.4%)	42
Stool	0	0	3 (33.3%)	0	0	3 (33.3%)	1 (11.1%)	1 (11.1%)	1 (11.1%)	9
Vaginal discharge	0	0	1 (5.5%)	2 (11.1%)	6 (33.3%)	5 (27..8%)	2 (11.1%)	1 (5.5%)	1 (5.5%)	18
Burn exudate	1 (7.1%)	0	3 (21.5%)	1 (7.1%)	8 (57.2%)	1 (7.1%)	0	1 (7.1%)	0	14
Diabetic foot ulcer	0	0	0	1 (50%)	0	1 (50%)	0	0	0	2
Total	1(1.2%)	1(1.2%)	11(12.8%)	16(18.6%)	22(25.6%)	19(22%)	9(10.5%)	4(4.6%)	3(3.5%)	86

Table (3-13) Frequency of different β-lactamase producing *E.coli* isolates (MDR) to antibiotics resistant classes

Sample Types	MDR									
	Number of Classes									
	3 Classes	4 Classes	5 Classes	6 Classes	7 Classes	8 Classes	9 Classes	10 Classes	11 Classes	Total
Urine samples	0	1(1.8)	6(10.7)	12(21.5)	8(14.3)	13(23.2)	10(17.8)	4(7.1)	2(3.6)	56
Stool samples	0	1(7.7)	3(23)	0	2(15.4)	3(23)	2(15.4)	1(7.7)	1(7.7)	13
Vaginal discharge	0	0	1(5.3)	2(10.5)	6(31.6)	5(26.3)	2(10.5)	2(10.5)	1(5.3)	19
Burin exudate	1	0	3(18.7)	1(6.2)	8(50)	2(12.5)	0	1(6.2)	0	16
Diabetic foot ulcer	0	0	0	1(14.3)	0	1(14.3)	5(71.4)	0	0	7
Ear swabs	0	0	0	0	1	0	0	0	0	1
Total	1 (0.9%)	2 (1.8%)	13 (11.5%)	16 (14.3%)	25 (22.3%)	24 (21.4%)	19 (17%)	8 (7.1%)	4 (3.6%)	112

Table (3-14) Frequency of AmpC and non- AmpC producing *E.coli* isolates resistant to several antibiotics (30 types).

NO. of antibiotics types (30)	NO. of non-AmpC β-lactamase producing *E.coli* isolates n=86(%)	NO. of AmpC β-lactamase producing *E.coli* isolates n=26(%)	Total (β- lactamase producing *E.coli* isolates) n=112(%)
6	1(1.2%)	0	1 (0.9)
7	2(2.4%)	0	2 (1.8)
8	5(5.8%)	0	5 (4.5)
9	7(8%)	1(3.85%)	8 (6.5)
10	8(9.3%)	0	8 (7.15)
11	4(4.6%)	1(3.85%)	5 (4.5)
12	8(9.3%)	0	8 (6.5)
13	8 (9.3%)	0	8 (6.5)
14	9(10.5%)	1(3.85%)	10 (9.0)
15	10(11.6%)	0	10 (9.0)
16	4(4.6%)	1(3.85%)	5 (4.5)
17	9(10.5%)	0	9 (8.8)
18	5(5.8%)	3(11.5%)	8 (6.5)
19	2(2.4%)	4(15.4%)	7 (6.2)
20	3(3.5%)	1(3.85%)	4 (3.6)
21	1(1.2%)	4(15.4%)	5 (4.5)
22	0	6(23.1%)	6 (5.3)
23	0	4(15.4%)	4 (3.6)

Jacoby (2009) and Ibrahim *et al*. (2016) found AmpC β-lactamases exhibit resistant to a broader spectrum of β-lactam antibiotics than ESBL.

3.10. Prevalence of ESBL Producing *E.coli* Isolates and Associated with Clinical Samples.

BPEC isolates, were examined by vitek 2 system, 74(66%) isolates revealed ESBL positive results (Table 3-9). McDanel *et al*. (2017) reported that the incidence of ESBL-*E. coli* and ESBL-*Klebsiella* infections in the United States has increased. Ajuja et al. (2021) reported the *Escherichia coli* and *Klebsiella pneumoniae* have been described as the two predominant groups of bacteria associated with ESBL production.

Chaudhary *et al*. (2023) found that (66.5%) of MDR *E.coli* isolates were ESBL positive, theses result compatible with current study (all isolates MDR). Mustafai *et al.* (2023) in cross sectional study found that 28 *E.coli* isolated out of 35 (80%) isolates were ESBL. A cross sectional study in Mosul city/ Iraq by Aiber *et al* (2022) found that (93.3%) of *E.coli* isolates were ESBL producer, Adegoke *et al.* (2021) found that all *E.coli* isolates (100%) were ESBL producer, these results were elevated when compared with current study.

High prevalence of ESBL producing *E.coli* my due to contaminated food with *E.coli* from animal origin, Casella *el al.* (2017) reported the possible transmission to humans may occur via direct contact with animals or the environment, or via the consumption of contaminated food

Regarding to clinical isolates, high resistance isolates were from vaginal discharge (82.4%), followed by urine (80.4%), (Table 3-9). Kaczmarek *et al*. (2018) Found that none of the tested isolates was positive for ESBL, this result different from current study and most studies.

A study in Babylon/ Iraq Hussein (2023) found that (72.7%) of *E.coli* isolated from urine were ESBL, in Iran sadeghi *et al*. (2022) reported (46%) of urine isolates produce ESBL, Al-Tamimi *et al.* (2022) in Jordan reported the ESBL screening test

was positive in (81.3%) of *E. coli* isolates, Pootong *et al.* (2018) in Thailand reported that (38.7%) of UPEC isolates were ESBL-producers, Mashhad/ Iran (72.9%) Mood *et al.* (2015), France (69.4%) Chevert *et al.* (2015), and Nepal (91.7%) Shakya *et al.* (2017). The difference observed in the prevalence of ESBL in *Escherichia coli* isolates in Iran and other parts of the world is probably due to differences in geographical distribution, infection control policies, source and size of the sample (Halaji *et al.,* 2020).

3.10.1. Comparison between ESBL and Non-ESBL β-Lactamase Producing Isolates for Resistance to Several Antibiotics

To detect the important of ESBL, the comparison between of ESBL and non-ESBL *E.coli* isolates resistance is very important, in (Table 3-15) the statistical analysis revealed *p*-value were high significant for some antibiotics belong to ESBL producing isolates that included ceftriaxone, aztreonem, nitrofurantoin, doxycycline, azithromycin, and low significant for cefazoline.

Mustafai *et al.* (2023) reported that the extended-spectrum β-lactamases (ESBLs) have been seen as a serious threat to public health since the beginning of the century.

The emergence of ESBL-producing *E. coli* isolates is becoming a critical issue globally (Chotinantakul *et al*., 2021).

Munita and Arias (2016) found that acquired resistance resulting from mutations impacts resistance against several classes of antibiotics, including β-lactam and non-β-lactam antibiotics.

Peralta *et al.* (2007), found that extended spectrum β-lactamase (ESBL) -producing *E. coli* are much concern because of their increased frequency and their resistance to a wide range of β-lactams and to other groups of antimicrobial factors.

Table (3-15) Frequency of ESBL and non- ESBL producing *E.coli* isolates resistant to several antibiotics.

No.	Class	antibiotics	Total *E.coli* No.= 112		Non-ESBL producing *E.coli* No.= 38		ESBL producing *E.coli* No.= 74		*p*-value
			NO.	%	NO.	%	NO.	%	
1	B-lactam/penicillins	Ampicillin	112	100	38	100	74	100	1
2	B-lactam/penicillins	Piperacillin	111	99.1	37	97.4	74	100	0.955
3	B-lactam/penicillins	Amoxicillin	112	100	38	100	74	100	1
4	B-lactam/ B-lactam combination	Pipereacillin/ tazobactam	25	22.5	19	50	6	8.1	<0.001
5	B-lactam/ B-lactamase inhibitor	Amoxicillin/ clavulonate	112	100	38	100	74	100	1
6	B-lactam/cephems	Cefazolin	100	89.3	28	73.7	72	97.3	0.034
7	B-lactam/cephems	Ceftazidim	64	57.2	32	84.2	32	43.2	0.002
8	B-lactam/cephems	Ceftriaxone	88	78.5	15	39.5	73	98.6	<0.001
9	B-lactam/cephems	Cefepime	28	25	10	26.3	18	24.3	0.853
10	B-lactam/cephems	Cefoxitin	26	23.2	21	24.4	5	19.2	0.012
11	B-lactam/ carbapenems	Ertapenem	0	0	0	0	0	0	0
12	B-lactam/ carbapenems	Imipenem	4	3.5	4	3.5	0	0	0.001
13	B-lactam/ carbapenems	Meropenem	0	0	0	0	0	0	0
14	B-lactam/ monobactams	Aztreonam	76	67.9	19	20	57	77.0	0.001
15	Aminoglycosides	Amikacin	0	0	0	0	0	0	0
16	Aminoglycosides	Gentamicin	37	33	12	31.5	25	33.8	1
17	Aminoglycosides	Isepamicin	2	1.8	1	0.9	1	0.9	1
18	Quinolones and fluoroquinolones	Ciprofloxacin	73	65.2	33	86.8	40	54.0	<0.001
19	Quinolones and fluoroquinolones	Levofloxacin	73	65.2	33	86.8	40	54.0	<0.001
20	Quinolones and fluoroquinolones	Sparfloxacin	73	65.2	33	86.8	40	54.0	<0.001
21	Fluoroquinolones	Norfloxacin	70	62.5	22	57.9	48	64.9	0.001
22	Quinolones and fluoroquinolones	Nalidixic acid	90	80.4	34	89.5	56	75.7	0.012
23	Nitrofurans	Nitrofurntoin	9	8.0	7	18.5	2	27.0	<0.001
24	Folate pathway antagonists	Trimethoprim/sulfamethoxazole	76	67.9	33	86.8	43	58.1	0.013
25	Phenicols	chloramphenicole	48	42.9	25	65.8	23	31.0	<0.001
26	Fosfomycins	Fosfomycin	27	24.1	12	31.6	15	20.3	<0.001
27	Tetracyclines	Doxycycline	76	67.9	20	52.6	56	75.7	0.0016
28	Macrolides	Azithromycin	58	51.8	14	36.8	44	59.5	0.001
29	Lipopeptides	Colistin	27	24.1	9	10.5	18	69.2	0.047
30	Glycycline	Tigycycline	0	0	0	0	0	0	0

High significant (P < 0.001), Significant (P value < 0.05), Non-significant (P value > 0.05)

3.10.2. Comparison between AmpC and ESBL β- lactamase Producing Isolates for Resistance to Several Antibiotics.

The comparison between AmpC and ESBL producer is very important clinically. In present study there were 21 isolates out of 26 AmpC producer were ESBL negative, and there were 69 isolates out of 74 ESBL producer were non-AmpC producer (Table 3-16). Statistical analysis revealed *p*- value for AmpC producer isolates were high significant for several types of antibiotics that includes piperacillin/ tazobactam, ceftazidim, cefoxitin, impeneme, ciprofloxacin, levofloxacin, sparfloxacin, norfloxacin, nitrofloxacin, chlorofloxacin, azithrom, colistin and significant for cefepime, nalidixic acid, Trimethoprim/ sulfamethoxazole and fosfomycin.

Shayan and Bokaeian (2015) reported that resistance to extended-spectrum cephalosporins can also be associated in *E. coli* with the production of plasmid class C β-lactamases, such as CMY-2 enzymes. Plasmid-mediated AmpC β-lactamases represent a new threat since they confer resistance to cephamycins and are not affected by β-lactamase inhibitors. This resistance mechanism has been found around the world, can cause nosocomial outbreaks, and appears to be increasing in prevalence, Sadeghi *et al*. (2021) reported that, unlike ESBLs, plasmid-encoded AmpC β-lactamases are effectively active against cephamycins and are not inhibited by a β-lactamase inhibitor such as Clavulanic acid, this enzyme is usually associated with multiple antibiotic resistance (MDR) means that there are fewer antibiotic options available to treat.

Table (3-16) Frequency of AmpC and ESBL producing *E.coli* isolates resistant to several antibiotics.

No.	Class	antibiotics	AmpC producing *E.coli* isolates (without ESBL) No.= 21		ESBL producing *E.coli* isolates (without AmpC) No.= 69		*p*-value
			NO.	%	NO.	%	
1	B-lactam/penicillins	Ampicillin	21	100	69	100	1
2	B-lactam/penicillins	Piperacillin	21	100	69	100	1
3	B-lactam/penicillins	Amoxicillin	21	100	69	100	1
4	B-lactam/ B-lactam combination	Pipereacillin/ tazobactam	7	33.3	1	1.4	< 0.001
5	B-lactam/ B-lactamase inhibitor	Amoxicillin/ clavulonate	21	100	69	100	1.0
6	B-lactam/cephems	Cefazolin	21	100	67	97.1	0.96
7	B-lactam/cephems	Ceftazidim	21	100	27	39.1	< 0.001
8	B-lactam/cephems	Ceftriaxone	21	100	68	98.5	0.95
9	B-lactam/cephems	Cefepime	11	52.4	16	23.2	0.019
10	B-lactam/cephems	Cefoxitin	21	100	0	0	< 0.001
11	B-lactam/ carbapenems	Ertapenem	0	0	0	0	1
12	B-lactam/ carbapenems	Imipenem	4	19.0	0	0	< 0.001
13	B-lactam/ carbapenems	Meropenem	0	0	0	0	1
14	B-lactam/ monobactams	Aztreonam	15	71.4	52	75.4	0.096
15	Aminoglycosides	Amikacin	0	0	0	0	1
16	Aminoglycosides	Gentamicin	7	33.3	23	33.3	1
17	Aminoglycosides	Isepamicin	0	0	0	0	1
18	Quinolones and fluoroquinolones	Ciprofloxacin	20	95.2	37	53.6	< 0.011
19	Quinolones and fluoroquinolones	Levofloxacin	20	95.2	37	53.6	< 0.001
20	Quinolones and fluoroquinolones	Sparfloxacin	20	95.2	37	53.6	< 0.001
21	Fluoroquinolones	Norfloxacin	18	85.7	44	63.8	< 0.001
22	Quinolones and fluoroquinolones	Nalidixic acid	20	95.2	52	75.4	0.0023
23	Nitrofurans	Nitrofurntoin	6	9.5	2	2.9	< 0.001
24	Folate pathway antagonists	Trimethoprim/sulfa methoxazole	16	77.2	43	62.3	0.012
25	Phenicols	chloramphenicole	15	71.4	21	30.4	< 0.001
26	Fosfomycins	Fosfomycin	10	47.6	15	21.7	0.012
27	Tetracyclines	Doxycycline	15	71.4	52	75.4	0.93
28	Macrolides	Azithromycin	19	90.5	39	56.5	< 0.001
29	Lipopeptides	Colistin	19	90.5	17	24.6	<0.001
30	Glycycline	Tigycycline	0	0	0	0	1

High significant (*P* value < 0.001), Significant (*P* value < 0.05), Non-significant (*P* value > 0.05)

3.11. Prevalence of Carbapenemase-Producing *E.coli* Isolates and Associated with Clinical Samples

The results of antibiotic resistance by Vitek 2 system, revealed that out of 112 BPEC only 4 (3.6%) isolates were resistant to carbapeneme (Table 3-9).

Liu and Liu (2016) explained *E.coli* susceptibility to carbapenem antibiotics because high stability of carbapenem against β-lactamase and reported there is no resistance to impenem (0%), a study to Halaji *et al*. (2020) revealed resistance to impenem and meropenem (0%) that consistent with present study, but a study in Wuhan city /china by Lin *et al*. (2021) founded *E.coli* resistant to meropenem, etrapenem and impenem were (81.5%), (100%), (72.2) respectively, these results disagreement with our findings, Ghaffoori and Suleiman, (2022) in Anbar city/ Iraq pointed resistance to carbapeneme (etrapeneme and impeneme) were (2.9%).

3.12. Prevalence of *E.coli* that Resistant to non- β-Lactam Antibiotics and Associated with Clinical Samples

In (Table 3-9) showed the prevalence of resistance to three main classes aminoglycosides, quinolones and colistin that study genetically in current study.

Regarding to aminoglycosides 37(33%) isolates of BPEC were aminoglycosides (gentamicin) resistant (Table 3-9). Jain *et al*. (2021) noted resistance to gentamicin (39%), relatively similar the results in current study. A cross sectional study in Mosul city/ Iraq by Aiber *et al* (2022) found that (60%) of *E.coli* isolates were gentamicin resistant.

Regading prevalence of quinolone resistance, 73(65.1%) isolates were resistant (Table 3-9). The results of *E.coli* isolated from vaginal discharge and DFU revealed resistant to quinolone (100%) and (85.7%) respectively, mohammed *et al*. (2021) in Erbil city/ Iraq reported the susceptible for *E.coli* isolated from vaginal swab

(40%), Naqid *et al*. (2020) in Duhok city / Iraq reported resistance to ciprofloxacin (57.1%), in Benghazi / Libya Al-awkally *et al*. (2022) noted (15%) of *E.coli* isolates resistant to ciprofloxacin, Adegoke *et al*. (2011) in South Africa published about (48%) of *E.coli* resistant to ciprofloxacin, from these different results, the resistance results of vaginal *E.coli* to ciprofloxacin in current study was high.

E.coli isolated from diabetic foot ulcer (DFU) were resistant to quinolone (85.7%) (Table 3-9), a study in Kufa / Iraq by Wahid *et al*. (2022) reported the resistance to ciprofloxacin, levofloxacin and norfloxacin were (89.4%), (79%) and (84.2%) respectively, these results compatible with present study.

Prevalence of colistin resistant isolates, 27(24.1%) were resistant and mostly from DFU(57.2%) (Table 3-9). Saseedharan *et al.* (2018) found that no resistance to colistin. A review study in Asia countries by Sultana *et al*.(2023) reported that colistin is effective drugs to treatment DFU caused by *E.coli*, this results different from findings in current study.

3.13. Prevalence of Integron Class 1(*Int1*) Gene Among β-Lactamase Producing *E.coli* I Isolates

All BPEC isolates (No. 112) were screened for *Int1* detection, PCR results revealed that 111(99.1%) β- lactamase producing *E.coli* isolates carried *Int1*, Rao *et al*. (2006) Reported that from Asian countries also have noted a high prevalence of *Int1* in gram-negative clinical isolates and reported *Int1* which are highly associated with resistance to antimicrobial agents. Mohammed *et al*. (2020) reported *Int1* was detected in all *E.coli* isolates (100%).

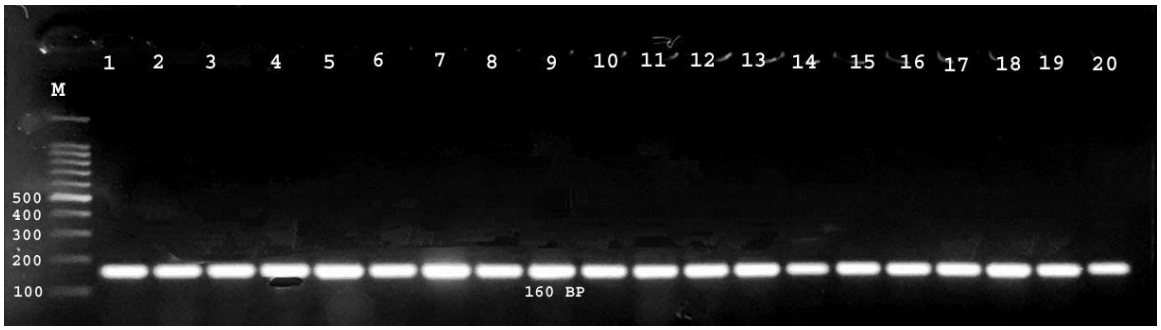

Figure (3-4): Agarose gel with ethidium bromid stained of monoplex PCR amplified product from extract DNA of *E.coli* isolates with class 1 integron (*Int1*) gene primer. Lane (L) is DNA molecular size marker (1000-bp ladder). Lanes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, show positive results with *IntI* gene (160 bp).

3.14. Prevalence of ESBL Genes Among β-Lactamase Producing *E.coli* Isolates

Conventional PCR technique were used for detection of ESBL genes, the results revealed high frequency of $bla_{\text{CTX-M}}$ (100%), followed by bla_{SHV} (87.8%), bla_{OXA} (85.1) and bla_{TEM} (79.7%) (Table 3-17) , (Figures 3-5, 3-6, 3-7, and 3-8). Al-Mayahie (2013) found that the prevalence ESBL genes were $bla_{\text{CTX-M}}$ followed by bla_{SHV}, bla_{OXA} and bla_{TEM}. Estaleva *et al.* (2021) reported that the worldwide dissemination of CTX-M type β-lactamases has been dramatic and greater than the impact of the TEM- and SHV-type ESBLs. Ibrahim *et al.*(2023) reported that prevalence $bla_{\text{CTX-M}}$(65.7%) and bla_{TEM} (37.1%). Chaudhary *et al.*(2023) found that among the ESBL genotypes, the most common were bla_{TEM} (63.4%) followed by $bla_{\text{CTX–M}}$ (36.6%), these results lower than in current study, Shaikh *et al.* (2015) noted the less commonly reported ESBLs include the bla_{OXA} type, bla_{PER} type and bla_{GES} type, in conclution the result of bla_{OXA} was discordance with most studies.

Table (3-17) Prevalence ESBL genes among beta lactamase producer *E.coli* (No. 112)

ESBL positive *E.coli* No.: total (%)	*Bla*$_{TEM}$ Frequency (%)	*Bla*$_{SHV}$ Frequency (%)	*Bla*$_{CTX-M}$ Frequency (%)	*Bla*$_{OXA}$ frequency (%)
74(66)	**59(79.7)**	**65(87.8)**	**74(100)**	**63(85.1)**

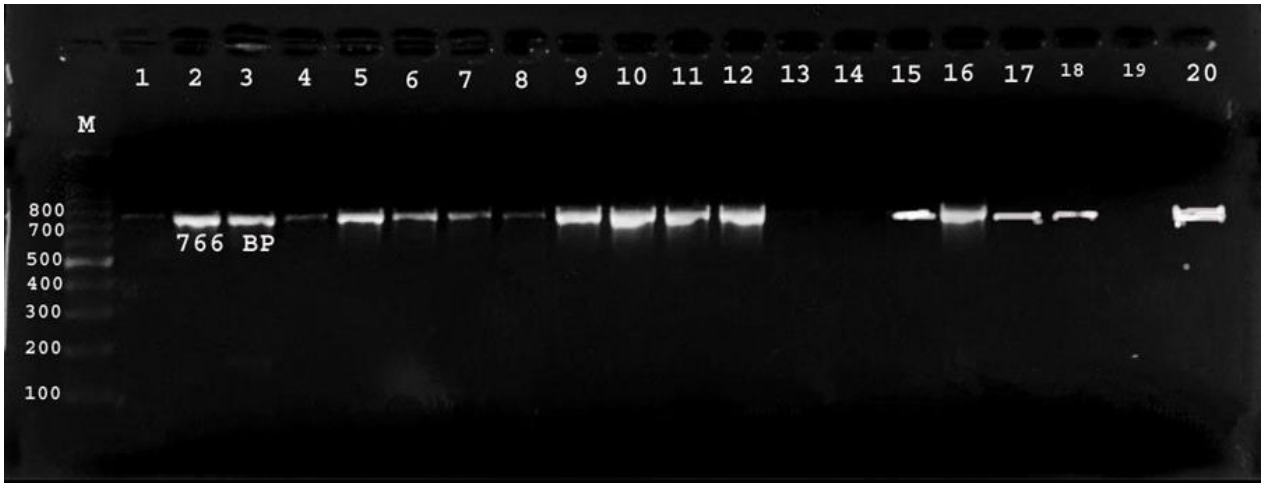

Figure (3-5) Ethidium bromide-stained agarose gel of monoplex PCR amplified products from extracted DNA of ESBL positive *E.coli* isolates that amplified with primers for *Bla* $_{TEM}$ genes forward and reverse, the electrophoresis was performed in 1.5% agarose , lanes M, 1500-bp DNA ladder, lanes (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 15, 16, 17, 18, 20) show positive result for *Bla*$_{TEM}$ (766 bp), lanes (13, 14, 19) show negative result for *Bla*$_{TEM}$.

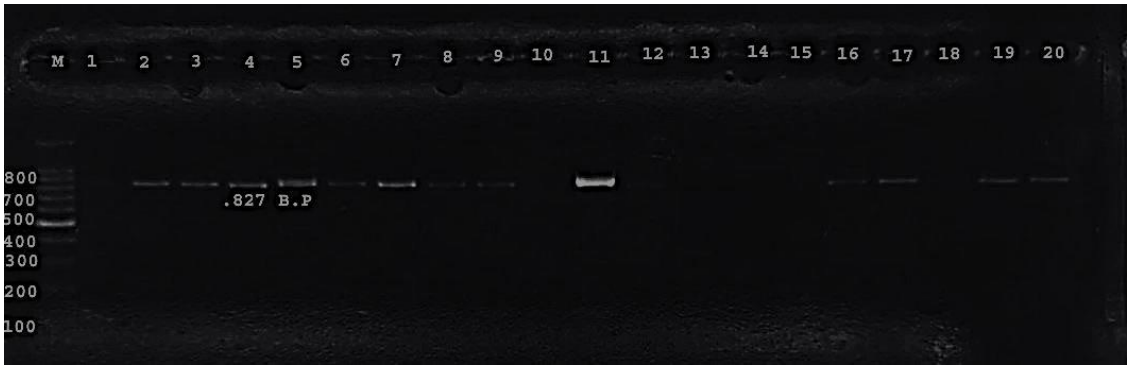

Figure (3-6) Ethidium bromide-stained agarose gel of monoplex PCR amplified products from extracted DNA of ESBL positive *E.coli* isolates that amplified with primers for *bla* $_{SHV}$ genes forward and reverse, the electrophoresis was performed in 1.5% agarose, lanes M, 1500-bp DNA ladder, lanes (2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 16, 17, 19, 20) show positive result for *bla*$_{SHV}$ (827bp), lanes (1, 10, 13, 14, 15, 18) show negative result for *bla*$_{SHV}$.

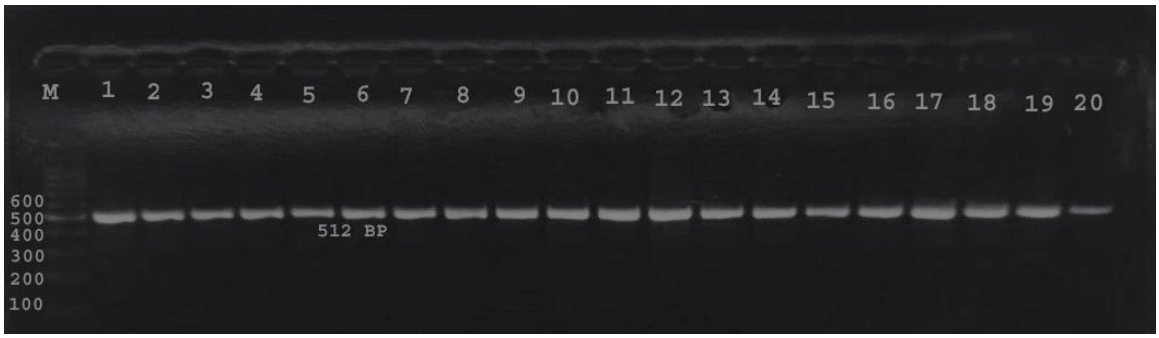

Figure (3-7) Ethidium bromide-stained agarose gel of monoplex PCR amplified products from extracted DNA of ESBL positive *E.coli* isolates that amplified with primers for *bla* CTX-M genes forward and reverse, the electrophoresis was performed in 1.5% agarose , lanes M, 1500-bp DNA ladder, all lanes show positive result for *bla*CTX-M(1000bp).

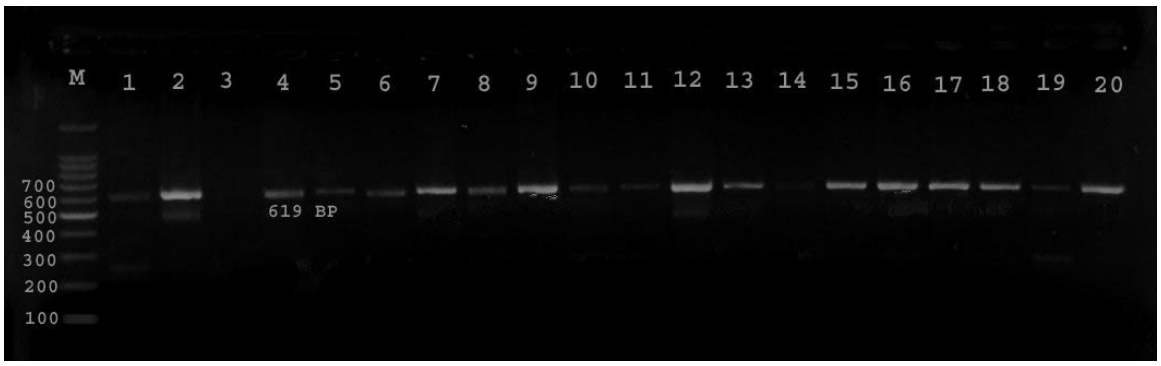

Figure (3-8) Ethidium bromide-stained agarose gel of monoplex PCR amplified products from extracted DNA of ESBL positive *E.coli* isolates that amplified with primers for bla_{OXA} genes forward and reverse, the electrophoresis was performed in 1.5% agarose, lanes M, 1500-bp DNA ladder, lanes (1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20) show positive result for bla_{OXA}(619 bp), lanes (3) show negative result for bla_{OXA}.

3.15. Prevalence Carbapenemase Genes in β-Lactamase Producing *E.coli* Isolates

Only 4 (3.6%) isolates from β- lactamase producing *E.coli* isolates were resistant to carbapeneme, the results of genetic study revealed (100%) of isolates were carry bla_{OXA} genes followed by bla_{NDM} (75%) of isolates, $bla_{VIM,}$ bla_{KPC} and bla_{IMP} were not detected (Table 3-18),(Figures 3-9, 3-10).

Table (3-18) Frequency of carbapenemase genes among β- lactamase producer *E.coli* isolates (No. 112)

Carbapenemase producing *E.coli* isolates No.: total (%)	***bla*$_{OXA}$ Frequency (%)**	***bla*$_{NDM}$ Frequency (%)**	***bla*$_{VIM}$ Frequency (%)**	***bla*$_{KPC}$ Frequency (%)**	***bla*$_{IMP}$ Frequency (%)**
4(3.6)	**4(100)**	**3(75)**	**0**	**0**	**0**

Al-Hasso (2023) in Mosul city/ Iraq reported *bla*$_{VIM}$ and *bla*$_{NDM}$ were not detected in clinical samples, Dadashi *et al.* (2019) reported in Asia the bla$_{NDM}$ were detected in (82.6%) of carbapenemase resistance isolates, this result similar findings in current study, Taha *et al.*(2023) found that *bla*$_{NDM}$ (95%), *bla*$_{IMP}$ (0%) and *bla*$_{OXA}$ (95%), these results compatible with current study, but they were detected *bla*$_{VIM}$ (100%) and *bla*$_{KPC}$ (85%), these results disagreed with current study, Miao *et al.(*2019) in china were detected *bla*$_{KPC-2}$ in (1.25%) of carbapenemase producing isolates, Athanasakopoulou *et al*. (2022) reported *bla*$_{NDM}$ and *bla* $_{OXA-1}$ were detected (100%) in carbapenemase resistance isolates, Nosheen *et al.* (2021) noted that *bla*$_{NDM}$ genes exhibited minimal variation compared to other *E. coli* strains isolated from different countries that possessed the same genes

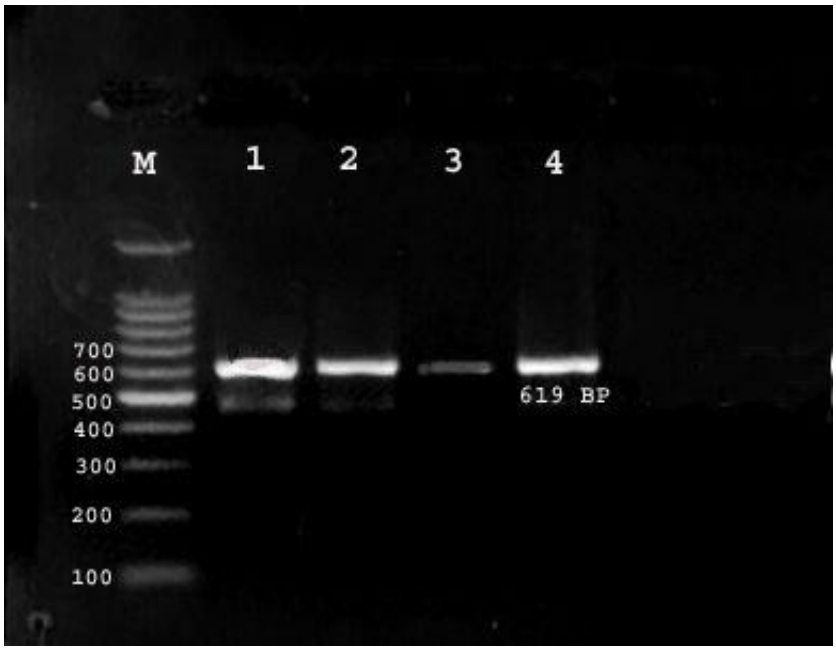

Figure (3-9) Ethidium bromide-stained agarose gel of monoplex PCR amplified products from extracted DNA of carbapeneme resistant, β- lactamase producing *E.coli* isolates that amplified with primers for bla_{OXA} genes forward and reverse, the electrophoresis was performed in 1.5% agarose, lanes M, 1500-bp DNA ladder, lanes (1, 2, 3, 4) show positive result for bla_{OXA} (619 bp).

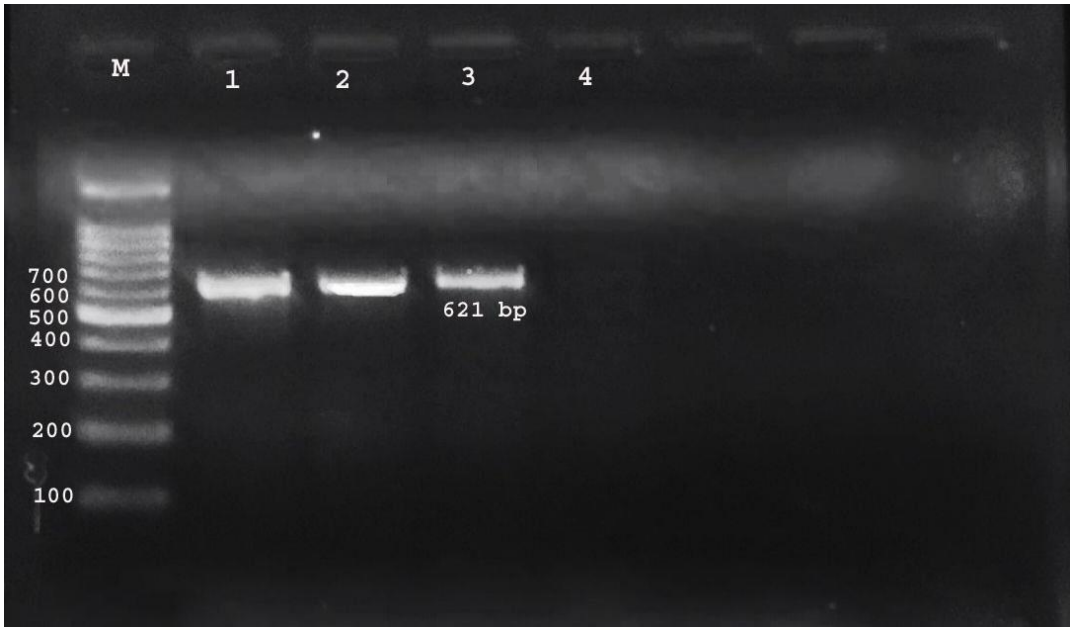

Figure (3-10) Ethidium bromide-stained agarose gel of monoplex PCR amplified products from extracted DNA of carbapeneme resistant, β- lactamase producing *E.coli* isolates that amplified with primers for bla_{NDM} genes forward and reverse, the electrophoresis was performed in 1.5% agarose, lanes M, 1500-bp DNA ladder, lanes (1, 2, 3) show positive result for bla_{NDM} (621bp), lanes (4) show negative result for bla_{NDM}.

3.16. Prevalence Aminoglycosides Gene (*ACC(6)Ib-cr*) Among β-Lactamase Producing *E.coli* Isolates

ACC(6)Ib-cr gene causes resistant to both aminoglycosides and fluoroquinolones (Guan *et al*. 2013 ; Robicsek *et al.* 2006), in present study *ACC(6)Ib-cr* gene used for detection aminoglycosides resistance.

The results of phenotypic revealed 37(33%) isolates were resistant to aminoglycosides, PCR results revealed all (100%) isolates carry *ACC(6)Ib-cr*, (Figure 3-11), Jones *et al*. (2008) found that *ACC(6) Ib-cr* were detected in (45%) of gentamicin-resistant isolates, this result lower than findings in present study, Guan *et al.* (2013) *ACC(6)-Ib-cr* is a variant of ACC(6)-Ib and a common aminoglycoside acetyltransferase, and reported, the -cr variant of ACC(6)-Ib has two amino acid changes, Nordmann and Poirel (2005) found that *ACC(6)-Ib-cr* is common in clinical isolates of Gram negative bacteria.

Jacoby *et al.* (2014) reported that *ACC(6)Ib-cr* usually found in a cassette as part of integrons in multi-resistance plasmids, which may contain other PMQR genes. In present study *Int1* were prevalence in (99.1%) of β- lactamase producer, and this main cause for high prevalence of *ACC(6) Ib-cr*.

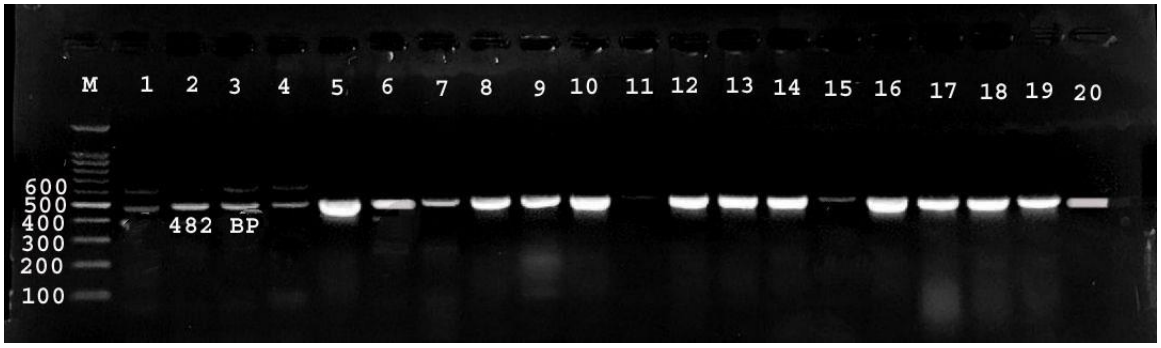

Figure (3-11) Ethidium bromide-stained agarose gel of monoplex PCR amplified products from extracted DNA of aminoglycosides resistant, β- lactamase producing *E.coli* isolates that amplified with primers for *ACC(6) Ib-cr* genes forward and reverse, the electrophoresis was performed in 1.5% agarose, lanes M, 1500-bp DNA ladder, all lanes show positive result for *ACC(6) Ib-cr* (482 bp).

3.17. Prevalence Quinolones Resistant Genes in β-Lactamase Producing *E.coli* Isolates

PCR technique were used for detection *qnr A* and *qnr B* , the results revealed out of 74(66%) quinolone resistant β-lactamase producing *E.coli* isolates 42(37.5%) isolates carry *qnr A* and 36 (32.2%) isolates carry *qnr B* (Figures 3-12 and 2-13)

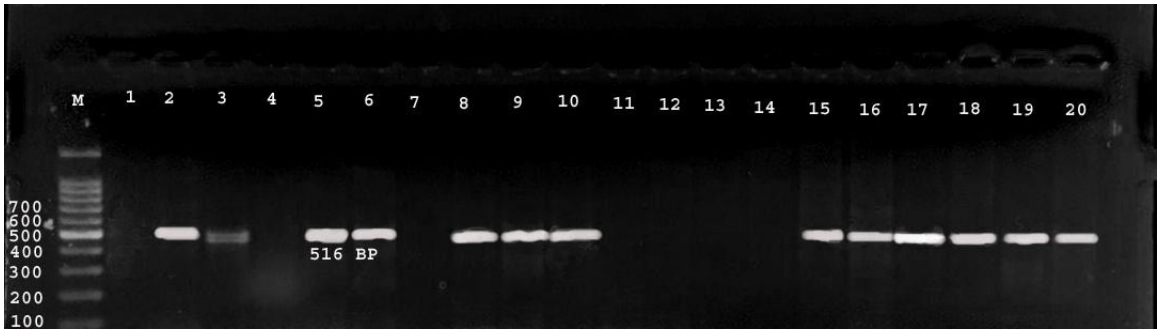

Figure (3-12) Ethidium bromide-stained agarose gel of monoplex PCR amplified products from extracted DNA of quinolones resistant, β- lactamase producing *E.coli* isolates that amplified with primers for *qnr A* genes forward and reverse, the electrophoresis was performed in 1.5% agarose, lanes M, 1500-bp DNA ladder, lanes (2, 3, 5, 6, 8, 9, 10, 15, 16, 17, 18, 19, 20) show positive result for *qnr A*(516 bp), lanes (1, 4, 7, 11, 12, 13, 14) show negative result for *qnr A.*

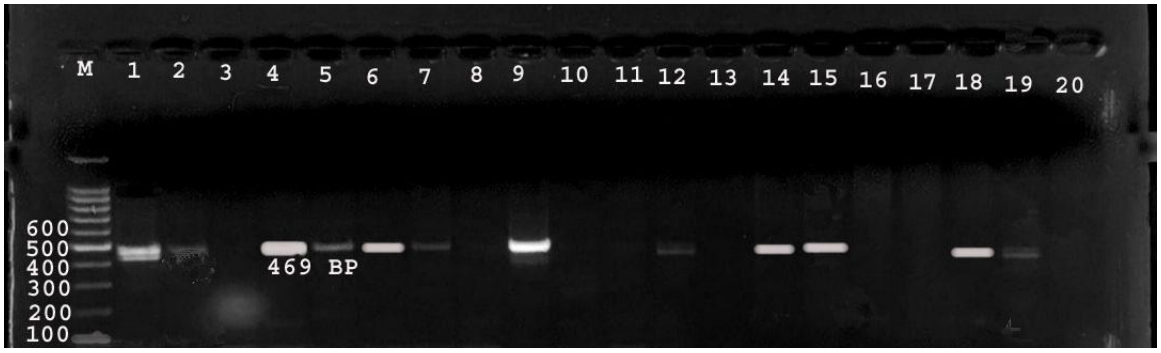

Figure (3-13) Ethidium bromide-stained agarose gel of monoplex PCR amplified products from extracted DNA of quinolones resistant, β- lactamase producing *E.coli* isolates that amplified with primers for *qnr B* genes forward and reverse, the electrophoresis was performed in 1.5% agarose , lanes M, 1500-bp DNA ladder, lanes (1, 2, 4, 5, 6, 7, 9, 12, 14, 15, 18, 19) show positive result for *qnr B*(469 bp), lanes (3, 8, 10, 11, 13, 16, 17, 20) show negative result for *qnr B*.

Across sectional study in Iran by Hadizadeh *et al.* (2017) that found (7.2%) of β-lactamase *E.coli* carry qnr genes. FarajzadehSheikh *et al.* (2019) found that *qnr A* and *qnr B* were detected in (10%) and (21%) respectively. Taha *et al.* (2019) in Eygpt found that the prevalence of *qnr A* and *qnrB* in plasmid were (10%) for each

one. Most studies revealed the results of *qnr A* and *qnr B* lower than results in present study, may be due to drug abuser.

3.17.1. Antibiotics Resistance for β-lactamase Producing *E.coli* Isolates Positive for *ACC(6)Ib-cr* and *qnr* Genes.

PBEC isolates positive for *ACC(6)Ib-cr* and qnr genes revealed high resistance for several types of antibiotics, all isolates resistant for 13 types of antibiotics or more, high number 5(19.2%) isolates were resistant to 18 types of antibiotics (Table 3-19).

Table (3-19) Frequency of numbers for antibiotic resistance types in BPEC isolates positive for both *ACC(6)Ib-cr* and *qnr* genes.

No. : (Total) of isolates positive for qnr genes and *ACC(6)Ib-cr*	**No.(%) of antibiotics types to isolates carry both *qnr* genes and *ACC(6)Ib-cr***							
	13 types	**14 types**	**15 types**	**17 types**	**18 types**	**19 types**	**20 types**	**22 types**
26	**4 (15.4)**	**2 (7.7)**	**4 (15.4)**	**4(15.4)**	**5 (19.2)**	**3 (11.5)**	**2 (7.7)**	**2 (7.7)**

When compared this isolates with BPEC isolates (Table 3-14) show high number 10(9%) isolates were resistant to 14 and 15 types of antibiotics, and there were 37(33%) isolates resistant for 12 types of antibiotics or below.

Park *et al.* (2006) reported that When both *qnrA* and *ACC(6)Ib-cr* are present in the same cell, the level of resistance is increased fourfold more than that conferred by *qnrA* alone, In addition, the presence of *ACC(6)Ib-cr* alone increased substantially the frequency of selection of chromosomal mutants upon exposure to ciprofloxacin, Robicsek *et al.* (2006) reported that when both *qnr* and *ACC(6)Ib-cr* are present in the same cell, the level of resistance reaches clinical significance.

3.18. Prevalence of Colistin Resistant Genes in β- Lactamase Producing *E.coli*

Regarding to colistin resistance of β- lactamase producing *E.coli* isolates, the results revealed 27(24.1%) of *E.coli* isolates were colistin resistant. The results of multiplex PCR technique for mcr genes detection, revealed *mcr 2* and *mcr 3* were detected in (55.5%) and (22.2%) respectively of β- lactamase producing *E.coli* isolates (No. 112), (Figure 3-14).

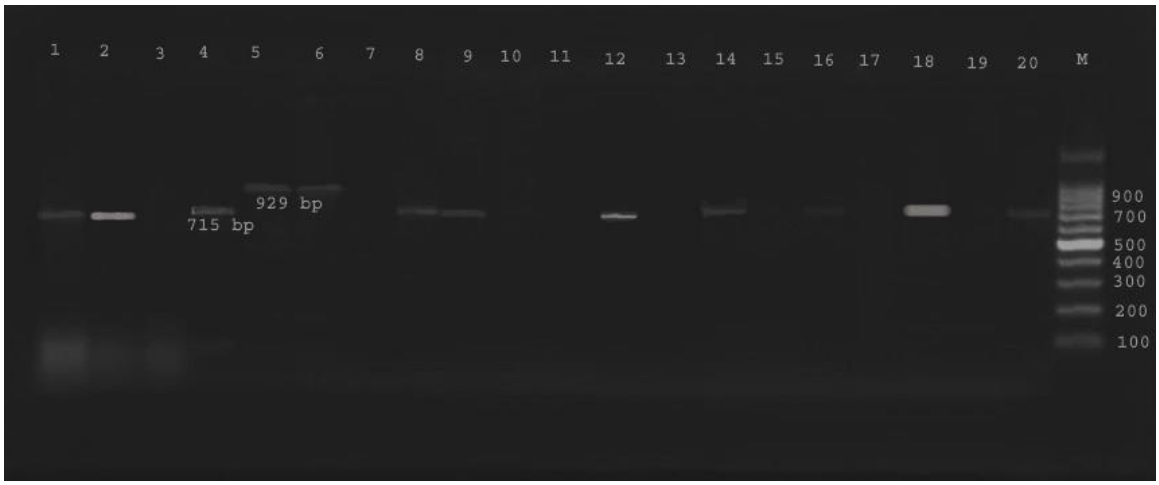

Figure (3-14) Ethidium bromide-stained agarose gel of multiplex PCR amplified products from extracted DNA of colistin resistant, β- lactamase producing *E.coli* isolates that amplified with primers for (*mcr 2* and *mcr 3*) genes forward and reverse, the electrophoresis was performed in 1.5% agarose , lanes M, 1500-bp DNA ladder, lanes (1, 2, 4, 8, 9, 12, 14, 16, 18, 20) show positive result for *mcr 2* (715 bp), lanes (5, 6) show positive result for *mcr 3*(929 bp), lanes (3, 7, 10, 11, 13, 15, 17, 19) show negative results for both genes.

A review study by Dadashi *et al.* (2022) reported the prevalence of mcr gene in Asia, Europe, America, Africa and Oceania from colistin-resistant *E. coli* was 66.72%, 25.49%, 5.19%, 2.27% and 0.32 %, respectively, this result (Asia) were elevated as in present study.

Ejaz *et al.*(2021) reported that mcr genes were deteted in (33.3%) of gram negative bacteria, this result consistent with present study, Whereas Rabie *et al.* (2020) reported that mcr 3 were not detected, this result disagreement with present study.

Ling *et al*. (2020) noted several *mcr* genes (*mcr-1* to *mcr-9*) have been described during the last four years from different countries

Colistin has occurred as a response to the limited alternative treatment options available against MDR bacteria, ultimate antimicrobial drug, colistin is impaired by the emergence of mobile colistin resistance (*mcr*) genes. Plasmid-mediated colistin resistance (Col-R) conferred by *mcr* genes endangers the last therapeutic option for multifarious β-lactamase-producing bacteria (Ejaz *et al.*, 2021).

3.19. Prevalence of *pampC* Genes Among β-Lactamase Producing *E.coli* Isolates.

In present study the detection of AmpC isolates depending on cefoxitin resistance, out of 112 BPEC isolates, 26 isolates were cefoxitin resistance, PCR technique was used for detection of *pampC* genes that included $bla_{CMY,}$ bla_{CIT}, bla_{DHA}, bla_{FOX}, $bla_{MOX,}$ $bla_{EBC.}$and $bla_{ACC.}$

Monoplex PCR were used for detection $bla_{CMY,}$ whereas Multiplex PCR were used for detection 6 *pampC* genes that included bla_{CIT}, bla_{DHA}, bla_{FOX}, bla_{MOX} , $bla_{EBC.}$ and $bla_{ACC.}$

The results revealed AmpC genes were detected in 25 isolates out of 26 AmpC isolates, high prevalence of bla_{CMY} (88.5%) Followed by bla_{CIT} 57.7% and bla_{DHA} 42.3% bla_{FOX}, $bla_{MOX,}$ bla_{ACC} and $bla_{EBC,}$ were not detected (Table 3-20) and (Figures 3-15, 3-16, 17, 3-18)

Table (3-20) Frequency of *pampC* genes in AmpC producing *E.coli* isolates

AmpC producing *E.coli* isolates No.(%)	*bla*$_{CMY}$ Frequency (%)	*bla*$_{CIT}$ Frequency (%)	*bla*$_{DHA}$ Frequency (%)	*bla*$_{FOX}$ Frequency (%)	*bla*$_{MOX}$ Frequency (%)	*bla*$_{EBC}$ Frequency (%)	*bla*$_{ACC}$ Frequency (%)
26	**23(88.5%)**	**15(57.7%)**	**10(42.3%)**	**0.00**	**0.00**	**0.00**	**0.00**

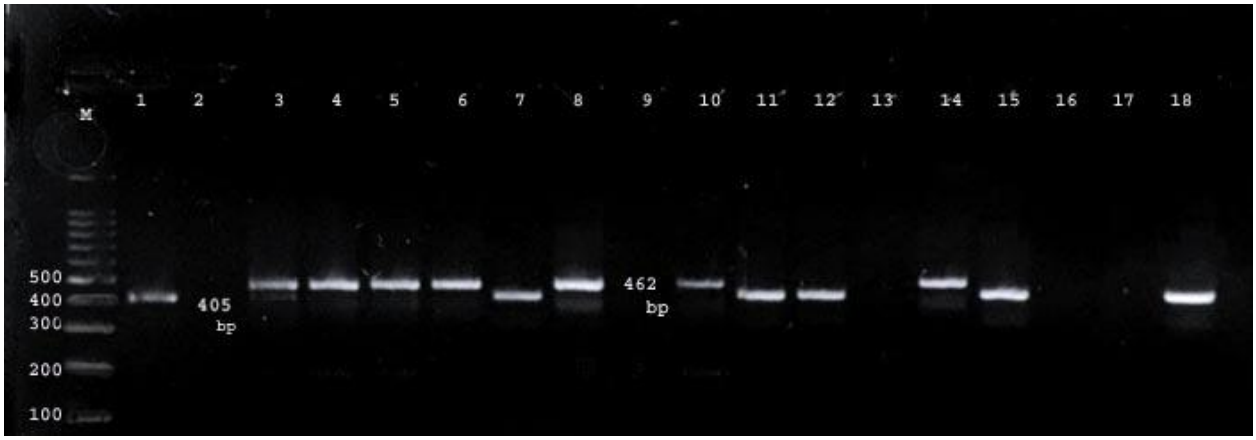

Figure (3-15) Ethidium bromide-stained agarose gel of multiplex PCR amplified products from extracted DNA of AmpC positive *E.coli* isolates that amplified with six primers forward and reverse to genes (*bla*$_{DHA}$, *bla*$_{CIT}$, *bla*$_{ACC}$, *bla*$_{FOX}$, *bla*$_{MOX}$, *bla*$_{CEA}$), the electrophoresis was performed in 1.5 % agarose, lance M, 1500-bp DNA ladder , lanes (1, 7, 11, 12, 15, 18) show positive results for *bla*$_{DHA}$ (405 bp), lanes (3, 4, 5, 6, 8, 10, 14) show positive results for *bla*$_{CIT}$(462 bp), all these lanes belong to cefoxitin resistance *E.coli*. lanes (2,16,17) were cefoxitin-intermediate not carry any one from 6 genes.

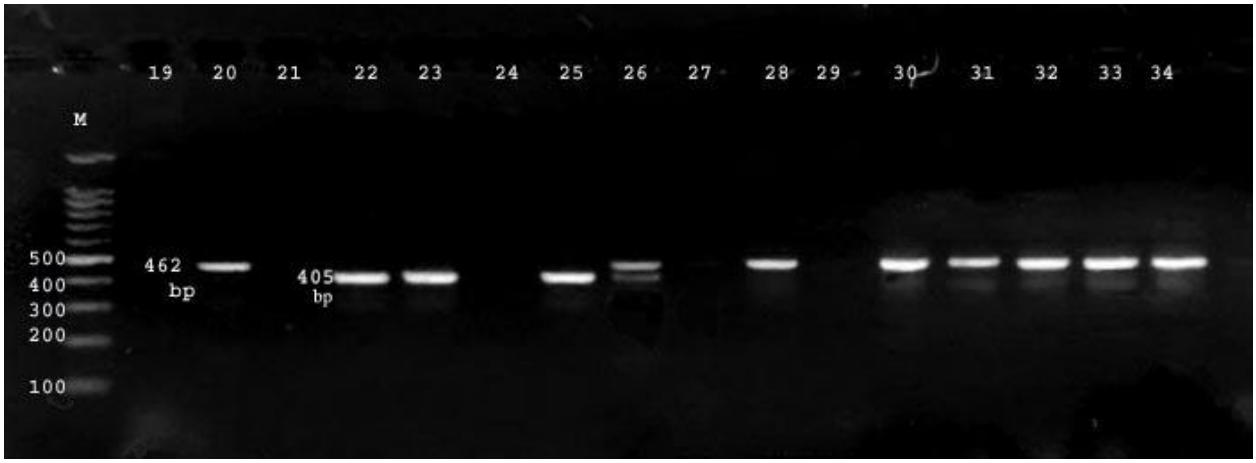

Figure (3-16) Ethidium bromide-stained agarose gel of multiplex PCR amplified products from extracted DNA of AmpC positive *E.coli* isolates that amplified with six primers forward and reverse to genes (*bla*$_{DHA}$, *bla*$_{CIT}$, *bla*$_{ACC}$, *bla*$_{FOX}$, *bla*$_{MOX}$, *bla*$_{CEA}$), the electrophoresis was performed in 1.5%, lanes M, 1500-bp DNA ladder lanes (22, 23, 25,26) show positive results for *bla*$_{DHA}$ (405 bp), lanes (20, 26, 28, 30, 31, 32, 33, 34) show positive results for *bla*$_{CIT}$(462 bp), all these lanes belong to cefoxitin resistance *E.coli*. lanes (19, 21, 24, 27, 29), were cefoxitin-intermediate not carry any one from 6 genes.

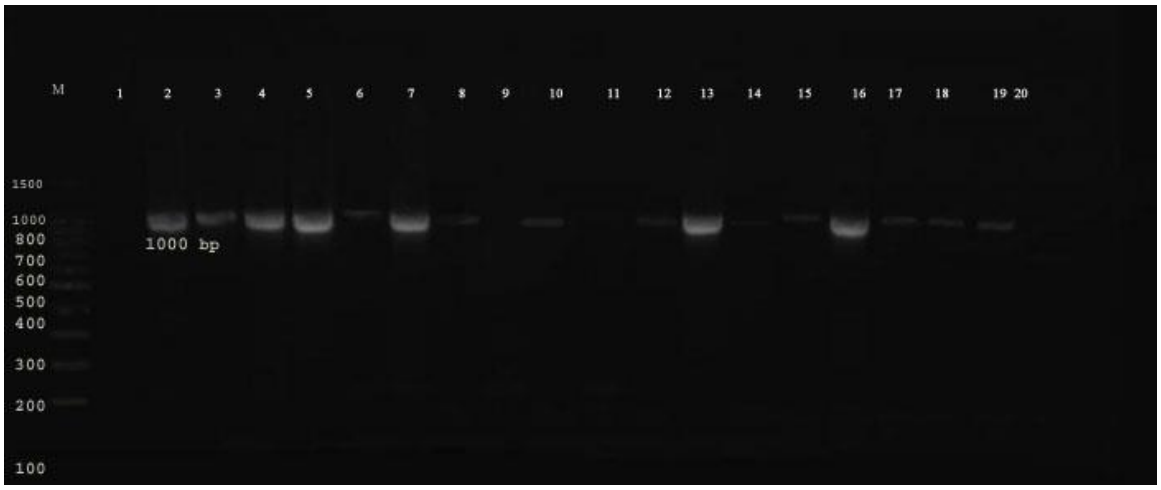

Figure (3-17) Ethidium bromide-stained agarose gel of monoplex PCR amplified products from extracted DNA of AmpC positive *E.coli* isolates that amplified with primers for *bla* $_{CMY}$ genes forward and reverse, the electrophoresis was performed in 1.5% agarose , lanes M, 1500-bp DNA ladder, lanes (3, 4, 5, 6, 7, 8, 10, 12, 13, 14, 15, 18, 19) show positive result for *bla*$_{CMY}$(1000bp), lanes (1, 9, 11, 20) show negative result for bla$_{CMY}$, lanes (2,16,17) belong to cefoxitin-intermediate isolates were appeared positive results.

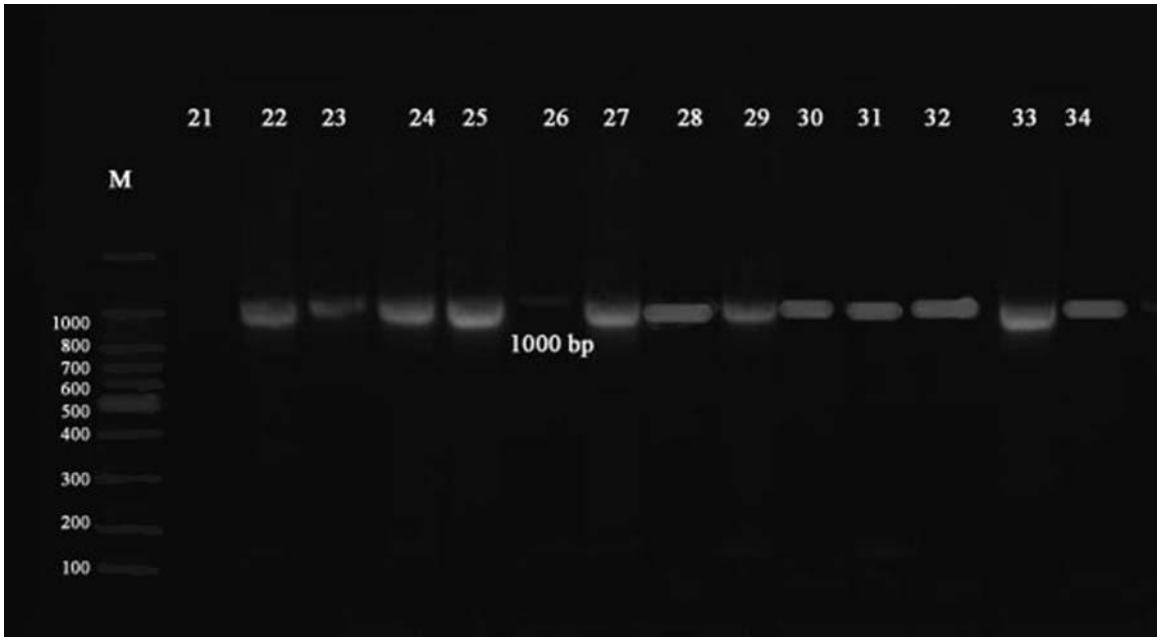

Figure (3-18) Ethidium bromide-stained agarose gel of monoplex PCR amplified products from extracted DNA of AmpC positive *E.coli* isolates that amplified with primers for *bla* $_{CMY}$ genes forward and reverse, the electrophoresis was performed 1.5% agarose , lanes M, 1500-bp DNA ladder, lanes (22, 23, 25, 28, 30, 31, 32, 33, 34) show positive result for *bla*$_{CMY}$(1000bp), lanes (26) show negative result for *bla*$_{CMY}$, lanes (21, 24, 27, 29) belong to cefoxitin-intermediate isolates were appeared positive results.

Coolen *et al.* (2019) reported *bla*$_{CMY}$ that as the most common AmpC encoding resistance gene. And noted depending on the type of pampC β-lactamase, the hydrolysing capability might vary. Helmy and Wasfy (2014) reported that *bl*$_{CMY}$ was

the most predominant gene (86.9%), a study by Sadeghi *et al*., (2022) in Iran reported *bla*$_{MOX}$ (14.6%), *bla*$_{EBC}$ (8.3%) and *bla*$_{CIT}$ (18.8%) , while, *bla*$_{DHA}$, *bla*$_{FOX}$, and *bla*$_{ACC}$ genes were not found in any isolates. Jojy *et al*. (2021) in Manama city, Kingdom of Bahrain, revealed *bla*$_{ACC}$, *bla*$_{EBC}$, *bla*$_{FOX}$ and *bla*$_{MOX}$ were not detected by PCR methods, the results of last four genes of this study compatible with results of the same genes, *bla*$_{CIT}$ (31%), *bla*$_{DHA}$(10.3%) of cefoxitin-resistant isolates, these results were lower than results in present study, Studies in different areas of the world have revealed geographical diversity in the molecular subtypes of pAmpC genes. In this regard, CIT subtype of AmpC has been widely detected in the United States and Canada in recent years.

3.19.1. Frequency of *pampC* Genes in the Same *E.coli* Isolate

The results revealed high frequency for *bla*$_{CIT}$ +*bla*$_{CMY}$ genes (53.85%) followed by *bla*$_{DHA}$ + *bla*$_{CMY}$ (26.92%) and *bla*$_{DHA}$(7.7%), whereas, *bla*$_{DHA}$ + *bla*$_{CIT}$ + *bla*$_{CMY}$ (3.85%) and there were only one AmpC-producing isolates without *pampC* genes (Table 3-21)

The frequency of *pampC* genes in the same isolates depending on the prevalence of *pampC* genes that depending on the geographic area, Woodford *et al*. (2007) also described the predominance of *bla*$_{CIT}$ in AmpC‑producing *E. coli*, in the UK and Ireland. In contrast to these studies, Wassef *et al*. (2014) reported *bla*$_{MOX}$ and *bla*$_{FOX}$ families as the most prevalent *pampC* subtypes in Egypt, followed by *bla*$_{EBC}$ and *bla*$_{CIT}$ subtypes. Adding to the geographical complexity of AmpC strains, Ingram *et al*. (2011), in North Africa and Australia have reported *bla*$_{CMY}$, *bla*$_{DHA}$, and *bla*$_{EBC}$ as the most frequent subtypes of AmpC producers, Abdalhamid *et al.* (2017) reported the prevalence of *bla*$_{CMY-4}$/ *bla*$_{CMY-6}$ in the United Arab Emirates and Kuwait and *bla*$_{DHA-1}$/ *bla*$_{CMY-2}$ in Saudi Arabia. Collectively, these discrepancies are of particular scientific interest about the evolution of *pampC* subtypes worldwide.

Estaleva *et al.* (2020) found that Bla_{FOX} +Bla_{MOX} high frequency, that detected in 10 isolated from 25 AmpC producer *E.coli* isolates.

Table (3-21) The occurrence patterns of genes encoding AmpC beta lactamase

No.	Pattern	Cefoxitin –Resistance (AmpC) isolate No.=26	
		No.	%
1	Bla_{DHA}	2	7.7
2	Bla_{CMY}	1	3.85
3	Bla_{DHA} +Bla_{CMY}	7	26.92
4	Bla_{CIT} +Bla_{CMY}	14	53.85
5	Bla_{DHA} + Bla_{CIT} + Bla_{CMY}	1	3.85
6	Without study genes	1	3.85

3.20. AmpC β-lactamase Producing Isolates and Associated with other Antibiotics Resistance Genes.

3.20.1. AmpC Producing *E.coli* Isolates Positive for ESBL

Out of 26 isolates positive for cefoxitin (AmpC producing isolates) only 5 (19.2%) isolates positive for ESBL, the PCR results revealed all isolates carry bla_{CTX-M} (100%) followed by bla_{TEM} and bla_{OXA} (80%) and bla_{SHV} (60%) (Table 3-22) and (Figures 3-19, 3-20, 3-21, 3-22). The frequency of ESBL genes in Amp C producing isolates were not different from frequency of ESBL genes in β-lactamase producing isolates.

Regarding to antibiotics resistance in AmpC/ ESBL producing isolates (Table 3-27) shown there were mild elevated in antibiotics resistant for AmpC isolates alone.

AmpC producers may appear susceptible to extended-spectrum cephalosporins when initially tested (Thomson 2010; Tracz *et al.* 2007). Cherif *et al.* (2016) reported that Co-presence of ESBLs and AmpC may occur and significant in antibiotics resistance. Peter-Getzlaff *et al.* (2011) reported that bacterial strains

producing AmpC beta-lactamases and/ or extended-spectrum beta-lactamases (ESBLs) are of particular concern.

Table (3-22) Frequency of ESBL genes in AmpC β-lactamase producing *E.coli* isolates

AmpC positive isolates, positive for ESBL	**ESBL genes**			
	***bla*$_{TEM}$**	***bla*$_{SHV}$**	***bla*$_{CTX-M}$**	***bla*$_{OXA}$**
1	+	+	+	+
9	-	-	+	+
10	+	-	+	+
12	+	+	+	-
16	+	+	+	+
Total (5)	**4(80%)**	**3(60%)**	**5(100%)**	**4(80%)**

+ detected by PCR, - Non- detected by PCR

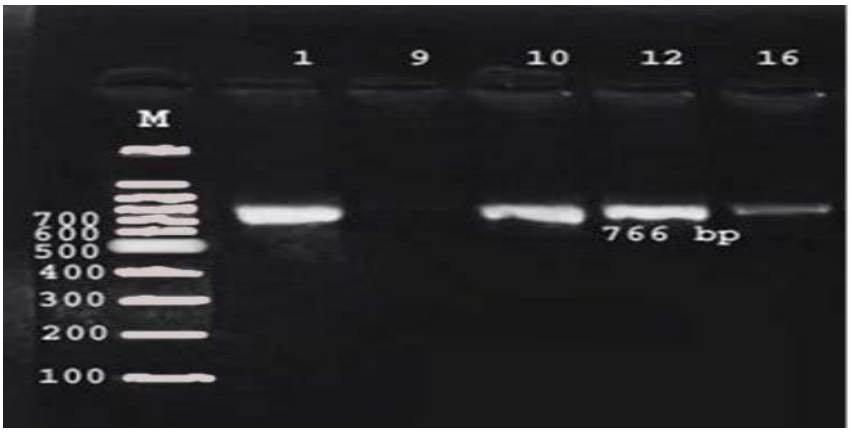

Figure (3-19) Ethidium bromide-stained agarose gel of monoplex PCR amplified products from extracted DNA of AmpC positive *E.coli* isolates that amplified with primers for *bla*$_{TEM}$ genes forward and reverse, the electrophoresis was performed 1.5% agarose, lanes M, 1500-bp DNA ladder, lanes (1,10,12,16) show positive result for *bla*$_{TEM}$ (766 bp), lanes (9) show negative result for *bla*$_{TEM}$.

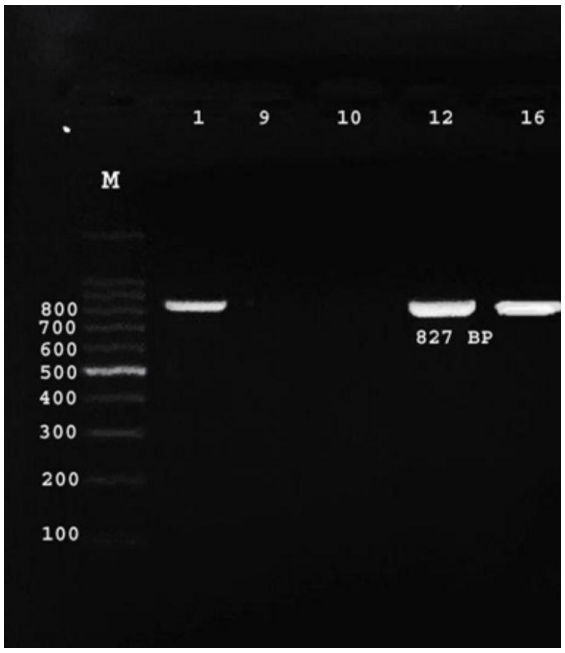

Figure (3-20) Ethidium bromide-stained agarose gel of monoplex PCR amplified products from extracted DNA of AmpC positive *E.coli* isolates that amplified with primers for *bla* $_{SHV}$ genes forward and reverse, the electrophoresis was performed 1.5% agarose , lanes M, 1500-bp DNA ladder, lanes (1,12,16) show positive result for *bla*$_{SHV}$(827 bp), lanes (9,10) show negative result for *bla*$_{SHV}$.

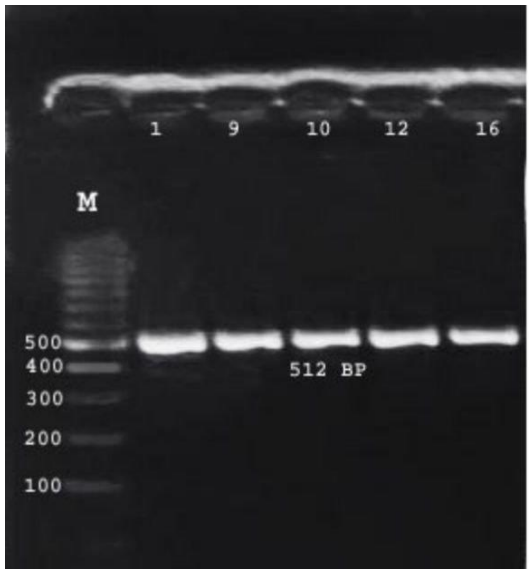

Figure (3-21) Ethidium bromide-stained agarose gel of monoplex PCR amplified products from extracted DNA of AmpC positive *E.coli* isolates that amplified with primers for *bla*$_{CTX-M}$ genes forward and reverse, the electrophoresis was performed 1.5% agarose, lanes M, 1500-bp DNA ladder, lanes (1,9,10,12,16) show positive result for *bla*$_{CTX-M}$ (512 bp).

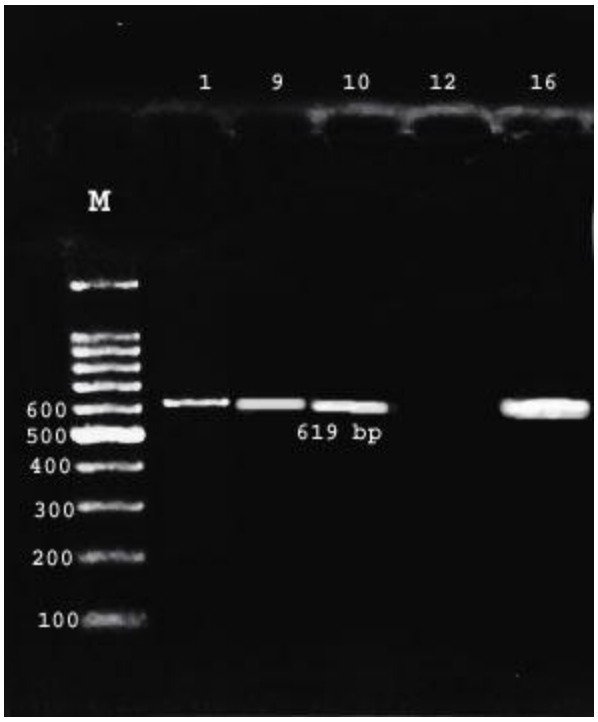

Figure (3-22) Ethidium bromide-stained agarose gel of monoplex PCR amplified products from extracted DNA of AmpC positive *E.coli* isolates that amplified with primers for *bla* $_{OXA}$ genes forward and reverse, the electrophoresis was performed 1.5% agarose, lanes M, 1500-bp DNA ladder, lanes (1,9,10,16) show positive result for *bla*$_{OXA}$(619 bp), lanes (12) show negative result for *bla*$_{OXA}$.

3.20.2. AmpC Producing Isolates Resistant for Carbapeneme.

Out of 26 AmpC producing isolates 4(15.4%) isolates resistant for carbapeneme, all carbapenems resistant isolates in current study belong to AmpC producing *E.coli* isolates, the PCR results revealed *bla*$_{OXA}$ (100%), *bla*$_{NDM}$ (75%) , *bla*$_{KPC}$, *bla*$_{VIM}$ and *bla*$_{IMP}$ were not detected in carbapeneme- resistant isolates (Table 3-23) (Figures 3-23, 3-24).

Table (3--23) Frequency of carbapenemase genes in AmpC producing *E.coli* isolates resistant to carbapeneme.

AmpC positive isolates positive for carbapenemase	Carbapenemase genes				
	***bla*$_{KPC}$**	***bla*$_{NDM}$**	***bla*$_{VIM}$**	***bla*$_{OXA}$**	***bla*$_{IMP}$**
14	**-**	**+**	**-**	**+**	**-**
15		**+**	**-**	**+**	**-**
21	**-**	**+**	**-**	**+**	**-**
23	**-**	**-**	**-**	**+**	**-**
Total (4)	**0**	**3(75%)**	**0**	**4(100%)**	**0**

+ detected by PCR, - Non- detected by PCR

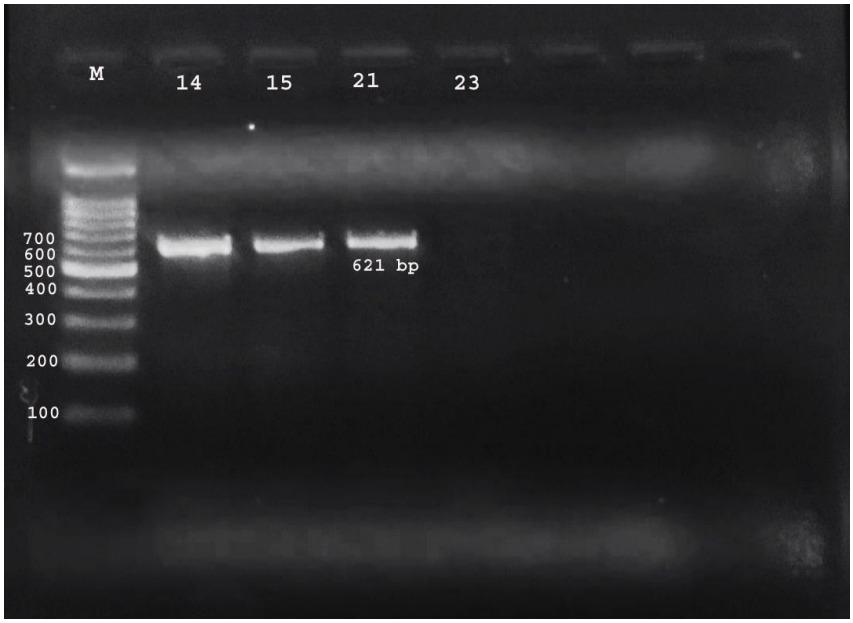

Figure (3-23) Ethidium bromide-stained agarose gel of monoplex PCR amplified products from extracted DNA of AmpC positive *E.coli* isolates and resistance to carbapeneme, that amplified with primers for *bla* $_{NDM}$ genes forward and reverse, the electrophoresis was performed 1.5% agarose, lanes M, 1500-bp DNA ladder, lanes (14, 15 21) show positive result for *bla*$_{NDM}$ (621 bp), lanes (23) show negative result for *bla*$_{NDM}$.

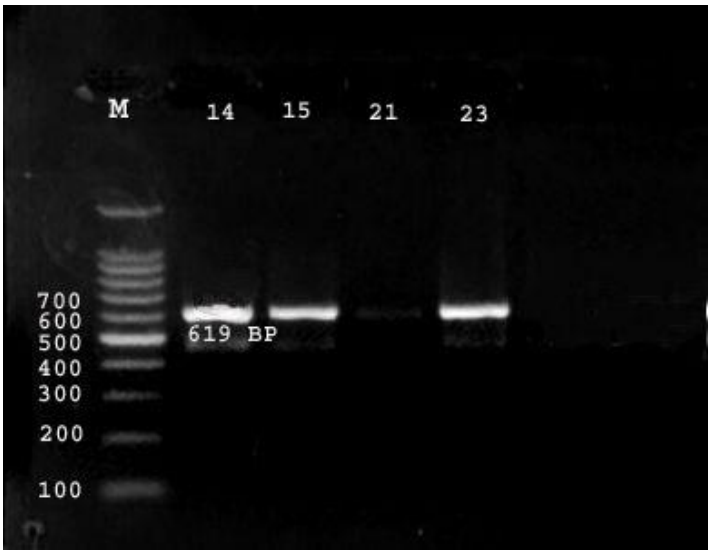

Figure (3-24) Ethidium bromide-stained agarose gel of monoplex PCR amplified products from extracted DNA of AmpC positive *E.coli* isolates and resistance to carbapeneme, that amplified with primers for bla_{OXA} gene forward and reverse, the electrophoresis was performed 1.5% agarose, lanes M, 1500-bp DNA ladder, lanes (14, 15, 21, 23) show positive result for bla_{OXA} (619 bp).

3.20.3. AmpC β-lactamase Producing *E.coli* Isolates Resistant to Quinolone.

In present study there are strong relationship between AmpC producing *E.coli* isolates and quinolone resistance from 26 AmpC-producing isolates, 23 (88.5%) isolates resistant to quinolone, PCR technique used for detection of quinolone resistance genes, *qnr A* and *qnr B*, the results revealed (69.5%) and (47.8%) respectively. The prevalence of quinolone resistance genes elevated when compared with prevalence in β- lactamase producing *E.coli* (BPEC) isolates (Table 3-24), (Figures 3-25 and 3-26).

Table (3-24) Frequency of quinolone resistance genes (*qnr A* and *qnr B*) in AmpC producing *E.coli* isolates resistant to quinolones

AmpC positive isolates resistant for quinolone	Quinolone resistance genes	
	qnr A	***qnr B***
2	+	+
3	-	-
4	+	-
5	+	-
6	+	+
7	+	-
8	-	+
9	+	-
10	-	+
11	+	+
12	-	-
13	+	-
14	+	-
15	+	-
17	-	+
18	-	+
19	+	+
21	+	+
22	+	+
23	-	-
24	+	+
25	+	-
26	+	-
Total (23)	**16 (69.5%)**	**11 (47.8%)**

+ detected by PCR, - Non- detected by PCR

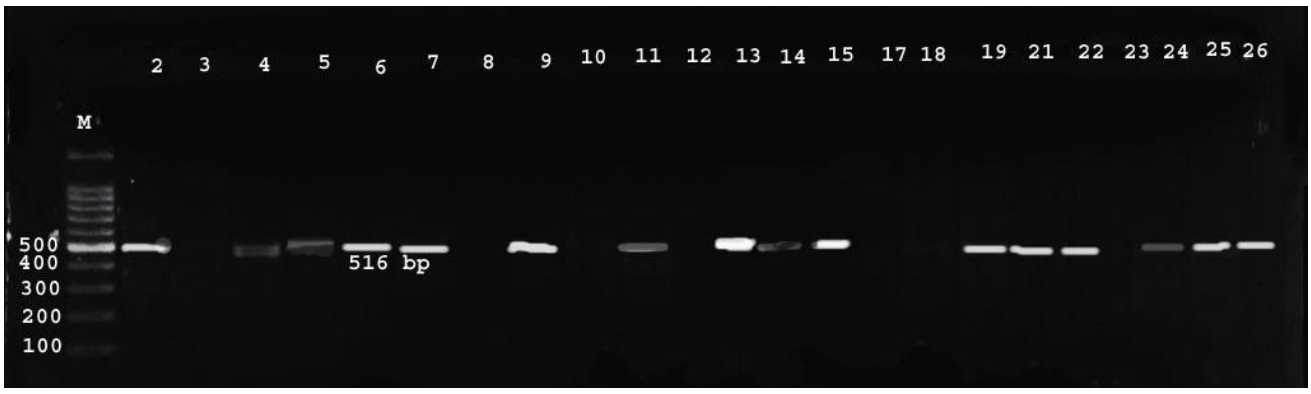

Figure (3-25) Ethidium bromide-stained agarose gel of monoplex PCR amplified products from extracted DNA of AmpC positive *E.coli* isolates and quinolone resistant that amplified with primers for *qnr A* genes forward and reverse, the electrophoresis was performed 1.5% agarose , lanes M, 1500-bp DNA ladder, lanes (2, 4, 5, 6 , 7, 9,11, 13, 14, 15, 19, 21, 22, 24, 25, 26) show positive result for *qnr A*(516 bp), lanes (3, 8, 10, 12, 17, 18, 23) show negative result for *qnr A*.

Figure (3-26) Ethidium bromide-stained agarose gel of monoplex PCR amplified products from extracted DNA of AmpC positive *E.coli* isolates and quinolone resistant that amplified with primers for *qnr B* genes forward and reverse, the electrophoresis was performed 1.5% agarose , lanes M, 1500-bp DNA ladder, lanes (2, ,6 , 8,10, 11, 17, 18, 19, 21, 22) show positive result for *qnr B* (469 bp), lanes (3, 4, 5, 7, 9, 12, 13, 14, 15, 23) show negative result for *qnr B*.

The association of *qnr* genes with AmpC beta-lactamases was noteworthy; some strains containing a *qnrA* gene produce AmpC beta-lactamase (Wang *et al.* 2008; Nordman and Poirel 2005) . Some reports show an association of *qnrB* with an ESBL and AmpC beta-lactamase (Pai and Choi 2007; Jacoby *et al* 2006).

3.20.4. AmpC β-lactamase Isolates Resistant to Aminoglycosides

Out of 26 AmpC producing isolates there were 8 (33.3%) isolates resistant to aminoglycosides, the PCR results revealed all aminoglycosides isolates were positive for *ACC(6)Ib-cr* (100%) (Table 3-25), (Figure 3-11), from these results the prevalence of *ACC(6)Ib-cr* were even in AmpC and BPEC isolates.

Table (3-25) Prevalence of *ACC(6)Ib-cr* gene in Amp C-producing isolates resistant to aminoglycosides

AmpC positive isolates resistant to aminoglycosides	Aminoglycosides resistance gene (*ACC(6)Ib-cr*)
2	**+**
4	**+**
8	**+**
9	**+**
11	**+**
12	**+**
13	**+**
20	**+**
Total (8)	**8 (100%)**

+ detected by PCR, - Non- detected by PCR

3.20.5. AmpC producing *E.coli* isolates resistant to colistin.

The antibiotic susceptibility results revealed from 26 AmpC isolates only 5(19.2%) isolates were colistin resistant, PCR results for colistin resistance genes *mcr2* and *mcr3* were 60% and 40% respectively (Table 3-26), there were slightly elevated in prevalence of *mcr 3* genes in AmpC producing isolates when compared with BPEC isolates, this elevated non-significant because low isolates numbers.

Table (3-26) Prevalence of colistin resistant - genes among AmpC-producing isolates were resistant to colistin

AmpC positive isolates resistant for colistin	Colistin resistance genes	
	mcr2	***mcr3***
11	**+**	**-**
16	**+**	**-**
19	**+**	**-**
24	**-**	**+**
26	**-**	**+**
Total (5)	**3 (60%)**	**2 (40%)**

+ detected by PCR, - Non- detected by PCR

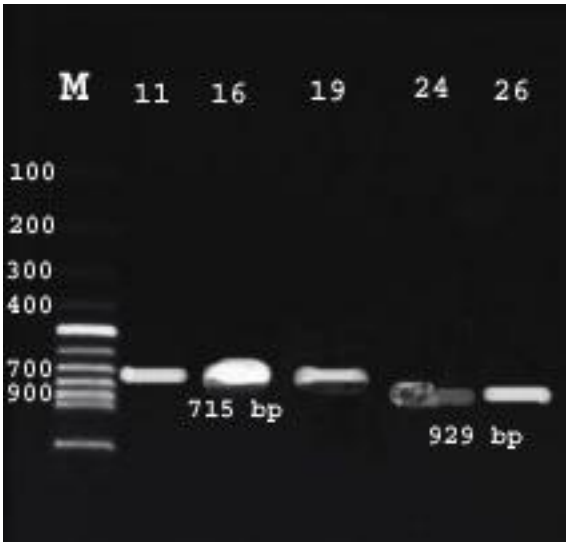

Figure (3-27) Ethidium bromide-stained agarose gel of multiplex PCR amplified products from extracted DNA of colistin resistant, AmpC β- lactamase producing *E.coli* isolates that amplified with primers for (*mcr 2* and *mcr 3*) genes forward and reverse, the electrophoresis was performed in 1.5% agarose , lanes M, 1500-bp DNA ladder, lanes (11, 16, 19) show positive result for *mcr 2* (715 bp), lanes (24, 26) show positive result for *mcr 3* (929 bp).

3.21. Association Between AmpC β- lactamase Producing Isolates and Other Antibiotics Resistance Genes

The frequency of *pampC* genes *bla*$_{CMY}$, *bla*$_{CIT}$ and *bla*$_{DHA}$ were relatively equal except AmpC producing *E.coli* isolates that isolated from diabetic foot ulcer all isolates carry *bla*$_{CMY}$ and *bla*$_{CIT}$ (Table 3-27).

Urine AmpC production *E.coli* isolates, were high frequency for ESBL genes, whereas AmpC produced *E.coli* isolates from burn, stool, vaginal exudate and diabetic foot ulcer were low frequency for ESBL genes (Table 3-27), these results consistent with sadeghi *et al*. (2022) in Iran that reported high prevalence of ESBL genes in cefoxitin resistant *E.coli* isolated from urine, A study in Hilla city by Hussein (2023) found that high prevalence of ESBL genes in *E.coli* isolated from urine, *bla*$_{CTX-M}$ (76%), *bla*$_{TEM}$ (75%), and *bla*$_{SHV}$ (73%).

Table (3-27) The frequency of *ampC* genes and other antibiotics resistant genes in AmpC producing *E.coli* isolates

Source	Isolates No.	Resistance style	No.of β-lactam antibiotics resistance	No. of non-β- lactam antibiotics resistance	No. of classes	No. (types) of *ampC* genes	No.(types) of other resistance genes in AmpC producing *E.coli* isolates
Urine	1 *	Resistance	9	2	5	1 (bla_{DHA})	4 (*bla*SHV, *bla*CTX-M, *bla*OXA, Int1)
	2	Intermediate	9	9	7	1 (*bla*CMY)	7 (*bla*SHV, *bla*TEM, *bla*CTX-M, *bla*OXA, integron 1,qnr A, qnr B)
	3	Resistance	12	9	8	2(*bla*CIT, *bla*CMY)	4 (*qnr A, qnr B, ACC(6) Ib-cr, ,Int1*)
	4	Resistance	12	9	9	2 (*bla*CIT, *bla*CMY)	1 (*Int1*)
	5	Resistance	12	10	9	2 (*bla*CIT, *bla*CMY)	3 (*qnr A, ACC(6) Ib-cr, Int1*)
	6	Resistance	12	10	10	2 (*bla*CIT, *bla*CMY)	2 (*qnr A, Int1*)
	7	Resistance	12	6	5	2 (*bla*DHA, *bla*CMY)	3 (*qnr A, qnr A, Int1*)
	8	Resistance	11	8	8	2 (*bla*CIT, *bla*CMY)	4 (*bla*SHV, *bla*CTX-M, *bla*OXA, *qnr A, Int1*)
	9 *	Resistance	9	9	8	-	3 (*bla*CTX-M, *bla*OXA, *Int1*)
	10 *	Resistance	9	9	8	2 (*bla*CIT, *bla*CMY)	6 (*bla*TEM, *bla*CTX-M, *bla*OXA, *qnr A, ACC(6) Ib-cr, Int1*)
	11	Resistance	10	9	9	1 (*bla*DHA)	5 (*bla*TEM, *bla*CTX-M, *bla*OXA, *qnr B, Int 1*)
	12 *	Resistance	10	10	10	2 (*bla*DHA, *bla*CMY)	8 (*bla*SHV, *bla*TEM, *bla*CTX-M, *qnr A, qnr B, ACC(6)Ib-cr, Int 1,mcr 2*)
	13	Resistance	10	9	9	1 (*bla*CMY)	5 (*bla*SHV, *bla*CTX-M, *bla*TEM, *ACC(6)Ib-cr, Int1*)
	14	Resistance	12	11	11	2 (*bla*CIT, *bla*CMY)	4 (*qnr A, ACC(6)Ib-cr, bla*NDM, *Int1*)
	15	Resistance	12	10	10	2 (*bla*DHA, *bla*CMY)	3 (*qnr A, bla*NDM, *Int1*)
Burn	16 *	Intermediate	5	9	7	1 (*bla*CMY)	2 (*qnr B, Int1*)
	17	Intermediate	5	9	7	1 (*bla*CMY)	4 (*qnr A, qnr B, ACC(6)Ib-cr, Int1*)
	18	Resistance	11	9	8	2 (*bla*DHA, *bla*CMY)	2 (*qnr A, Int1*)
	19	Intermediate	5	8	8	1(*bla*CMY)	4 (*qnr A, qnr B, ACC(6)Ib-cr, Int1*)
	20	Resistance	9	4	9	2(*bla*CMY, *bla*CIT)	6(*bla*TEM, *bla*SHV, *bla*CTX-M, *bla*OXA, *Int1,mcr 2*)
Stool	21	Intermediate	6	11	11	-	4 (*qnr B, ACC(6)Ib-cr, Int1,mcr 2*)
	22	Resistance	7	9	7	2 (*bla*DHA, *bla*CMY)	2 (*qnr B, Int1*)
	23	Resistance	11	8	7	2 (*bla*DHA, *bla*CMY)	2 (*qnr B, Int1*)
	24	Intermediate	5	10	8	1(*bla*CMY)	4 (*qnr B, Int1, ACC(6)Ib-cr, mcr 2)*
	25	Resistance	11	7	9	2 (*bla*DHA, *bla*CMY)	4 (*qnr A, qnr B, Int1,mcr 2*)
	26	Resistance	7	2	4	3 (*bla*DHA, *bla*CIT, *bla*CMY)	2 (*ACC(6)Ib-cr, Int1*)
Vaginal exudate	27	Intermediate	8	6	7	1 (*bla*CMY)	6 (*bla*TEM, *bla*CTX-M, *bla*OXA, *qnr A, qnr B, Int1*)
	28	Resistance	10	12	10	2 (*bla*CMY, CIT)	5 (*qnr A, qnr B, bla*OXA, *bla*NDM, *Int1*)
	29	Intermediate	5	8	8	1 (*bla*CMY)	4 (*qnr A, qnr B, ACC(6)Ib-cr, Int1*)
Diabetic foot ulcer	30	Resistance	13	10	9	2 (*bla*CIT, *bla*CMY)	3 (*qnr A, qnr B, Int1*)
	31	Resistance	12	10	9	2 (*bla*CIT, *bla*CMY)	2 (*bla*OXA, *Int1*)
	32	Resistance	13	10	9	2 (*bla*CIT, *bla*CMY)	3(*qnr A, qnr B, Int1, mcr 3*)
	33	Resistance	13	10	9	2 (*bla*CIT, *bla*CMY)	2(*qnr A, Int1*)
	34	Resistance	13	9	9	5(*bla*CIT, *blaCMY*)	3(*qnr A, Int1, mcr 3*)

*isolate produce both AmpC and ESBL

ACC(6)Ib-cr, qnr A, qnr B, mcr2 and *mcr3* were equal in frequency, *Int1* were distributed in all isolates.

There were 5 *E.coli* isolates produce both AmpC and ESBL, these isolates were more resistance to antibiotics, 2 isolates resistant to 19 antibiotics types, 1 isolates resistant to 18 antibiotics types, 1 isolates resistant to 13 antibiotics types, and 1 isolates resistant to 11 antibiotic types (Table 3-27).

Weber *et al.* (2021) reported that resistance to broad-spectrum β-lactams can be mediated by ESBLs and AmpC β-lactamases (AmpC). Both can hydrolyze 3rd- and 4th-generation cephalosporins such as cefotaxime.

In present study only 8 *E.coli* isolates were cefoxitin intermediate, 7 isolates carry bla_{CMY} gene and one isolates without *pampC* genes (Table 3-25)

3.22. DNA Sequencing:

To confirmed PCR results, DNA sequencing was done for PCR amplicons, forward only, for bla_{DHA} and bla_{CIT} and the results were compared against the National Centre of Biotechnology Information (NCBI) GenBank sequence database using the BLASTn. The results of *bla*DHA gene revealed that gene was the same gene with purity 100% and bla_{CIT} sequence was similar 100% to bla_{CMY-42} (Alignment 1-12)

Ingti *et al* (2018.) found that sequencing of bla_{CIT} gene similar nucleotide sequence of bla_{CMY-42} variant. Lan *et al.* (2017) show the bla_{CIT} was the same name of bla_{CMY-42}. Oliveira *et al*. (2019) reported that bla_{CIT} variants includes bla_{LAT-1} to bla_{LAT-3}, bla_{BIL-1}, bla_{CMY-2} to bla_{CMY-7}, bla_{CMY-12} to bla_{CMY-18}, bla_{CMY-21} to bla_{CMY-23} and bla_{CMY-42}.

1-Alignment of DHA-1 with *E.coli* strain EF2 DHA family class C beta-lactamase (*bla*$_{DHA}$) gene, partial cds Sequence ID: OQ096360.1

Score	Expect	Identities	Gaps	Strand
634 bits(343)	7e-179	343/343(100%)	0/343(0%)	Plus/Plus

```
Query  1    CGGCGGCAAAATACCAGCCGGAGCTGGCTCTGCCGCAGTGGAAGGGGATCACATTGCTGG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  161  CGGCGGCAAAATACCAGCCGGAGCTGGCTCTGCCGCAGTGGAAGGGGATCACATTGCTGG  220

Query  61   ATCTGGCTACCTATACCGCAGGCGGACTGCCGTTACAGGTGCCGGATGCGGTAAAAAGCC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  221  ATCTGGCTACCTATACCGCAGGCGGACTGCCGTTACAGGTGCCGGATGCGGTAAAAAGCC  280

Query  121  GTGCGGATCTGCTGAATTTCTATCAGCAGTGGCAGCCGTCCCGGAAACCGGGCGATATGC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  281  GTGCGGATCTGCTGAATTTCTATCAGCAGTGGCAGCCGTCCCGGAAACCGGGCGATATGC  340

Query  181  GTCTGTATGCAAACAGCAGTATCGGCCTGTTTGGTGCTCTGACCGCAAACGCGGCGGGGA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  341  GTCTGTATGCAAACAGCAGTATCGGCCTGTTTGGTGCTCTGACCGCAAACGCGGCGGGGA  400

Query  241  TGCCGTATGAGCAGTTGCTGACTGCACGGATCCTGGCACCGCTGGGGTTATCTCACACCT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  401  TGCCGTATGAGCAGTTGCTGACTGCACGGATCCTGGCACCGCTGGGGTTATCTCACACCT  460

Query  301  TTATTACTGTGCCGGAAAGTGCGCAAAGCCAGTATGCGTACGG  343
            |||||||||||||||||||||||||||||||||||||||||||
Sbjct  461  TTATTACTGTGCCGGAAAGTGCGCAAAGCCAGTATGCGTACGG  503
```

2-Alignment of DHA-2 with *E.coli* strain EF2 DHA family class C beta-lactamase (*bla*$_{DHA}$) gene, partial cds Sequence ID: OQ096360.1

Score	Expect	Identities	Gaps	Strand
641 bits(347)	0.0	347/347(100%)	0/347(0%)	Plus/Plus

```
Query  1    GATCCGGCGGCAAAATACCAGCCGGAGCTGGCTCTGCCGCAGTGGAAGGGGATCACATTG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  157  GATCCGGCGGCAAAATACCAGCCGGAGCTGGCTCTGCCGCAGTGGAAGGGGATCACATTG  216

Query  61   CTGGATCTGGCTACCTATACCGCAGGCGGACTGCCGTTACAGGTGCCGGATGCGGTAAAA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  217  CTGGATCTGGCTACCTATACCGCAGGCGGACTGCCGTTACAGGTGCCGGATGCGGTAAAA  276

Query  121  AGCCGTGCGGATCTGCTGAATTTCTATCAGCAGTGGCAGCCGTCCCGGAAACCGGGCGAT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  277  AGCCGTGCGGATCTGCTGAATTTCTATCAGCAGTGGCAGCCGTCCCGGAAACCGGGCGAT  336

Query  181  ATGCGTCTGTATGCAAACAGCAGTATCGGCCTGTTTGGTGCTCTGACCGCAAACGCGGCG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  337  ATGCGTCTGTATGCAAACAGCAGTATCGGCCTGTTTGGTGCTCTGACCGCAAACGCGGCG  396

Query  241  GGGATGCCGTATGAGCAGTTGCTGACTGCACGGATCCTGGCACCGCTGGGGTTATCTCAC  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  397  GGGATGCCGTATGAGCAGTTGCTGACTGCACGGATCCTGGCACCGCTGGGGTTATCTCAC  456

Query  301  ACCTTTATTACTGTGCCGGAAAGTGCGCAAAGCCAGTATGCGTACGG  347
            |||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  457  ACCTTTATTACTGTGCCGGAAAGTGCGCAAAGCCAGTATGCGTACGG  503
```

3-Alignment of DHA-3 with *E.coli* strain EF2 DHA family class C beta-lactamase (*bla*$_{DHA}$) gene, partial cds Sequence ID: OQ096360.1

Score	Expect	Identities	Gaps	Strand
621 bits(336)	5e-175	338/339(99%)	0/339(0%)	Plus/Plus

```
Query  1    TGAATGATCCGGCGGCAAAATACCAGCCGGAGCTGGCTCTGCCGCAGTGGAAGGGGATCA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  152  TGAATGATCCGGCGGCAAAATACCAGCCGGAGCTGGCTCTGCCGCAGTGGAAGGGGATCA  211

Query  61   CATTGCTGGATCTGGCTACCTATACCGCAGGCGGACTGCCGTTACAGGTGCCGGATGCGG  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  212  CATTGCTGGATCTGGCTACCTATACCGCAGGCGGACTGCCGTTACAGGTGCCGGATGCGG  271

Query  121  TAAAAAGCCGTGCGGATCTGCTGAATTTCTATCAGCAGTGGCAGCCGTCCCGGAAACCGG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  272  TAAAAAGCCGTGCGGATCTGCTGAATTTCTATCAGCAGTGGCAGCCGTCCCGGAAACCGG  331

Query  181  GCGATATGCGTCTGTATGCAAACAGCAGTATCGGCCTGTTTGGTGCTCTGACCGCAAACG  240
            |||||||||||||||||||||| ||||||||||||||||||||||||||||||||||||
Sbjct  332  GCGATATGCGTCTGTATGCAAACAGCAGTATCGGCCTGTTTGGTGCTCTGACCGCAAACG  391

Query  241  CGGCGGGGATGCCGTATGAGCAGTTGCTGACTGCACGGATCCTGGCACCGCTGGGGTTAT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  392  CGGCGGGGATGCCGTATGAGCAGTTGCTGACTGCACGGATCCTGGCACCGCTGGGGTTAT  451

Query  301  CTCACACCTTTATTACTGTGCCGGAAAGTGCGCAAAGCC  339
            |||||||||||||||||||||||||||||||||||||||
Sbjct  452  CTCACACCTTTATTACTGTGCCGGAAAGTGCGCAAAGCC  490
```

4-Alignment of DHA-4 with *E. coli* strain EF2 DHA family class C beta-lactamase (*bla*$_{DHA}$) gene, partial cds Sequence ID: OQ096360.1

Score	Expect	Identities	Gaps	Strand
636 bits(344)	2e-179	344/344(100%)	0/344(0%)	Plus/Plus

```
Query  1    AATGATCCGGCGGCAAAATACCAGCCGGAGCTGGCTCTGCCGCAGTGGAAGGGGATCACA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  154  AATGATCCGGCGGCAAAATACCAGCCGGAGCTGGCTCTGCCGCAGTGGAAGGGGATCACA  213

Query  61   TTGCTGGATCTGGCTACCTATACCGCAGGCGGACTGCCGTTACAGGTGCCGGATGCGGTA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  214  TTGCTGGATCTGGCTACCTATACCGCAGGCGGACTGCCGTTACAGGTGCCGGATGCGGTA  273

Query  121  AAAAGCCGTGCGGATCTGCTGAATTTCTATCAGCAGTGGCAGCCGTCCCGGAAACCGGGC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  274  AAAAGCCGTGCGGATCTGCTGAATTTCTATCAGCAGTGGCAGCCGTCCCGGAAACCGGGC  333

Query  181  GATATGCGTCTGTATGCAAACAGCAGTATCGGCCTGTTTGGTGCTCTGACCGCAAACGCG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  334  GATATGCGTCTGTATGCAAACAGCAGTATCGGCCTGTTTGGTGCTCTGACCGCAAACGCG  393

Query  241  GCGGGGATGCCGTATGAGCAGTTGCTGACTGCACGGATCCTGGCACCGCTGGGGTTATCT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  394  GCGGGGATGCCGTATGAGCAGTTGCTGACTGCACGGATCCTGGCACCGCTGGGGTTATCT  453

Query  301  CACACCTTTATTACTGTGCCGGAAAGTGCGCAAAGCCAGTATGC  344
            |||||||||||||||||||||||||||||||||||||||||||
Sbjct  454  CACACCTTTATTACTGTGCCGGAAAGTGCGCAAAGCCAGTATGC  497
```

5-Alignment of DHA-5 with *E.coli* strain EF2 DHA family class C beta-lactamase (*bla*$_{DHA}$) gene, partial cds Sequence ID: OQ096360.1

Score	Expect	Identities	Gaps	Strand
640 bits(346)	1e-180	346/346(100%)	0/346(0%)	Plus/Plus

```
Query  1    ATCCGGCGGCAAAATACCAGCCGGAGCTGGCTCTGCCGCAGTGGAAGGGGATCACATTGC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  158  ATCCGGCGGCAAAATACCAGCCGGAGCTGGCTCTGCCGCAGTGGAAGGGGATCACATTGC  217

Query  61   TGGATCTGGCTACCTATACCGCAGGCGGACTGCCGTTACAGGTGCCGGATGCGGTAAAAA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  218  TGGATCTGGCTACCTATACCGCAGGCGGACTGCCGTTACAGGTGCCGGATGCGGTAAAAA  277

Query  121  GCCGTGCGGATCTGCTGAATTTCTATCAGCAGTGGCAGCCGTCCCGGAAACCGGGCGATA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  278  GCCGTGCGGATCTGCTGAATTTCTATCAGCAGTGGCAGCCGTCCCGGAAACCGGGCGATA  337

Query  181  TGCGTCTGTATGCAAACAGCAGTATCGGCCTGTTTGGTGCTCTGACCGCAAACGCGGCGG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  338  TGCGTCTGTATGCAAACAGCAGTATCGGCCTGTTTGGTGCTCTGACCGCAAACGCGGCGG  397

Query  241  GGATGCCGTATGAGCAGTTGCTGACTGCACGGATCCTGGCACCGCTGGGGTTATCTCACA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  398  GGATGCCGTATGAGCAGTTGCTGACTGCACGGATCCTGGCACCGCTGGGGTTATCTCACA  457

Query  301  CCTTTATTACTGTGCCGGAAAGTGCGCAAAGCCAGTATGCGTACGG  346
            |||||||||||||||||||||||||||||||||||||||||||||
Sbjct  458  CCTTTATTACTGTGCCGGAAAGTGCGCAAAGCCAGTATGCGTACGG  503
```

6-Alignment of DHA-6 with *E. coli* strain EF2 DHA family class C beta-lactamase (bla_{DHA}) gene, partial cds Sequence ID: OQ096360.1

Score	Expect	Identities	Gaps	Strand
636 bits(344)	2e-179	344/344(100%)	0/344(0%)	Plus/Plus

```
Query  1    CCGGCGGCAAAATACCAGCCGGAGCTGGCTCTGCCGCAGTGGAAGGGGATCACATTGCTG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  160  CCGGCGGCAAAATACCAGCCGGAGCTGGCTCTGCCGCAGTGGAAGGGGATCACATTGCTG  219

Query  61   GATCTGGCTACCTATACCGCAGGCGGACTGCCGTTACAGGTGCCGGATGCGGTAAAAAGC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  220  GATCTGGCTACCTATACCGCAGGCGGACTGCCGTTACAGGTGCCGGATGCGGTAAAAAGC  279

Query  121  CGTGCGGATCTGCTGAATTTCTATCAGCAGTGGCAGCCGTCCCGGAAACCGGGCGATATG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  280  CGTGCGGATCTGCTGAATTTCTATCAGCAGTGGCAGCCGTCCCGGAAACCGGGCGATATG  339

Query  181  CGTCTGTATGCAAACAGCAGTATCGGCCTGTTTGGTGCTCTGACCGCAAACGCGGCGGGG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  340  CGTCTGTATGCAAACAGCAGTATCGGCCTGTTTGGTGCTCTGACCGCAAACGCGGCGGGG  399

Query  241  ATGCCGTATGAGCAGTTGCTGACTGCACGGATCCTGGCACCGCTGGGGTTATCTCACACC  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  400  ATGCCGTATGAGCAGTTGCTGACTGCACGGATCCTGGCACCGCTGGGGTTATCTCACACC  459

Query  301  TTTATTACTGTGCCGGAAAGTGCGCAAAGCCAGTATGCGTACGG  344
            |||||||||||||||||||||||||||||||||||||||||||
Sbjct  460  TTTATTACTGTGCCGGAAAGTGCGCAAAGCCAGTATGCGTACGG  503
```

7- Alignment of CIT-1 with *Escherichia coli* class C beta-lactamase bla_{CMY-42} (bla_{CMY-42}) gene, complete cds Sequence ID: MF577055.1

Score	Expect	Identities	Gaps	Strand
769 bits(416)	0.0	416/416(100%)	0/416(0%)	Plus/Plus

```
Query  1    CCGCCTGCTGCACTTAGCCACCTATACGGCAGGCGGCCTACCGCTGCAGATCCCCGATGA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  372  CCGCCTGCTGCACTTAGCCACCTATACGGCAGGCGGCCTACCGCTGCAGATCCCCGATGA  431

Query  61   CGTTAGGGATAAAGCCGCATTACTGCATTTTTATCAAAACTGGCAGCCGCAATGGACTCC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  432  CGTTAGGGATAAAGCCGCATTACTGCATTTTTATCAAAACTGGCAGCCGCAATGGACTCC  491

Query  121  GGGCGCTAAGCGACTTTACGCTAACTCCAGCATTGGTCTGTTTGGCGCGCTGGCGGTGAA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  492  GGGCGCTAAGCGACTTTACGCTAACTCCAGCATTGGTCTGTTTGGCGCGCTGGCGGTGAA  551

Query  181  ACCCTCAGGAATGAGTTACGAAGAGGCAATGACCAGACGCGTCCTGCAACCATTAAAACT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  552  ACCCTCAGGAATGAGTTACGAAGAGGCAATGACCAGACGCGTCCTGCAACCATTAAAACT  611

Query  241  GGCGCATACCTGGATTACGGTTCCGCAGAACGAACAAAAAGATTATGCCTGGGGCTATCG  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  612  GGCGCATACCTGGATTACGGTTCCGCAGAACGAACAAAAAGATTATGCCTGGGGCTATCG  671

Query  301  CGAAGGGAAGCCCGTACACAGTTCTCCGGGACAACTTGACGCCGAAGCCTATGGCGTGAA  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  672  CGAAGGGAAGCCCGTACACAGTTCTCCGGGACAACTTGACGCCGAAGCCTATGGCGTGAA  731

Query  361  ATCCAGCGTTATTGATATGGCCCGCTGGGTTCAGGCCAACATGGATGCCAGCCACG  416
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  732  ATCCAGCGTTATTGATATGGCCCGCTGGGTTCAGGCCAACATGGATGCCAGCCACG  787
```

8-Alignment of CIT-2 with *E.coli* 1284128 *bla*CMY gene for class C beta-lactamase $bla_{\text{CMY-141}}$, complete CDS Sequence ID: NG_051215.1

Length: 1146Number of Matches: 1Score	Expect	Identities	Gaps	Strand
769 bits(416)	0.0	416/416(100%)	0/416(0%)	Plus/Plus

```
Query  4    CCGCCTGCTGCACTTAGCCACCTATACGGCAGGCGGCCTACCGCTGCAGCTCCCCGATGA  63
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  372  CCGCCTGCTGCACTTAGCCACCTATACGGCAGGCGGCCTACCGCTGCAGCTCCCCGATGA  431

Query  64   CGTTAGGGATAAAGCCGCATTACTGCATTTTTATCAAAACTGGCAGCCGCAATGGACTCC  123
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  432  CGTTAGGGATAAAGCCGCATTACTGCATTTTTATCAAAACTGGCAGCCGCAATGGACTCC  491

Query  124  GGGCGCTAAGCGACTTTACGCTAACTCCAGCATTGGTCTGTTTGGCGCGCTGGCGGTGAA  183
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  492  GGGCGCTAAGCGACTTTACGCTAACTCCAGCATTGGTCTGTTTGGCGCGCTGGCGGTGAA  551

Query  184  ACCCTCAGGAATGAGTTACGAAGAGGCAATGACCAGACGCGTCCTGCAACCATTAAAACT  243
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  552  ACCCTCAGGAATGAGTTACGAAGAGGCAATGACCAGACGCGTCCTGCAACCATTAAAACT  611

Query  244  GGCGCATACCTGGATTACGGTTCCGCAGAACGAACAAAAAGATTATGCCTGGGGCTATCG  303
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  612  GGCGCATACCTGGATTACGGTTCCGCAGAACGAACAAAAAGATTATGCCTGGGGCTATCG  671

Query  304  CGAAGGGAAGCCCGTACACAGTTCTCCGGGACAACTTGACGCCGAAGCCTATGGCGTGAA  363
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  672  CGAAGGGAAGCCCGTACACAGTTCTCCGGGACAACTTGACGCCGAAGCCTATGGCGTGAA  731

Query  364  ATCCAGCGTTATTGATATGGCCCGCTGGGTTCAGGCCAACATGGATGCCAGCCACG  419
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  732  ATCCAGCGTTATTGATATGGCCCGCTGGGTTCAGGCCAACATGGATGCCAGCCACG  787
```

9-Alignment of CIT-3 with *E. coli* strain 91 plasmid p91_*bla*$_{CMY-42}$, complete sequence Sequence ID: MN007140.1

Score	Expect	Identities	Gaps	Strand
780 bits(422)	0.0	422/422(100%)	0/422(0%)	Plus/Minus

```
Query  1      AGGGTATCCGCCTGCTGCACTTAGCCACCTATACGGCAGGCGGCCTACCGCTGCAGATCC
60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  41272  AGGGTATCCGCCTGCTGCACTTAGCCACCTATACGGCAGGCGGCCTACCGCTGCAGATCC
41213

Query  61     CCGATGACGTTAGGGATAAAGCCGCATTACTGCATTTTTATCAAAACTGGCAGCCGCAAT
120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  41212  CCGATGACGTTAGGGATAAAGCCGCATTACTGCATTTTTATCAAAACTGGCAGCCGCAAT
41153

Query  121    GGACTCCGGGCGCTAAGCGACTTTACGCTAACTCCAGCATTGGTCTGTTTGGCGCGCTGG
180
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  41152  GGACTCCGGGCGCTAAGCGACTTTACGCTAACTCCAGCATTGGTCTGTTTGGCGCGCTGG
41093

Query  181    CGGTGAAACCCTCAGGAATGAGTTACGAAGAGGCAATGACCAGACGCGTCCTGCAACCAT
240
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  41092  CGGTGAAACCCTCAGGAATGAGTTACGAAGAGGCAATGACCAGACGCGTCCTGCAACCAT
41033

Query  241    TAAAACTGGCGCATACCTGGATTACGGTTCCGCAGAACGAACAAAAAGATTATGCCTGGG
300
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  41032  TAAAACTGGCGCATACCTGGATTACGGTTCCGCAGAACGAACAAAAAGATTATGCCTGGG
40973

Query  301    GCTATCGCGAAGGGAAGCCCGTACACAGTTCTCCGGGACAACTTGACGCCGAAGCCTATG
360
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  40972  GCTATCGCGAAGGGAAGCCCGTACACAGTTCTCCGGGACAACTTGACGCCGAAGCCTATG
40913

Query  361    GCGTGAAATCCAGCGTTATTGATATGGCCCGCTGGGTTCAGGCCAACATGGATGCCAGCC
420
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  40912  GCGTGAAATCCAGCGTTATTGATATGGCCCGCTGGGTTCAGGCCAACATGGATGCCAGCC
40853

Query  421    AC  422
              ||
Sbjct  40852  AC  40851
```

10- Alignment of CIT-4 with Escherichia coli strain EK404 class C beta-lactamase *bla*$_{\text{CMY-42}}$ (*bla*$_{\text{CMY}}$) gene, *bla*$_{\text{CMY-42}}$ allele, complete cds Sequence ID: MG701323.1

Score	Expect	Identities	Gaps	Strand
654 bits(354)	0.0	354/354(100%)	0/354(0%)	Plus/Plus

```
Query  1    ATGACGTTAGGGATAAAGCCGCATTACTGCATTTTTATCAAAACTGGCAGCCGCAATGGA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  428  ATGACGTTAGGGATAAAGCCGCATTACTGCATTTTTATCAAAACTGGCAGCCGCAATGGA  487

Query  61   CTCCGGGCGCTAAGCGACTTTACGCTAACTCCAGCATTGGTCTGTTTGGCGCGCTGGCGG  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  488  CTCCGGGCGCTAAGCGACTTTACGCTAACTCCAGCATTGGTCTGTTTGGCGCGCTGGCGG  547

Query  121  TGAAACCCTCAGGAATGAGTTACGAAGAGGCAATGACCAGACGCGTCCTGCAACCATTAA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  548  TGAAACCCTCAGGAATGAGTTACGAAGAGGCAATGACCAGACGCGTCCTGCAACCATTAA  607

Query  181  AACTGGCGCATACCTGGATTACGGTTCCGCAGAACGAACAAAAAGATTATGCCTGGGGCT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  608  AACTGGCGCATACCTGGATTACGGTTCCGCAGAACGAACAAAAAGATTATGCCTGGGGCT  667

Query  241  ATCGCGAAGGGAAGCCCGTACACAGTTCTCCGGGACAACTTGACGCCGAAGCCTATGGCG  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  668  ATCGCGAAGGGAAGCCCGTACACAGTTCTCCGGGACAACTTGACGCCGAAGCCTATGGCG  727

Query  301  TGAAATCCAGCGTTATTGATATGGCCCGCTGGGTTCAGGCCAACATGGATGCCA  354
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  728  TGAAATCCAGCGTTATTGATATGGCCCGCTGGGTTCAGGCCAACATGGATGCCA  781
```

11-Alignment of CIT-5 with Escherichia coli strain EK404 class C beta-lactamase *bla*$_{\text{CMY-42}}$ (*bla*$_{\text{CMY}}$) gene, blaCMY-42 allele, complete cds Sequence ID: MG701323.1

Score	Expect	Identities	Gaps	Strand
699 bits(378)	0.0	378/378(100%)	0/378(0%)	Plus/Plus

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Query  1    GCGGCCTACCGCTGCAGATCCCCGATGACGTTAGGGATAAAGCCGCATTACTGCATTTTT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  404  GCGGCCTACCGCTGCAGATCCCCGATGACGTTAGGGATAAAGCCGCATTACTGCATTTTT  463

Query  61   ATCAAAACTGGCAGCCGCAATGGACTCCGGGCGCTAAGCGACTTTACGCTAACTCCAGCA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  464  ATCAAAACTGGCAGCCGCAATGGACTCCGGGCGCTAAGCGACTTTACGCTAACTCCAGCA  523

Query  121  TTGGTCTGTTTGGCGCGCTGGCGGTGAAACCCTCAGGAATGAGTTACGAAGAGGCAATGA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  524  TTGGTCTGTTTGGCGCGCTGGCGGTGAAACCCTCAGGAATGAGTTACGAAGAGGCAATGA  583

Query  181  CCAGACGCGTCCTGCAACCATTAAAACTGGCGCATACCTGGATTACGGTTCCGCAGAACG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  584  CCAGACGCGTCCTGCAACCATTAAAACTGGCGCATACCTGGATTACGGTTCCGCAGAACG  643

Query  241  AACAAAAAGATTATGCCTGGGGCTATCGCGAAGGGAAGCCCGTACACAGTTCTCCGGGAC  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  644  AACAAAAAGATTATGCCTGGGGCTATCGCGAAGGGAAGCCCGTACACAGTTCTCCGGGAC  703

Query  301  AACTTGACGCCGAAGCCTATGGCGTGAAATCCAGCGTTATTGATATGGCCCGCTGGGTTC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  704  AACTTGACGCCGAAGCCTATGGCGTGAAATCCAGCGTTATTGATATGGCCCGCTGGGTTC  763

Query  361  AGGCCAACATGGATGCCA  378
            ||||||||||||||||||
Sbjct  764  AGGCCAACATGGATGCCA  781
```

12-Alignment of CIT-6 with Escherichia coli strain EK404 class C beta-lactamase CMY-42 (*bla*$_{CMY}$) gene, *bla*$_{CMY-42}$ allele, complete cds Sequence ID: MG701323.1

Score	Expect	Identities	Gaps	Strand
654 bits(354)	0.0	354/354(100%)	0/354(0%)	Plus/Plus

```
Query  1    ATCCCCGATGACGTTAGGGATAAAGCCGCATTACTGCATTTTTATCAAAACTGGCAGCCG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  ATCCCCGATGACGTTAGGGATAAAGCCGCATTACTGCATTTTTATCAAAACTGGCAGCCG  480

Query  61   CAATGGACTCCGGGCGCTAAGCGACTTTACGCTAACTCCAGCATTGGTCTGTTTGGCGCG  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  481  CAATGGACTCCGGGCGCTAAGCGACTTTACGCTAACTCCAGCATTGGTCTGTTTGGCGCG  540

Query  121  CTGGCGGTGAAACCCTCAGGAATGAGTTACGAAGAGGCAATGACCAGACGCGTCCTGCAA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  541  CTGGCGGTGAAACCCTCAGGAATGAGTTACGAAGAGGCAATGACCAGACGCGTCCTGCAA  600

Query  181  CCATTAAAACTGGCGCATACCTGGATTACGGTTCCGCAGAACGAACAAAAAGATTATGCC  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  601  CCATTAAAACTGGCGCATACCTGGATTACGGTTCCGCAGAACGAACAAAAAGATTATGCC  660

Query  241  TGGGGCTATCGCGAAGGGAAGCCCGTACACAGTTCTCCGGGACAACTTGACGCCGAAGCC  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  661  TGGGGCTATCGCGAAGGGAAGCCCGTACACAGTTCTCCGGGACAACTTGACGCCGAAGCC  720

Query  301  TATGGCGTGAAATCCAGCGTTATTGATATGGCCCGCTGGGTTCAGGCCAACATG  354
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  721  TATGGCGTGAAATCCAGCGTTATTGATATGGCCCGCTGGGTTCAGGCCAACATG  774
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Conclusions and Recommendations

Conclusions

1-The current study disclosed high prevalence of ESBL followed by AmpC genes among β- lactamase producing *E. coli* isolates from different sources.

2- AmpC - producing *E. coli* isolates showed resistance to large numbers of antibiotics compared with ESBL-producing isolates

3-The analysis of the antibiotic resistance of β- lactamase *E. coli* isolates showed high level of resistance to ampicillin, amoxicillin, piperacillin, amoxicillin- clavulanate, cefazoline, aztreonem, and quinolones.

4- β- lactamase *E.coli* isolates showed high susceptibility to tigycycline, amikacin, isepamicin, and carpapenems.

5- All β-lactamase producing *E. coli* isolates showed MDR pattern of resistance.

6- High prevalence of bla_{CMY}, bla_{DHA} and bla_{CIT} genes was seen among AmpC- producing isolates while bla_{OXA}, bla_{SHV} and $bla_{CTX-M,}$ and bla_{TEM} genes were detected among ESBL- producing isolates.

7- High prevalence of *ACC(6)Ib-cr* gene in aminoglycoside resistance isolates and *qnr A* and *qnr B* genes in quinolon resistance isolates. However *E.coli* isolates carrying both *ACC(6)Ib-cr* and *qnr* genes were highly resistant to antibiotics when compared with other isolates, these isolates were resistant to at least 13 antibiotics.

8- High prevalence of *Int1* gene was detected among β- lactamase-producing *E. coli* isolates.

9- All Carbapeneme resistant *E. coli* isolates were sensitive to colistin.

Recommendations

1- Findings of this study suggested that systems for antibiotic susceptibility surveillance and the detection of molecular analysis of AmpC and ESBL-producing bacteria in Iraqi hospitals must be established as a part of assignments for the Ministry of health in Iraq.

2- Extensive study for *E.coli* isolates from vaginal exudate and DFU including AmpC and ESBL-producing isolates.

3- Comparison study to antibiotic resistance and prevalence of AmpC genes among enterobacteriaceae.

4-Study of the carbapenems and colistin resistance among enterobacteriaceae species especially colistin resistant in carbapenems resistant isolates.

5- Molecular study of the bacterial isolates that carry both *ACC(6)Ib-cr* and quinolone resistant gene (*qnr*) and their association with antibiotic resistance.

6- Extensive study of the bacterial isolates that carry both AmpC and ESBL and their association with antibiotic resistance.

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Appendices

AST report of *E.coli* isolates with VITEK 2 system

bioMérieux Customer: Microbiology Chart Report Printed July 31, 2022 12:08:57 PM CDT

Patient Name: 16, m j
Location:
Lab ID: 24720221

Patient ID: 24720221
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Escherichia coli

Source: **Collected:**

Comments:	

Susceptibility Information	**Analysis Time:** 10.93 hours	**Status:** Final

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
ESBL	POS	+	Imipenem	<= 0.25	S
+Amoxicillin		R	+Meropenem		S
Ampicillin	>= 32	R	+Panipenem		
+Piperacillin/Sulbactam			Amikacin	<= 2	S
Piperacillin/Tazobactam	64	I	Gentamicin	<= 1	S
Cefazolin	>= 64	R	+Isepamicin		S
+Cefotetan			+Tobramycin		S
Cefoxitin	<= 4	S	Ciprofloxacin	>= 4	R
+Cefoperazone			Levofloxacin	>= 8	R
Ceftazidime	>= 64	R	+Sparfloxacin		R
+Ceftizoxime			Tigecycline	<= 0.5	S
Ceftriaxone	>= 64	R	Nitrofurantoin	<= 16	S
Cefepime	>= 64	R	Trimethoprim/ Sulfamethoxazole	<= 20	S
Ertapenem	<= 0.5	S			

AES Findings	
Confidence:	Consistent

A

bioMérieux Customer:

Microbiology Chart Report

Printed July 31, 2022 12:08:58 PM CDT

Patient Name: 18, m j
Location:
Lab ID: 24720222

Patient ID: 24720222
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Escherichia coli

Source:

Collected:

Comments:	

Susceptibility Information	**Analysis Time:** 10.92 hours	**Status:** Final

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
ESBL	POS	+	Ertapenem	<= 0.5	S
+Amoxicillin		R	Imipenem	<= 0.25	S
Ampicillin	>= 32	R	+Meropenem		S
+Piperacillin/Sulbactam			+Panipenem		
Piperacillin/Tazobactam	<= 4	S	Amikacin	<= 2	S
Cefazolin	>= 64	R	Gentamicin	<= 1	S
+Cefotetan			+Isepamicin		S
Cefoxitin	<= 4	S	+Tobramycin		S
+Cefoperazone			Ciprofloxacin	<= 0.25	S
Ceftazidime	4	S	Levofloxacin	<= 0.12	S
+Ceftizoxime			Tigecycline	<= 0.5	S
Ceftriaxone	>= 64	R	Nitrofurantoin	32	S
Cefepime	2	S	Trimethoprim/ Sulfamethoxazole	<= 20	S

AES Findings	
Confidence:	Consistent

B

bioMérieux Customer:

Microbiology Chart Report

Printed July 31, 2022 12:08:59 PM CDT

Patient Name: 19, m j
Location:
Lab ID: 24720223

Patient ID: 24720223
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Escherichia coli

Source:

Collected:

Comments:	

Susceptibility Information	**Analysis Time:** 7.25 hours	**Status:** Final

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
ESBL	POS	+	Imipenem	<= 0.25	S
+Amoxicillin		R	+Meropenem		S
Ampicillin	>= 32	R	+Panipenem		
+Piperacillin/Sulbactam			Amikacin	<= 2	S
Piperacillin/Tazobactam	<= 4	S	Gentamicin	>= 16	R
Cefazolin	>= 64	R	+Isepamicin		S
+Cefotetan			+Tobramycin		
Cefoxitin	8	S	Ciprofloxacin	>= 4	R
+Cefoperazone			Levofloxacin	>= 8	R
Ceftazidime	16	R	+Sparfloxacin		R
+Ceftizoxime			Tigecycline	<= 0.5	S
Ceftriaxone	>= 64	R	Nitrofurantoin	<= 16	S
Cefepime	8	S	Trimethoprim/ Sulfamethoxazole	>= 320	R
Ertapenem	<= 0.5	S			

AES Findings	
Confidence:	Consistent

C

الخلاصه

من المعروف عالميا أن الإشريشيا القولونية هي بكتريا تعايشية مع البشر وكذلك في العديد من أنواع الحيوانات ، في الجهاز الهضمي البشري تفتقر إلى الضراوة. ومع ذلك ، عندما توجد خارج الأمعاء ، يمكن أن تسبب الإشريشيا القولونية عدة أمراض.

ظهرت مقاومة مضادات الميكروبات (AMR) كواحدة من مشاكل الصحة العامة الرئيسية في القرن الحادي والعشرين. تم اكتشاف إنزيمات الطيف الممتد (ESBLs) وإنزيمات AmpC في جميع أنواع العائلة المعويه تقريبًا.

تم جمع ١٨٧٤ عينه سريريه من اربع مستشفيات رئيسيه في مدينة الحله وهي مستشفى مرجان التعليمي ومستشفى الحله التعليمي العام ومستشفى بابل التعليمي للولاده والاطفال ومستشفى الامام الصادق (ع) للفتره من شباط الى نهاية حزيران ٢٠٢٢

خلال الدراسة الحالية ، تم عزل الاشريشيا القولونية من عينات سريرية مختلفة والتي تتضمن البول وإفرازات الحروق والإفرازات المهبلية والبلغم وقرحة القدم السكرية والبراز والسائل الدماغي الشوكي والدم ومسحة الأذن عن طريق الزراعة على MacConkey agar، Eosin Methylene blue agar بالإضافة إلى العديد من الاختبارات الكيميائية الحيوية لغرض تشخيص البكتريا.

تم الكشف عن قدرة إنتاج البيتالاكتميز لجميع عزلات الإشريشيا القولونية وذلك باستخدام النيتروسيفين ، وتم إجراء حساسية المضادات الحيوية لعزلات الاشريشيا المنتجة للبتالاكتميز وفقا ل CLSI، ٢٠٢٣ باستخدام جهاز الفايتك ٢ وطريقة انتشار الاقراص.

استخدم تفاعل البلمرة المتسلسل (PCR) للكشف الجيني عن جينات البلازميد, *pampC* وجينات ESBL وجينات الكاربابنيمز وجينات مقاومة الكوينولون المحموله على البلازميد (PMQR) وجينات مقاومة للامينوكلايكوسيد وجينات مقاومة الكوليستيين و integrin class 1 وأخيرا تم إجراء تسلسل الحمض النووي لبعض الجينات العائدة *pampC*.

وجدت النتائج أنه من بين ١٨٧٤ عينة سريرية ، كانت ٢٣١ عزلة للاشريشيا القولونية، ومنها ١١٢ (٤٨.٥٪) منتجه للبيتالاكتميز. كان معدل عزل الإشريشيا القولونية غالبا من البراز

٣٨(٦١.٤٪) يليه البول ١١٨(١٨.٤٪). فيما يتعلق بجنس المرضى ، تم جمع عزلات الإشريشيا القولونية من البول في الغالب من المرضى الإناث ٧٧(٦٥.٣٪)، في حين الحروق وقرحة القدم السكري في الغالب من الذكور ١٩ (٦٣.٣٪) و ٩(٦٩.٣٪) على التوالي.

بالنسبه لفحص المضادات الحيويه AST تم فحص الإشريشيا القولونية ل ٣٠ نوعا من المضادات الحيوية تنتمي إلى ١٥ فئة، ١٤ نوعا من البيتالاكتام و ١٦ نوعا من غير البيتالاكتام، وكشفت النتائج أن جميع العزلات كانت مقاومة للأمبيسيلين، أموكسيسيلين، أموكسيسيلين/كلافولونات، وبيبيراسيلين ١١١(٩٩.١٪). فيما يتعلق بالعينات السريرية ، كانت الاشريشيا القولونية المعزولة من البول عالية المقاومة للسيفازولين ٥٣(٩٤.٦٪) والسيفترياكسون ٥٢(٩٢.٨٪) والأستريونيم ٤٢(٧٥٪) وحمض الناليديكسيك ٥٠(٨٩.٣٪). وكانت الاشريشيا القولونية المعزولة من الإفرازات المهبلية عالية المقاومة للسيفازولي١٧ (١٠٠٪) ، والأستريونيم ١٧(١٠٠٪) وسيبروفلوكساسين ١٧(١٠٠٪) والليفوفلوكساسين ١٧(١٠٠٪) وسبارفلوكساسين ١٧(١٠٠٪) وحمض الناليديكسيك ١٧(١٠٠٪) وكانت الاشريشيا القولونية المعزولة من قرحة القدم السكري عالية المقاومة للسيفازولين (١٠٠٪) و سيفترياكسون ٧(١٠٠٪) و أزتريونيم ٧(١٠٠٪) و سيبروفلوكساسين ٦(٨٥.٧) وليفوفلوكساسين ٦(٨٥.٧٪) و سبارفلوكساسين ٦(٨٥.٧٪) ونورفلوكساسين ٦(٨٥.٧٪)

على الرغم من ذلك ، كانت عزلات الاشريشية القولونيه حساسة للميروبينيم ١١٢(١٠٠٪) وتيجيسيكلين ١١٢(١٠٠٪) وإرتابينيم ١٠٨(٩٦.٥٪) وإمبينيم ١٠٧(٩٥.٥٪) وأميكاسين ١٠٧(٩٢٪) وإيسيباميسين ١١٠(٩٨.٢). جميع العزلات اظهرت نمط MDR لمقاومة الاشريشيا القولونية. من بين ١١٢ عزلة إشريكية قولونية منتجه للبتالاكتميز ٧٤(٦٦٪) كانت ESBL ٤(٣,٥٪) مقاومة للكاربابينيم و٣٧(٣٣٪) كانت مقاومة للامينوكلايكوساید و٧٣(٦٥.١٪) كانت مقاومه للكوينولون ٢٧(٢٤.١٪) كانت مقاومة للكوليستين.

كشفت نتائج تفاعل البوليمر المتسلسل لجينات ESBL عن ارتفاع معدل انتشار bla_{CTX-M} (١٠٠٪) ، بين عزلات الاشريشيه القولونيه المنتجة ل ESBL ، تليها bla_{SHV} (٨٧.٨٪) ، bla_{OXA} (٨٥.١٪) و bla_{TEM} (٧٩.٧٪).

كانت نتائج تفاعل البوليمر المتسلسل لجينات الكاربابينيماز هي bla_{OXA} (١٠٠٪) و bla_{NDM} (٧٥٪) ، وجينات مقاومة الكوينولون بوساطة البلازميد *qnr A* (٣٥.٥٪) و *qnr B* (٣٢.٢٪)، وجين أمينوكليكوسيدات *ACC(6)Ib-cr* (١٠٠٪) ، وجينات مقاومة الكوليستين *mcr 2* (٥٥.٥٪) و *mcr 3* (٢٢.٢٪) ، وأخيرا تم اكتشاف *Int1* في (٩٩.١٪).

فيما يتعلق بجينات pampC ، أظهرت نتائج تفاعل البوليمر المتسلسل انتشارا مرتفعا لجينات bla_{CMY} (٨٨.٥٪) تليها bla_{CIT} (٥٧.٧٪) و bla_{DHA} (٤٢.٣٪) ،الجينات bla_{FOX} ، bla_{MOX} ، bla_{EBC} ، bla_{ACC} لم يعثر عليها في الدراسة الحالية.

من بين ٢٦ عزلة اشريشيه قولونيه منتجة ل AmpC ،هنالك عزله واحده (٣.٨٥٪) تحمل ٣ أنواع من جينات *pampC* ، و ١٩عزله (٧٣٪) تحمل نوعين من *pampC* ، و ٥ عزلات (١٩.٣٪) تحمل جين واحدة وعزله واحده (٣.٨٥٪) لا تحمل أي واحد من جينات الدراسة.

تم الكشف عن تسلسل الحمض النووي bla_{DHA} و bla_{CIT} ، وكلها متوافقة مع bla_{DHA} عند مقارنتها ب NCBI بنقاوة ١٠٠٪ ، bla_{CIT} مشابهة جدا bla_{CMY-42}.

من هذه الدراسه، يمكن ان نستنتج ان ESBL كانت اكثر انتشارا من AmpC في الاشريشيه القولونيه المنتجه للبيتالاكتميز لكن Amp C كانت اكثر مقاومة للمضادات الحيويه عند مقارنتها ب ESBL ، جميع عزلات الاشريشيه القولونيه كانت مقاومه للامبسيلين والاموكسيسلين و الاموكسيسلين / الكلافولونات ، في حين انها حساسه للاميكاسين و امبينيم وميرونيم و التيكيسايكلين. تم الكشف عن ارتفاع معدل انتشار جينات ESBL وجينات *pampC* مثل جين bla_{CMY} وجين *ACC(6) Ib-cr* وجين *Int1* بين عزلات الاشريشيا القولونيه MDR.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة بابل
كلية الطب

تواجد وانتشار جينات البيتالاكتاميز *ampC* بين العزلات السريريه لبكتريا *Echerichia coli* متعددة المقاومة للمضادات الحياتيه في محافظة بابل

أطروحة

مقدمة الى مجلس كلية الطب - جامعة بابل

وهي جزء من متطلبات نيل درجة دكتوراه فلسفة في

العلوم/ الاحياء المجهرية الطبية

من قبل

محمد جاسم خليف الطائي

بكالوريوس علوم حياة جامعة بابل ٢٠٠٦

ماجستير احياء مجهرية طبية – جامعة بابل ٢٠١٤

بأشراف

أ.د . علاء هاني الجراخ

٢٠٢٣م ١٤٤٤هـ