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Molecular Detection of Plasmid Mediated Amp C (pAmpC) β- lactamases and Associated Antibiotics Resistance Genes among *E.coli* Isolates in Iraq

Mohammed Jasim Altaee
Dept. of Microbiology/ College of Medicine / University of Babylon, Hilla, Iraq
E-mail: mohammedjasim293@gmail.com
Alaa H. Al-Charrakh*
Dept. of Microbiology/ College of Medicine / University of Babylon, Hilla, Iraq
email: ahani67@gmail.com
ORCID: http://orcid.org/0000-0002-6533-6395
*Corresponding author

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* **Corresponding author:**
Prof. Dr. Alaa H. Al-Charrakh
Department of Microbiology,
College of Medicine/ University of Babylon,
Hilla /Iraq
Email: ahani67@gmail.com
ORCID: http://orcid.org/0000-0002-6533-6395
Telephone: 00-964-7816658072

Abstract:

Background and Objectives: *Escherichia coli* is well known to be a universal commensal flora in humans as well as in several animal species. Antimicrobial resistance (AMR) has emerged as one of the principal public health problems of the 21st century. This study was undertaken for detection of the antibiotic resistance and Amp C β- lactamase genes for E.coli isolates. **Methods:** Different clinical samples (urine, diabetic food ulcer, vaginal discharge, burn exudate, stool, sputum, blood, ear swab and CSF) were collected from patients from February to June 2022. Out of 1874 clinical samples, 231 were belonged to E. coli isolates,112 (48.5%) were produce β-lactamase, E.coli were examined for list of antibiotics. **Results:** All isolates resistance to ampicillin, amoxicillin and amoxicillin/clavulonate, resistance to piperacillin (99.1%), E.coli sensitive for meropenem (100%), etrapenem (96.5%), impenem (95.5%), amikacin (92%) and isepamicin (98.2), According to pAmp C genes, show high prevalence BlCMY (88.5%), BlCIT (57.7%) and BlDHA(42.3%), BlFOX, BlMOX, BlEBC, BlACC were not detected in present study, From 26 pAmp C producing isolates, 1(3.85%) isolates carry 3 types of pAmp C genes, 19(73%) isolates carry 2 types of pAmp C, 5(19.3%) isolates carry 1 isolates

and 1 (3.85%) isolate do not carry any of the studied genes**. Conclusions:** E. coli isolated from various clinical specimens showed differences in antibiotic sensitivity patterns, with high resistance to commonly used antibiotics, high prevalence of BlCMY, BlCIT and BlDHA genes in E.coli isolates and class 1 integron leading cause for E.coli resistance.

Keywords: pAmpC β- lactamases, E.coli, Antibiotics Resistant Genes, Detection.

Introduction

Escherichia coli is well known to be a universal commensal flora in humans as well as in several animal species but is also reported to be one of the most common enterobacterial species which causes extra-intestinal infections in these hosts (1)**.** *E. coli* is the most common bacteria in the human gastrointestinal tract and lacks virulence in this setting. However, when found outside of the intestinal tract, *E. coli* can cause urinary tract infections (the most common pathogen leading to uncomplicated cystitis), pneumonia, bacteremia, and spontaneous bacterial peritonitis.(2,3). Enteropathogenic Escherichia coli (EPEC) has been a major cause of infant diarrhea in developing countries during the 20th century(4).

Antimicrobial resistance (AMR) has emerged as one of the principal public health problems of the 21st century that threatens the effective prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses and fungi no longer susceptible to the common medicines used to treat them. The problem of AMR is especially urgent regarding antibiotic resistance in bacteria.(5). In Gram-negative bacteria, AmpC beta-lactamase production is chromosome or plasmid mediated. Chromosomal ampC genes are expressed constitutively at a low level. Some Entero-bacteriaceae, such as Enterobacter spp., Citrobacter spp., and Serratia spp., carry an inducible ampC gene. In these cases, the gene is strongly induced by -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 (6). The regulation of chromosomal ampC expression in Escherichia coli differs considerably from that in other Enterobacteriaceae. E. coli lacks ampR, and thus ampC expression is not inducible (7). In E. coli, ampC is expressed constitutively at a low level (8). Various mutations in the Amp C promoter/attenuator region of E. coli have been identified that result in constitutive overexpression (9,10). 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. (6). Plasmid-based ampC genes are expressed constitutively in most cases. However, some plasmid-carried ampC genes, such as the 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 (11). 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.(6)

Materials and Methods

Sampling, Bacterial Isolation, and Identification:

A total of 1874 samples were collected from four central hospitals in Babylon province including Marjan Teaching Hospital, Alamam Al-Sadeq Hospital, Al-Hilla General Teaching Hospital and Babylon Hospital. The number and percentage of samples were collected from this hospitals, 590 (31.5%), 186 (9.9%), 483 (25.8%) and 615 (32.8) respectively. Samples were collected for five months from February to June 2022 including various sources of infections urine, burn exudate, vaginal discharge, sputum, diabetics foot ulcer, stool, CSF, blood, and ear swabs, present study include all ages male and female. samples cultured onto a range of general and selective bacterial culture media including blood agar, MacConkey, Eosin methylene blue (EMB). cultured media were incubated at 37°C for 24 hours aerobically. Bacterial identification was done using the physiological and biochemical tests.

Phenotypic testing for β-lactamase production:

Nitrocefin disk (HARDY diagnostic, Santa Maria, Califronia,USA) it used for detect beta-lactamase production, nitrocefin is a chromogenic cephalosporin that changes from yellow to red when the amide bonds in the beta-lactamase hydrolysed by beta-lactamase.

Antimicrobial Susceptibility Testing:

An antimicrobial susceptibility test was performed on all isolates positive for nitrocefin (substrate for beta lactamase) by vitek 2 system(bioMérieux,france) AST GN76 kit and disc diffusion method that compatible with Clinical and Laboratory Standards Institute (CLSI) guidelines 2021 (12). AST GN76 kit includes ESBL, ampicillin, piperacillin/tazobactam, cefazolin, cefoxitin, ceftazidime, ceftriaxone, cefepime, etrapenem, imipenem, merpenem, amikacin, gentamicin, isepamicin, ciprofloxacin, levofloxacin, sparfloxacin, tigycycline, nitrofurantoin, and trimethoprime/ sulfamethoxazole. The antibiotics tested by disc diffusion method include azithromycin, doxycycline, piperacillin,amoxicillin/clavulonate, aztreonem, colistin, nalidixic acid, norfloxacin, fosfomycin, and chloroamphenicole.

Phenotypic testing for pAmp-C β–lactamase:

A total of 112 *E. coli* isolates were screened for phenotypic pAmp C beta-lactamase production by vitek 2 compact system (bioMe´rieux, France) using (ASTGN 76), depends on cefoxitin resistance.

Phenotypic testing for extended spectrum β-lactamase production:

A total of 112 *E. coli* isolates were screened for ESBL production by vitek 2 compact system using (ASTGN 76) that compatible with CLSI recommended conditions.(12) ESBL screening also included the following criteria for *E. coli*, a cefepime(PEP) 1, cefotaxime (CTX) 0.5, ceftazidime(CAZ) 0.5, cefepime (PEP)/ clavulonic acid (CA)1/10, cefotaxime (CTX)/clavulonic acid (CA) 0.5/4, ceftazidime(CAZ)/clavulonic acid(CA) 0.5/4.

DNA extraction:

from pure overnight cultures, plasmid DNA templates of isolates were extracted and prepared by Plasmid Extraction Mini Kit (FAVORGEN comp., Taiwan, cat number FAPDE 100) according to the manufacturer's protocol. purity of DNA were determined by NanoDrop one (Thermo Scientific NanoDrop, United States) at 260 nm, and used as a template in PCR technique.

PCR protocol:

detection of genes occure by PCR using specific primers, The primers used, product size and search for PCR condition listed in Table 2. The PCR reactions were prepared in 25 µl total reaction mixture volume for monoplex and 50 µl for multiplex, in 25 µl, the volume comprising of 12.5 µl of Taq DNA Polymerase Master Mix green (promega,UK), primers, 2 µl (10 pmol/ µl) from each one with 6 µl (50 ng) of extracted DNA, and sterile deionized water to achieve a final volume of 25 µl, in 50 µl volume, each volume multiply by 2 The amplifications were carried out in a thermocycler (C1000 Touch, Bio-Rad). PCR products was analyzed by gel electrophoresis with 1.5% agarose and visualized by ultraviolet transillumination. A 1500 base-pair DNA ladder was used as the size reference.

Table (1): The pAmpC primers used in the study

Primer	Gene name	Oligo sequence (5-3)	Product size(bp)	Reference
FOX	blaFOX	F: AACATGGGGTATCAGGGAGATG R: CAAAGCGCGTAACCG GAT TGG	190	(12)
CIT	blaCIT	F: TGGCCAGAACTGACAGGCAAA R: TTTCTCCTGAACGTGGCTGGC	462	
DHA	blaDHA	F: AACTTTCACAGGTGTGCTGGGT R: CCGTACGCATACTGGCTTTGC	405	
EBC	blaEBC	F: TCGGTAAAGCCGATGTTGCGG R: CTTCCACTGCGGCTGCCAGTT	302	
ACC	blaACC	F: AACAGCCTCAGCAGCCGGTTA R: TTCGCCGCAATCATCCCTAGC	346	
MOX	blaMOX	F: GCTGCTCAAGGAGCACAGGAT R: CACATTGACATAGGTGTGGTGC	520	
CMY	blaCMY	F: GACAGCCTCTTTCTCCACA R: TGGAACGAAGGCTACGTA	1000	(13)
SHV	blaSHV	F:CTTTACTCGCCTTTATCG R:TCCCGCAGATAAATCACCA	827	(14)
TEM	blaTEM	F:TCAACATTTTCGTGTCGCCC R:AACTACGATACGGGAGGGCT	766	(15)
OXA	blaOXA	F: ATATCT CTACTGTTG CAT CTCC R: AAACCCTCTAAACCATCC	619	(16)
CTX-M	BlaCTX-M	F:ATGTGCAGYACCAGTAA R:ACCGCRATATCRTTGGT	536	(17)
KPC	blaKPC	F:CGTCTAGTTCTGCTGTCTTG	798	(18)

		R:CTTGTCATCCTTGTTAGGCG		
Acc(6)Ib	acc(6) Ib-cr	F :TTGCGATGCTCTATGAGTGGCTA R :CTCGAATGCCTGGCGTGTTT	482	(19)
mcr-2	mcr-2	F :CAAGTGTGTTGGTCGCAGTT R :TCTAGCCCGACAAGCATACC	715	(20)
mcr-3	mcr-3	F :AAATAAAAATTGTTCCGCTTATG R :AATGGAGATCCCCGTTTTT	929	
Int 1	Int 1	F: CAGTGGACATAAGCCTGTTC R: CCCGAGGCATAGACTGTA	160	(21)
qnr A	qnr A	F:ATTTCTCACGCCAGGATTTG R:GATCGGCAAAGGTTAGGTCA	516	(22)
qnr B	qnr B	F:GATCGTGAAAGCCAGAAAGG R:ACGATGCCTGGTAGTTGTCC	469	

Results

During the 5-month cross-sectional study period, a total of 1874 clinical samples were collected from four main hospitals in Babylon province. The clinical sample type's collected during the study period included 642 (34.3%) urine samples from patients suspected with UTIs, 422 (22.5%) burin exudate, 253(13.5%) vaginal discharge, 240 (12.8%) sputum samples, 118 (6.3%) diabetic foot ulcer, 62 (3.3%) stool samples, 57(3%) CSF, 53 (2.9%) blood specimens, 27(1.4%) ear swab in (Figure 1).

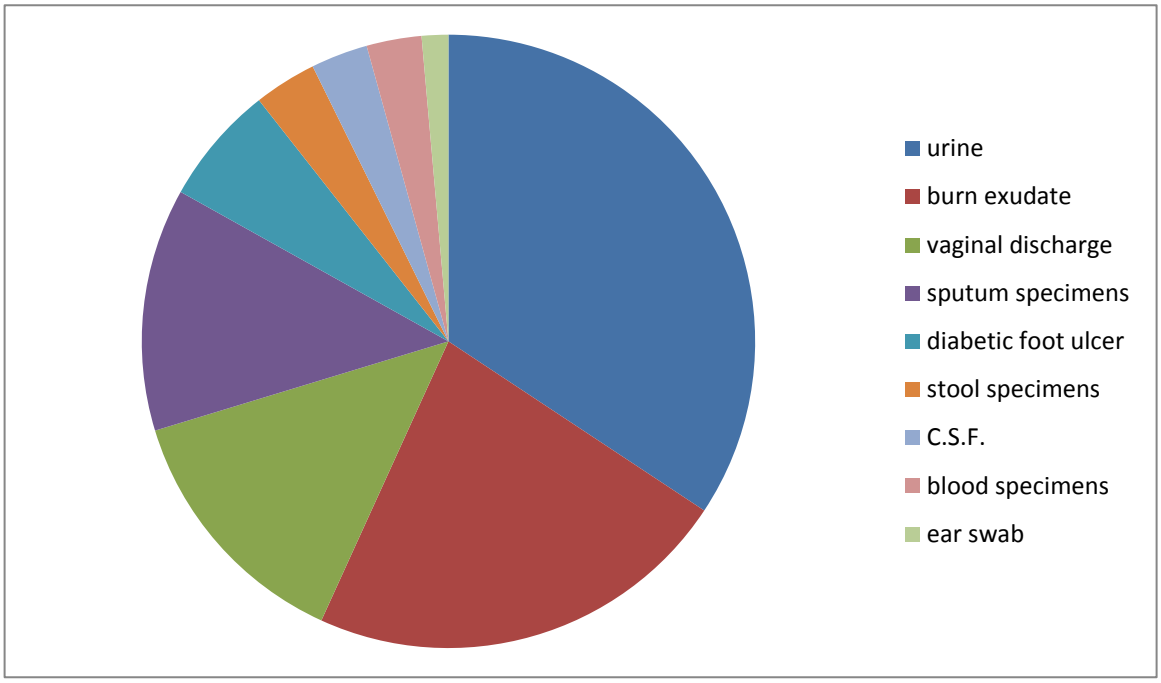

Figure (1): Types and percentage of various clinical samples

Antibiogram results show that the highest resistance is related to the antibiotics ampicillin (100%), amoxicillin (100%), amoxicillin/clavulonic acid (100%), piperacillin (99.1%), cephazolin (89.3%) as in (Figure 2).

E.coli isolated from urine were given high resistance to ceftriaxone (92.8%), aztreonem (75%). E.coli isolated from vaginal discharge revealed highest resistance to ciprofloxacin (100%), levofloxacin (100%), sparfloxacin(100%), aztreonem (100%), trimethoprime/sulfamethazole (94.1%). Highest resistance to aztreonem (100%), ceftriaxone (100%) were appeared in *E.coli* isolated from diabetic food ulcer as mention in (Table 2).

pAmp C producing E.coli isolates were revealed high resistance to antibiotics types(23.1%) of isolates were resistance to 22 types whereas, highest percentage (11.6%) of non- Amp C producing E.coli isolates resistance to 15 antibiotics types as mentioned in (Table 3).

Frequency of pAmp C producing E.coli isolates to antibiotics resistances classes, urine were high resistance to 8 classes (15.4%), 9 classes (15.4%) and 10 classes (11.5 %), all diabetic food ulcer were resistance to 9 classes, from total number were 42.5% of isolates resistance to 9 classes table (4).

According to pAmp C genes, show high prevalence BlCMY(88.5%), BlCIT (57.7%) and BlDHA (42.3%), BlFOX, BlMOX, BlEBC, BlACC were not detected in present study as in table(5).

From 26 pAmp C producing isolates, 1 (3.85%) isolates carry 3 types of pAmp C genes, 19(73%) isolates carry 2 types of pAmp C, 5(19.3%) isolates carry 1 isolates and 1(3.85%) isolates do not carry any one from study genes. Most isolates that carry 2 types of pAmp C genes were resistance to 12 or 13 types of β- lactam antibiotics, in present study there are 8 intermediate isolates for antibiotics resistance, 7 from 8 (87.5%) isolates carry 1 types of genes, (Table 6).

Urine pAmp C producing E.coli isolates mostly carry ESBL genes (BlaSHV, BlaTEM BlaCTX-M and BlaOXA), ACC(6)Ib-cr, qnr A and qnr B, burn pAmpC producing *E.coli* isolates mostly carry ACC(6)Ib-cr, qnr A and qnr B, stool pAmp C producing E.coli isolates carry ACC(6)Ib-cr, qnr B and mcr 2, E.coli isolated from vaginal exudate carry various genes and E.coli isolated from diabetic foot ulcer contain mcr3, qnr A and qnr B, all pAmpC-producing *E.coli* isolates contain class 1 integron (Table 6).

Only 1 isolates (3.85%) of pAmpC producing *E.coli* isolates carry 3 types of genes (BlaDHA + BlaCIT + BlaCMY), 14 (53.85) isolates carry (BlaCIT + BlaCMY) and 7 isolates (26.9%) carry (BlaDHA + BlaCMY) (Table 7).

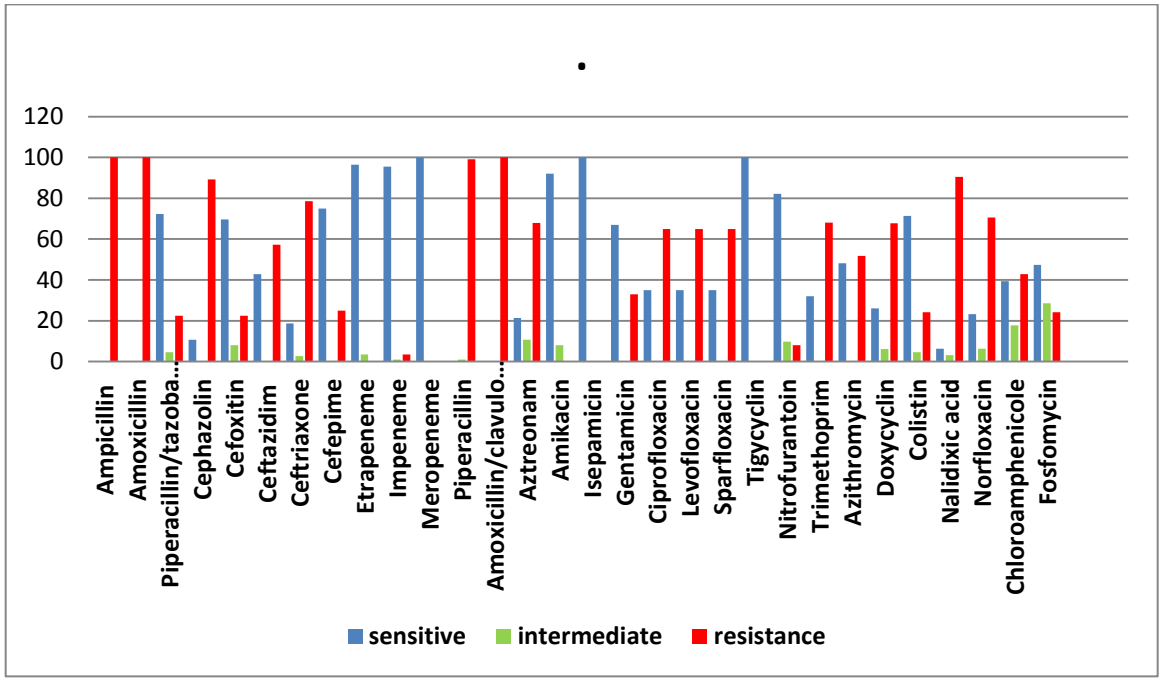

Figure (2): Antibiotics resistance profiles of *E. coli* isolated from various clinical sources

Table (2): Antibiotics susceptibility profile of E.coli isolated from different clinical samples

NO.	Antibiotics	No. of isolates recovered from clinical sources (% 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
1	Ampicillin	56(100)	16(100)	15(100)	17(100)	7(100)	1(100)
2	Amoxicillin	56(100)	16(100)	15(100)	17(100)	7(100)	1(100)
3	Piperacillin/tazobactam	16(28.5)	1(6.2)	2(13.3)	1(5.9)	5(71.4)	-
4	Piperacillin	56(100)	16(100)	15(100)	17(100)	7(100)	1(100)
5	Amoxicillin-clavulonate	56(100)	16(100)	15(100)	17(100)	7(100)	1(100)
6	Cephazolin	53(94.6)	9(60)	17(100)	7(100)	7(100)	1(100)
7	Cefoxitin	14(25)	2(12.5)	4(26.7)	1(5.9)	5(71.4)	-
8	Ceftazidim	38(67.8)	5(31.2)	5(33.3)	10(58.8)	6(85.7)	-
9	Ceftriaxone	52(92.	11(68.7)	4(26.7)	13(76.5)	7(100)	1(100)

		8)					
10	**Cefepime**	**24(42.8)**	**-**	**-**	**2(11.8)**	**2(28.6)**	**-**
11	**Etrapeneme**	**-**	**-**	**-**	**-**	**-**	**-**
12	**Impenem**	**2(3.5)**	**-**	**-**	**1(5.9)**	**1(14.3)**	**-**
13	**Meropeneme**	**-**	**-**	**-**	**-**	**-**	**-**
14	**Aztreonem**	**42(75)**	**5(31.2)**	**5(33.3)**	**17(100)**	**7(100)**	**-**
15	**Amikacin**	**-**	**-**	**-**	**-**	**-**	**-**
16	**Isepamicin**	**-**	**-**	**-**	**-**	**-**	**-**
17	**Gentamicin**	**19(33.9)**	**5(31.2)**	**3(20)**	**10(58.8)**	**-**	**-**
18	**Ciprofloxacin**	**32(57.1)**	**9(56.2)**	**8(53.3)**	**17(100)**	**6(85.7)**	**1(100)**
19	**Levofloxacin**	**32(57.1)**	**9(56.2)**	**8(53.3)**	**17(100)**	**6(85.7)**	**1(100)**
20	**Sparfloxacin**	**32(57.1)**	**9(56.2)**	**8(53.3)**	**17(100)**	**6(85.7)**	**1(100)**
21	**Tigycycline**	**-**	**-**	**-**	**-**	**-**	**-**
22	**Nitrofurntion**	**-**	**2(12.5)**	**3(20)**	**-**	**4(57.1)**	**-**
23	**Trimethoprime/sulfamethazole**	**31(55.3)**	**10(62.2)**	**12(80)**	**16(94.1)**	**6(85.7)**	**1(100)**
24	**Azithromycin**	**30(53.5)**	**5(31.2)**	**5(33.3)**	**14(82.4)**	**4(57.1)**	**-**
25	**Doxycycline**	**45(80.3)**	**10(62.2)**	**7(46.7)**	**12(70.6)**	**2(28.6)**	**-**
26	**Colistin**	**9(16)**	**6(37.5)**	**3(20)**	**5(29.4)**	**4(57.2)**	**-**
27	**Nalidixic acid**	**50(89.3)**	**15(93.7)**	**13(86.7)**	**17(100)**	**6(85.7)**	**-**
28	**Norfloxacin**	**38(67.8)**	**12(56.2)**	**10(66.7)**	**12(70.6)**	**6(85.7)**	**-**
29	**Chloroamphenicol**	**22(39.3)**	**9(75)**	**6(40)**	**6(35.3)**	**5(71.4)**	**-**
30	**Fosfomycin**	**21(37.5)**	**3(18.7)**	**3(20)**	**-**	**-**	**-**

Table (3): Frequency of AmpC and non-AmpC β-lactamase producing *E.coli* isolates

NO. of antibiotic resistance	**NO. of non AmpC β-lactamase producing isolates n=86 (%)**	**NO. of AmpC β-lactamase producing isolates n=26 (%)**
6	1(1.2%)	-
7	2(2.4%)	-
8	5(5.8%)	-
9	7(8%)	1(3.85%)
10	8(9.3%)	-
11	4(4.6%)	1(3.85%)
12	8(9.3%)	-
13	8 (9.3%)	-
14	9(10.5%)	1(3.85%)
15	10(11.6%)	-
16	4(4.6%)	1(3.85%)
17	9(10.5%)	-
18	5(5.8%)	3(11.5%)
19	2(2.4%)	5(19.2%)
20	3(3.5%)	1(3.85%)
21	1(1.2%)	4(15.4%)
22	-	6(23.1%)
23	-	4(15.4%)

Table (4) Frequency of different Amp C producing *E.coli* isolates to antibiotics resistance classes

Samples types	**MDR**						
	Number of classes						
	4 classes	5 classes	7 classes	8 classes	9 classes	10 classes	11 classes
Urine samples	-	2(7.7%)	-	4(15.4%)	4(15.4%)	3(11.5%)	1(3.8%)
Stool samples	1(3.8%)		2(7.7%)		1(3.8%)		
Vaginal discharge						1(3.8%)	
Burn exudate				1(3.8%)	1(3.8%)		
Diabetic food ulcer					5(19.5%)		
Total	1(3.8%)	2(7.7%)	2(7.7%)	5(19.2%)	11(42.5%)	4(15.3)	1(3.8%)

Table (5): Frequency of AmpC genes

Gene	Frequency	%
BlaCMY	23	88.5
BlaCIT	15	57.7
BlaDHA	10	42.3
BlaFOX	0	0
BlaMOX	0	0
BlaEBC	0	0
BlaACC	0	0

Table (6): Frequency of antibiotics resistance, AmpC genes and other antibiotics resistance genes in AmpC-producing *E.coli* isolates

Source	NO.	Resistance style	NO. of β-lactam antibiotic resistance	NO. of non-β-lactam antibiotic resistance	Total NO. of antibiotics resistance	NO. of classes	NO. of AmpC genes	NO. of other resistance genes in AmpC producing isolates
Urine	1	Resistance	9	2	11	5	1 (DHA)	4 (SHV, CTX-M, OXA, integron 1)
	2	Intermediate	9	9	18	7	1 (CMY)	7 (SHV, TEM, CTX-M, OXA, integron 1,qnr A, qnr B)
	3	Resistance	12	9	21	8	2 (CIT, CMY)	4 (qnr A, qnr B, ACC(6) Ib-cr, ,integron 1)
	4	Resistance	12	9	21	9	2 (CIT, CMY)	1 (integron 1)
	5	Resistance	12	10	22	9	2 (CIT, CMY)	4 (qnr A, ACC(6) Ib-cr, integrin 1)
	6	Resistance	12	10	22	10	2 (CIT, CMY)	2 (qnr A, integron 1)
	7	Resistance	12	6	18	5	2 (DHA, CMY)	3 (qnr A, qnr A, integron 1)
	8	Resistan	11	8	19	8	2 (CIT,	4 (SHV, CTX-M, OXA,

		ce					CMY)	qnr A, integron 1)
	9	Resistance	9	9	18	8	-	3 (CTX-M, OXA, integron 1)
	10	Resistance	9	9	18	8	2 (CIT, CMY)	7 (TEM, CTX-M, OXA, qnr A, ACC(6) Ib-cr, integrin 1)
	11	Resistance	10	9	19	9	1 (DHA)	5 (TEM, CTX-M, OXA, qnr B, integron 1)
	12	Resistance	10	10	20	10	2 (DHA, CMY)	8 (SHV, TEM, CTX-M, qnr A, qnr B, ACC(6)Ib-cr, integron 1,mcr 2)
	13	Resistance	10	9	19	9	1 (CMY)	5 (SHV, CTX-M, TEM, ACC(6)Ib-cr, integron 1)
	14	Resistance	12	11	23	11	2 (CIT, CMY)	4 (qnr A, ACC(6)Ib-cr, KPC, integron 1)
	15	Resistance	12	10	22	10	2 (DHA, CMY)	3 (qnr A, KPC, integron 1)
Burn	16	Intermediate	5	9	14	7	1 (CMY)	2 (qnr B, integron 1)
	17	Intermediate	5	9	14	7	1 (CMY)	4 (qnr A, qnr B, ACC(6)Ib-cr, integrin 1)
	18	Resistance	11	9	20	8	2 (DHA, CMY)	2 (qnr A, integrin 1)
	19	Intermediate	5	8	13	8	1(CMY)	4 (qnr A, qnr B, ACC(6)Ib-cr, integrin 1)
	20	Resistance	9	4	13	9	1(CMY)	6(TEM, SHV, CTX-M, OXA, integrin 1,mcr 2)
Stool	21	Intermediate	6	11	17	11	-	4 (qnr B, ACC(6)Ib-cr, integrin 1,mcr 2)
	22	Resistance	7	9	16	7	2 (DHA, CMY)	2 (qnr B, integrin 1)
	23	Resistance	11	8	19	7	2 (DHA, CMY)	2 (qnr B, integrin 1)
	24	Intermediate	5	10	15	8	1(CMY)	4 (qnr B, integrin 1, ACC(6)Ib-cr, mcr 2)
	25	Resistance	11	7	18	9	2 (DHA, CMY)	4 (qnr A, qnr B, integrin 1,mcr 2)
	26	Resistan	7	2	9	4	3 (DHA,	2 (ACC(6)Ib-cr, integrin

		ce					CIT, CMY)	1)
Vaginal exudate	27	Intermediate	8	6	14	7	1 (CMY)	6 (TEM, CTX-M, OXA, qnr A, qnr B, integrin 1)
	28	Resistance	10	12	22	10	1 (CMY)	5 (qnr A, qnr B, OXA, Kpc, integrin 1)
	29	Intermediate	5	8	13	8	1 (CMY)	4 (qnr A, qnr B, ACC(6)Ib-cr, integrin 1)
Diabetic foot ulcer	30	Resistance	13	10	23	9	2 (CIT, CMY)	3 (qnr A, qnr B, integrin 1)
	31	Resistance	12	10	22	9	2 (CIT, CMY)	2 (OXA, integrin 1)
	32	Resistance	13	10	23	9	2 (CIT, CMY)	3(qnr A, qnr B, integrin 1, mcr 3)
	33	Resistance	13	10	23	9	2 (CIT, CMY)	2(qnr A, integron 1)
	34	Resistance	13	9	22	9	5(CIT, CMY)	3(qnr A, integrin 1, mcr 3)

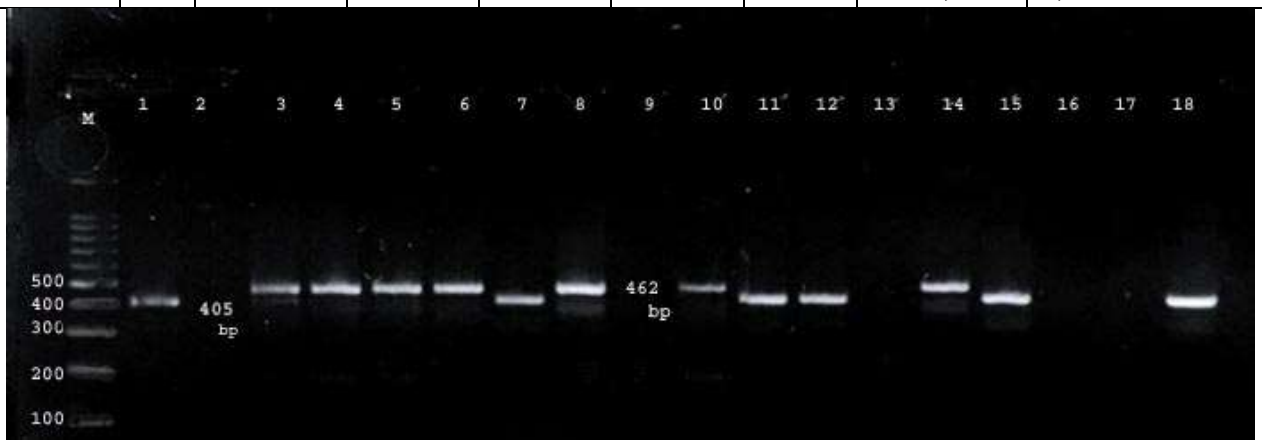

Figure (3): Ethidium bromide-stained agarose gel of multiplex PCR amplified products from extracted DNA of pAmp C positive *E.coli* isolates that amplified with primers for genes (BlaDHA, BlaCIT, BlaACC, BlaFOX, BlaMOX, BlaCEA), the electrophoresis was performed in 1.5% agarose at 70 volt for 2 hours, lance M, 1500-bp DNA ladder, lanes (1, 7, 11, 12, 15, 18) showed positive results for BlaDHA (405 bp), lanes (3, 4, 5, 6, 8, 10, 14) showed positive results for Bla CIT (462 bp). All these lanes belong to cefoxitin resistant *E.coli*. Lanes (2,16,17) are cefoxitin-intermediate; not carry any of the 6 genes.

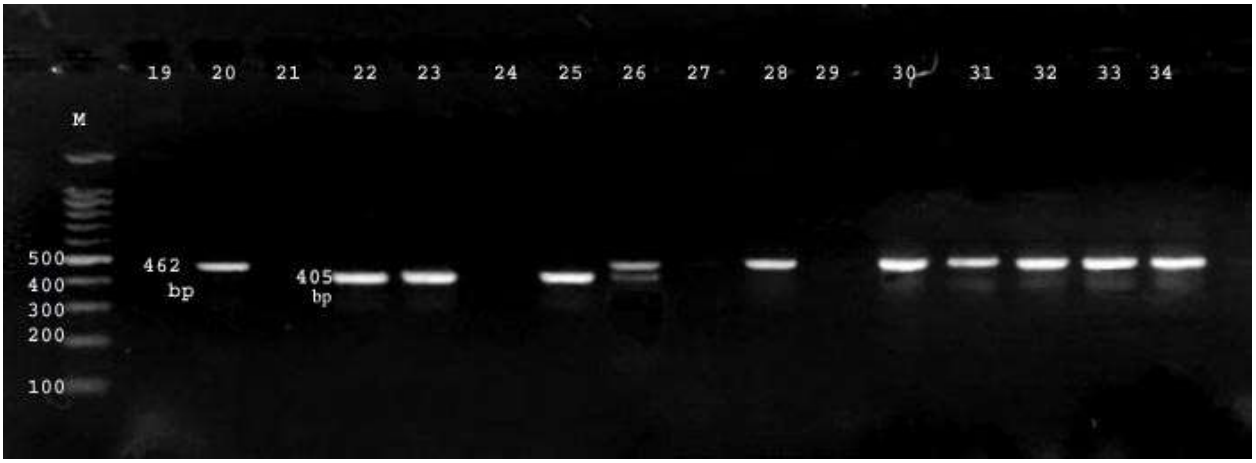

Figure (4): Ethidium bromide-stained agarose gel of multiplex PCR amplified products from extracted DNA of pAmpC positive *E.coli* isolates that amplified with primers for genes (BlaDHA, BlaCIT, BlaACC, BlaFOX, BlaMOX, BlaCEA), the electrophoresis was performed in 1.5% agarose at 70 volt for 2 hours, lanes M, 1500-bp DNA ladder lanes (22, 23, 25) show positive results for BlaDHA (405 bp), lanes (20, 26, 28, 30, 31, 32, 33, 34) showed positive results for Bla CIT (462 bp). All these lanes belonged to cefoxitin resistant *E.coli*. lanes (19, 21, 24, 26, 27, 29) were cefoxitin-intermediate not carry any of 6 genes except lanes 26 which carry BlaDHA and BlaCIT.

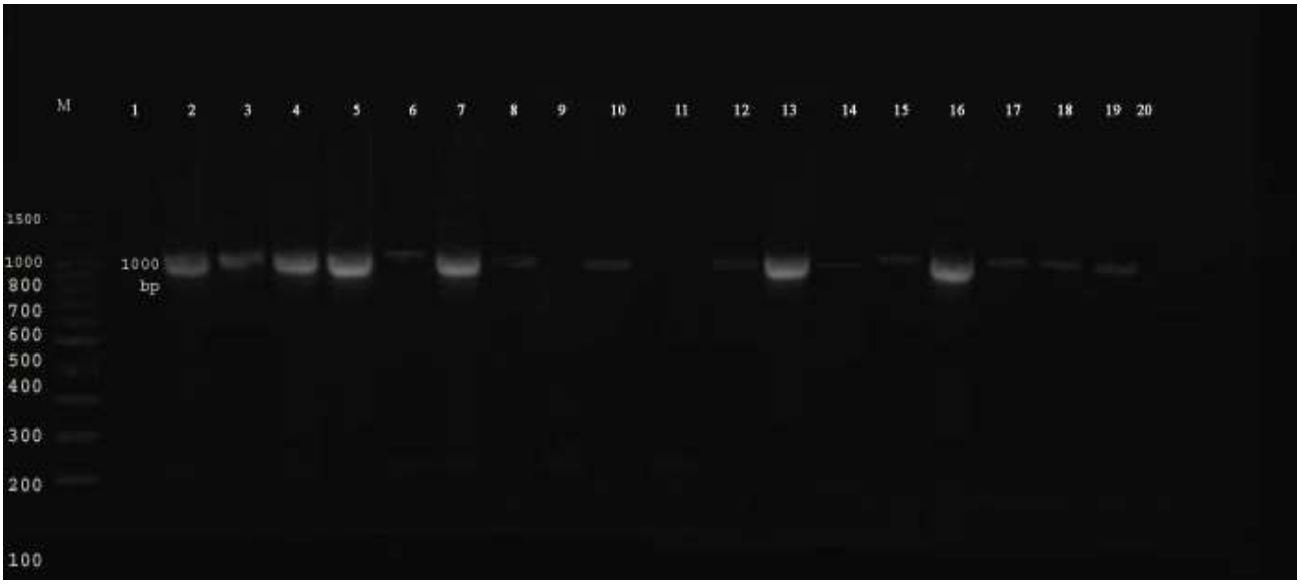

Figure (5): Ethidium bromide-stained agarose gel of monoplex PCR amplified products from extracted DNA of pAmpC positive *E.coli* isolates that amplified with primers for Bla CMY genes forward and reverse, the electrophoresis was performed in 1.5% agarose at 70 volt for 2 hours, lanes M, 1500-bp DNA ladder, lanes (2, 3, 4, 5, 6, 7, 8, 10, 12, 13, 14, 15, 16, 17, 18, 19) showed positive result for BlaCMY(1000bp), lanes (1, 9, 11,20) showed negative result for BlaCMY.

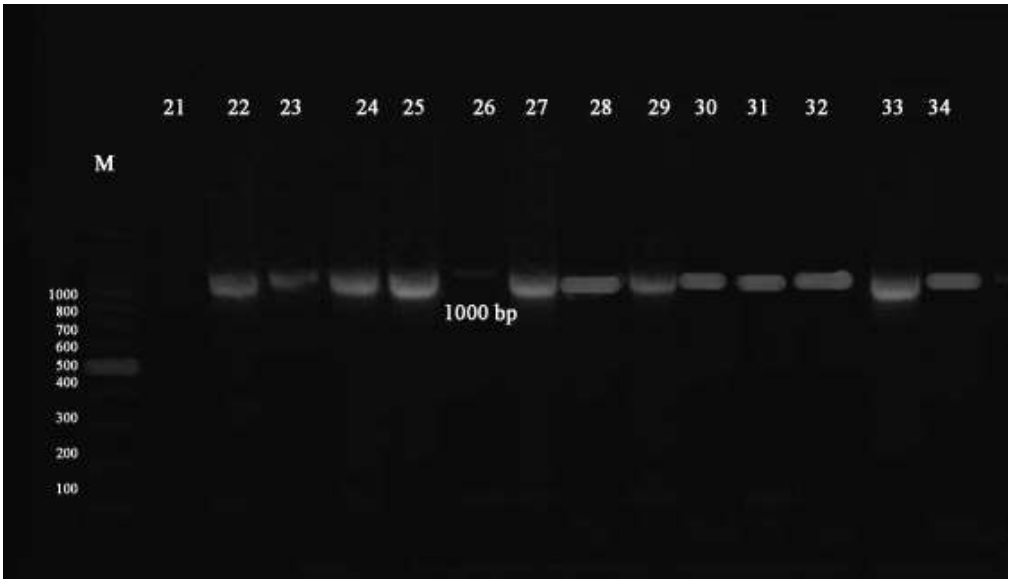

Figure (6): Ethidium bromide-stained agarose gel of monoplex PCR amplified products from extracted DNA of pAmpC-positive *E.coli* isolates that amplified with primers for Bla CMY genes forward and reverse, the electrophoresis was performed 1.5% agarose at 70 volt for 2 hours, lanes M, 1500-bp DNA ladder, lanes (22, 23, 24, 25, 27, 28, 29,30, 31, 32, 33, 34) showed positive result for BlaCMY (1000bp), lanes (21, 26) showed negative result for BlaCMY.

Table (7): The occurrence patterns of genes encoding AmpC betalactamases

Pattern	**Cefoxitin –Resistance (Amp C) isolate n=26**	
	NO.	**%**
BlaDHA	2	7.7
BlaCMY	1	3.85
BlaDHA +BlaCMY	7	26.92
BlaCIT +BlaCMY	14	53.85
BlaDHA + BlaCIT + BlaCMY	1	3.85
Without study genes	1	3.85

Discussion

Trends of resistance to β-lactam antibiotics in Gram-negative bacteria isolated from clinical samples have been increased over recent years.(24). pAmp C β- lactamase are dramatically recognized as a growing clinical problem (25) Detection of pAmpC type beta-lactamases in *E.coli* challenges microbiological laboratories. For better treatment, and molecular diagnosis, the use of PCR and other sequencing techniques is necessary, but these techniques are not always available (26). One of the objectives of this study was to determine the productive strains of beta-lactamase and pAmpC enzymes.

In the present study, the results found that all *E.coli* isolates were resistant to ampicillin (100%), amoxicillin (100%), amoxicillin-clavulonate (100%), piperacillin (99.1%), nalidixic acid (90.1%), cephazolin (89.3%), ceftriaxone(78.5), norfloxacin (70%), trimethoprim/sulfamethazole (68%),

aztreonem (67.9%), doxycycline (67.8%), ciprofloxacin (65%) figure (1), a study reported by bahramian et al., 2021 in Iran consistent with our results that found E.coli resistance to ampicillin (100%), ciprofloxacin (61.7%), ceftriaxone (85.1%) (27), in Duhok city north of Iraq, Naqid et al, (88.3%) of E.coli resistance to ceftriaxone (28). in Nigeria, Medugo et al, a study revealed E.coli resistance to ceftriaxone (80.4%). This result was compatible with our study (29) and in Iran were reported resistance to ceftriaxone (85.1%) (27), in Bangladesh, Jain et al. revealed resistance to amoxicillin (98%),(19) resistance to amoxicillin-clavulonate in contrast with Dan We et al (30) in china, were founded (6%) of isolate resistance to amoxiclave, Khatum et al (32) in Bangladesh revealed that 75% of isolates were resistant to amoxicillin-clavulonate which to our study. Xiangqun liu and Yongrui liu (33) 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.(34) revealed resistance to impenem and meropenem (0%) that consistent with our study, but Lin et al.(35) founded *E.coli* resistance to meropenem, etrapenem and impenem were (81.5%), (100%), (72.2) respectively and founded resistance to tigycycline (0%) as in our study, resistance to trimethoprim-sulfamethazole (77.8%), fosfomycin (22.2%), gentamicin (40.7%), amikacin (11.1%), aztreonem (66.7%) all these results were compatible with our study. Khatun et al.(32) revealed (70%) of *E.coli* isolates were resistance to ciprofloxacin, relatively similar our study.

E.coli isolated from vaginal discharge more resistance to antibiotics mentioned in (Tables 1 & 2), a study in Iraq reported vaginal *E.coli* were resistant to ampicillin, ceftriaxone, Trimethoprim/ Sulfamethoxazole, and cephazolin (36). Ahmad Atia (37) in Libya, reported that *E.coli* isolated from vagina were sensitive to several antibiotics including ceftriaxone, ciprofloxacin, and gentamicin in contrast with our study the resistance to ceftriaxone (76.5%), ciprofloxacin (100%), and gentamicin (58.8%).

According to genotypic study of the current study, out of 112 *E.coli* isolates, 26 AmpC-producing isolates (23.2%) and 75 ESBL producing isolates (67%), were identified. Deylamdeh and, Iran, reported that *E.coli* isolated from different clinical sources produced AmpC (40.5%) and ESBL (59.5%) of *E.coli* isolates (38). Soltan-Dallal et al. in Tehran, revealed that out of 128 *E.coli* isolates, 115 isolates (89.8%) were ESBL generators and 13 isolates (10.2%) were AmpC generators (39). In a study conducted in Kerman, Iran, on clinical specimens including urine, blood, and body fluids, 39.3% were AmpC-producers and (43.76%) were ESBL-producers (40) despite the heterogeneity in reported rates, our results were consistent with the average reported in these studies.

Among the 26 cefoxitin resistant isolates, plasmid encoded AmpC genes were detected by PCR in 25 (96.2%) isolates.This result was compatible with the result of (41) who found (88.4%) of cefoxitin resistance isolates were carry plasmid encoded AmpC genes. Tan et al., reported the sensitivity and specificity of detection AmpC by cefoxitin were 89% and 90% respectively.(42)

Plasmid mediated AmpC (pAmp c) genes were detected in our study included Bl_{CMY}, Bl_{CIT}, Bl_{DHA}, Bl_{ACC}, Bl_{EBC}, Bl_{FOX} and Bl_{MOX}, the results revealed Bl_{CMY} (88.5%), Bl_{CIT} (57.7%), Bl_{DHA} (42.3%), the genes Bl_{ACC}, Bl_{EBC}, Bl_{FOX} and Bl_{MOX} were not detected table (6), Helmy and Wasfy reported that Bl_{CMY} was the most predominant gene (86.9%). a study by Sadeghi et al, in iran reported Bl_{MOX} (14.6%), Bl_{EBC} (8.3%) and Bl_{CIT} (18.8%). Meanwhile, bla_{DHA}, bla_{FOX}, and bla_{ACC} genes were not found in any isolates (24).

Jojy et al., in Manama city, Kingdom of Bahrain, revealed Bl_{ACC}, Bl_{EBC}, Bl_{FOX} and Bl_{MOX} were not detected by PCR. The results of last four genes of this study were compatible with results of the same genes, Bl_{CIT} (31%), Bl_{DHA} (10.3%) of cefoxitin-resistant isolates. These results were lower than results in our study (43). Studies in different areas of the world have revealed geographical diversity in the molecular subtypes of pAmpC genes (44, 45). In contrast to these studies, Wassef et al. reported bla_{MOX} and bla_{FOX} families as the most prevalent AmpC subtypes in Egypt, followed by EBC and CIT subtypes (32). Adding to the geographical complexity of AmpC strains, studies in North Africa and Australia have reported bla_{CMY}, bla_{DHA}, and bla_{EBC} as the most frequent subtypes of pAmpC producers.(46,47)

Urine pAmp C producing *E.coli* isolates mostly carry ESBL genes (Bla_{SHV}, Bla_{TEM} Bla_{CTX-M} and Bla_{OXA}) (Table 6). These results were consistent with Sadeghi et al. who reported high prevalence of ESBL genes in cefoxitin resistant *E.coli* isolated from urine (24).

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