PHENOTYPIC & MOLECULAR CHARACTERIZATION OF AMPC B-LACTAMASES AMONG *ESCHERICHIA COLI, KLEBSIELLA* SPP. & *ENTEROBACTER* SPP. ISOLATED FROM AL-HUSSEIN TEACHING HOSPITAL IN THI-QAR GOVERNORATE [+]

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Abstract:

In this study,101 *Escherichia coli, Klebsiella* spp. and *Enterobacter* spp. isolated from clinical and hospital environment specimens of Al-Hussein Teaching Hospital in Dhi-Qar governorate, were analyzed for AmpC B-lactamase using phenotyping methods and polymerase chain reaction(PCR).

The purpose of this study was to detect and characterize AmpC β-lactamases in these isolates were not study in Al-Hussein Teaching Hospital by another researchers.

AmpC β-Lactamase was initially detected by phenotyping methods. Out of 101 of isolates, 80 (79.2%) were β-lactam resistant, 32 (31.6 %) isolates were deemed resistant and intermediate to cefoxitin were suspected as AmpC producer, 23 (22.7 %) of isolate were identified as AmpC β- lactamase producer, whereas, 5 (4.9 %) isolates were identified as inducible AmpC β- lactamase .

Plasmid mediated AmpC genes were detected by conventional PCR. Only 11 (10.9 %) of *Escherichia coli*, 2 (1.9 %) of *Klebsiella* spp. and 5 (4.9 %) of *Enterobacter* spp. gave positive results with AmpC gene. Results of antibiotic susceptibility revealed that AmpC carrying isolates had high resistance to Cefotaxime, Aztreonam, Amoxiclave, Carbencillin, Pipercilin, Nalidixic acid, Ampicillin, Cotrimethoxazole, Ciprofloxacin and Levofloxacin. The study also revealed that Norfloxacin, Amikacin, Ciprofloxacin and Levofloxacin was found to be effective against 100% of the AmpC harboring *Enterobacter* spp. Additionally, Imipenem and Meropenem were found to be effective against 60% of the AmpC harboring *E. coli*, 50% of *Klebsiella* spp. and 80% of *Enterobacte*r spp.

Key words: **AmpC β-Lactamase; phenotyping methods; Cefoxitin; PCR**

الصفات المظهرية والجزيئية لإنزيمات البيتالاكتاميز من نوع AmpC في جراثيم الاشرشيا القولونية والكلبسيلا والانتيروباكتر والمعزولة من مستشفى الحسين التعليمي في محافظة ذي قار

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المستخلص :

تضمنت هذه الدراسة 101 عزله لبكتريا *Escherichia coli* و *Klebsiella* spp. و *Enterobacter* spp. تم عزلها من مرضى وبيئة مستشفى الحسين التعليمي في محافظة ذي قار وتم الكشف عن AmpC β – Lactamase بالطرق المظهريه وال conventional PCR .

الهدف من هذه الدراسة هو الكشف ودراسة خصائص جينات AmpC β –Lactamase في هذه العزلات والتي لم تدرس سابقا من قبل الباحثين من محافظة ذي قار في مستشفى الحسين التعليمي.

أبتداءا تم الكشف عن AmpC β –Lactamase بالطرق المظهريه وظهر من مجموع 101 عزله ان هنالك (79.2%) 80 من تلك العزلات تبدي مقاومه للبيتالاكتاميز وأن (31.6 %) 32 من تلك العزلات نكون مقاومه ومتوسطة المقاومه لل cefoxitin وان هناك (22.7 %) 23 من تلك العزلات هي منتجه لل AmpC B – lactamase كذلك أظهرت النتائج بأنه هنالك (4.9 %) 5 هي Inducible AmpC β– lactamase .

تم الكشف عن المتوسط البلازمي لجينات AmpC في العزلات المشتبه بها باستخدام conventional PCR ووجد ان هناك (10.9 %) 11 من عزلات *Escherichia coli* و (1.9 %) 2 من عزلات *Klebsiella* spp. و 4.9) 5 (% من عزلات *Enterobacter* spp. تمتلك AmpC B–lactamase.

تم اختبار حساسية جميع العزلات الحامله *AmpC* genes تجاه المضادات الحياتيه وأظهرت هذه الدراسة مقاومه عاليه من قبل العزلات التي تمتلك AmpC B–lactamase باتجاه كل من Cefotaxime , Aztreonam , Ampicillin , Amoxiclave , Carbencillin , Pipercilin , Nalidixic acid , Ciprofloxacin , Cotrimethoxazole وال Levofloxacin . كذلك اظهرت هذه الدراسة فعاليه عاليه من المضادات Amikacin, Norfloxacin , Ciprofloxacin وال Levofloxacin ضد عزلات *Enterobacter* spp والمضادات Imipenem Meropenem , ضد العزلا ت *Escherichia coli* و *Klebsiella* spp., وال *Enterobacter* spp .

Introduction :

Ambler class C (AmpC) Beta-lactamases (β-lactamases) are clinically important cephalosporinases produced by many Enterobacteriaceae strains and mediate resistance to cephalothin, cefazolin, cefoxitin, most penicillins and β-lactam/β-lactam inhibitor combinations[1]. AmpC β-lactamases have gained importance since the late 1970s as one of the mediators of antimicrobial resistance in Gram negative bacilli. These enzymes are cephalosporinases capable of hydrolyzing all β-lactams to some extent [2,3].

AmpC β-lactamases are two types: plasmid-mediated and chromosomal or inducible AmpC[1]. The most common cause of AmpC overexpression in clinical isolates is a mutation in *AmpD* leading to AmpC hyper inducibility or constitutive hyperproduction [4] .

Most strains with plasmid-mediated AmpC enzymes have been isolated from patients after several days of hospitalization, but recently, AmpC producing isolates in cultures from long-term care facilities, rehabilitation centers, and outpatient clinics have been reported[5,6].

The plasmid-mediated AmpC genes are derived from inducible chromosomal genes that have become mobilized. Commonly reported genotypes are *ACC, FOX, MOX, DHA, CIT* and *EBC* [3,7]. These enzymes confer a resistance pattern similar to the overproduction of

chromosomal AmpC β-lactamases, which may involve all β-lactam antibiotics except for carbapenems and cefepime [8].
Plasmid-mediated *AmpC* genes are of special interest because their mobility allows them to emerge in one genus or species and spread to different organisms. The prevalence of plasmid mediated AmpC-type resistance at the national level in most countries is unknown because studies have not examined the strains at the molecular level required to elucidate the different mechanisms involved. A 2004 report from the United States documented 7 to 8.5 per cent of the *Klebsiella* spp. and 4 per cent of the *Escherichia coli* isolates contained plasmid mediated AmpC type enzymes [9].
The inducible chromosomal *AmpC* genes were detected on plasmids of *Klebsiella* spp., *Escherichia coli*, or *Salmonella* spp. In *Escherichia coli* AmpC is poorly expressed, while in *Klebsiella* and *Salmonella* species, the *AmpC* gene is missing from the chromosome and found on the plasmids [10].
Nevertheless, knowledge of the AmpC β-lactamases is still limited in Iraq. However, in Dhi-Qar governorate, no information regarding the molecular studies of the occurrence of plasmid mediated AmpC β- lactamases-producing bacteria are available from clinical cases and hospital environments. Therefore, there is an increase demand to investigate the role of these isolates in hospital infections.

Materials & Methods :

In this prospective laboratory based surveillance study (from first October 2012 till February 2013). A number of 101 non repeat Gram-negative strains [*Escherichia coli* (n=64), *Klebsiella* spp. (n=28) and *Enterobacter* spp. (n=9)] were isolated from a total of 705 specimens, 200 clinical samples (include urine, wound swabs and burn swabs) and 505 samples from hospital environment. The identities of all strains submitted were reconfirmed by conventional biochemical methods and API (Biomerieux, France) system. All the 101 bacterial isolates (*Escherichia coli*, *Klebsiella* spp. and *Enterobacter* spp.) were primarily screened for β- lactams resistance by growing on Muller-Hinton agar supplemented with Ampicillin and Amoxicillin (each alone) at final concentrations of 100 and 50μg/ml, separately, The isolates were then tested for extended-spectrum β-lactamase (ESBL) production using the screening criteria described by Clinical and Laboratory Standards Institute (CLSI) [11]. A positive result from initial screening was followed up with a phenotypic confirmatory test. ESBL production was carried out by the disk approximation method.
All 101 bacterial isolates in our study were tested for cefoxitin susceptibility for initial screening of AmpC B-lactamase according to CLSI by using standard disk diffusion method . The resistant isolates (≤18mm inhibition zone diameter) were considering as initially AmpC β- lactamase producers. Conventional PCR was used to detect the most common plasmid mediated *AmpC* genes *ACC, FOX, MOX, DHA, CIT* and *EBC* reported in literature using a protocol previously conducted [12]. All DNA was extracted from all suspected bacterial isolates using a commercial purification system (Genomic DNA Mini Kit (Geneaid, Thailand). Nanodrop was used to determine the purity, concentrations of the DNA and the degree of contamination with protein.
The technique of PCR was performed by using Master Mix (Accupower, Bionear Korea) with a final volume of 20 μl in 0.2-ml thin-walled tubes. The primers used for PCR amplification were synthesized by (AccuOligo, Korea) and provided in Lyophilized form (Table 1). Each reaction contained 17 ul of water, 2 ul of DNA template, 0.5 ul of Primer forward (10 picomole/ μl) MOXMF, CITMF, DHAMF, ACCMF, FOXMF and EBCMF, 0.5 ul of Primer

reverse (10 picomole/ µl) MOXMR, CITMR, DHAMR, ACCMR, FOXMR and EBCMR. The PCR programme consisted of an initial denaturation step at 94°C for 5 minutes, followed by 30 cycles of DNA denaturation at 94°C for 30 seconds, primer annealing at 60 °C for 45 sec, and primer extension at 72 °C for 45 sec. After the last cycle, a final extension step at 72°C for 5 min was added. PCR product was analyzed by gel electrophoresis with 2 per cent agarose. Gels were stained with ethidium bromide at 5µg/ml and visualized by UV transillumination. A DNA ladder of 1500-bp was used as a molecular ladder. Negative controls were PCR reagents mixed with water in place of template DNA.

Table (1): The primers used in this study

Product size (bp)	Oligo sequence (3'-5')	Primer name
190	F: AAC ATG GGG TAT CAG GGA GAT G R: CAA AGC GCG TAA CCG GAT TGG	*FOX*
462	F: TGG CCA GAA CTG ACA GGC AAA R: TTT CTC CTG AAC GTG GCT GGC	*CIT*
405	F: AAC TTT CAC AGG TGT GCT GGG T R: CCG TAC GCA TAC TGG CTT TGC	*DHA*
302	F: TCG GTA AAG CCG ATG TTG CGG R: CTT CCA CTG CGG CTG CCA GTT	*EBC*
346	F: AAC AGC CTC AGC AGC CGG TTA R: TTC GCC GCA ATC ATC CCT AGC	*ACC*
520	F: GCT GCT CAA GGA GCA CAG GAT R: CAC ATT GAC ATA GGT GTG GTG C	*MOX*

Results :

Results show that 80 (79.2%) of the 101 bacterial isolates were β-lactam resistant. It was shown that more than half β-lactam resistant bacterial isolates 62 (61.4%) were able to produce β-lactamase. Out of 62 β-lactamase producing isolates, 44 (43.5%) isolates were ESBL positive during the initial screening, and 27 (26.7%) of them were indicating the presence of an ESBL by disk approximation test (Table-2).

Table (2): β-lactam resistant bacteria, β-lactamase producing bacteria, suspected of producing ESBL isolates , and ESBL producing of bacterial isolates

Bacteria	No.(%) of isolates were β-lactam resistant	No.(%) of B-lactamase producing isolates	No.(%) of suspected ESBL isolates	No.(%) of ESBL producing isolates
***E. coli* (n=64)**	52 (51.5%)	38 (37.6%)	27 (26.7%)	20 (19.9%)
***Klebsiella* spp.(n=28)**	19 (18.8%)	15 (14.9%)	12 (11.9%)	6 (5.9%)
***Enterobacter* spp.(n=9)**	9 (8.9%)	9 (8.9%)	5 (4.9%)	1 (0.9%)
Total (n=101)	80 (79.2%)	62 (61.4 %)	44 (43.5%)	27 (26.7 %)

Overall, among eighty of isolates were β-lactam resistant, 32 isolates were deemed resistant and intermediate to cefoxitin by Kirby Bauer disc diffusion test were suspected as AmpC producers. This included *E. coli* (n=16), *Klebsiella* spp. (n=9) and *Enterobacter* spp. (n=7) (Table-3).

Table (3): Cefoxitin susceptibility of bacterial isolates

Bacteria	Susceptible	Intermediate	Resistant	No. of Bacterial isolate were deemed resistant and intermediate to cefoxitin
***E. coli* (n=64)**	48 (47.5%)	8 (7.9%)	8 (7.9%)	16 (15.9 %)
***Klebsiella* spp.(n=28)**	19 (18.8%)	2 (1.9%)	7 (6.9%)	9 (8.8 %)
***Enterobacter* spp.(n=9)**	2 (1.9%)	0 (0.0%)	7 (6.9%)	7 (6.9 %)
Total (n=101)	69 (68.2%)	10 (9.8%)	22(21.7%)	32 (31.6 %)

Out of 32 cefoxitin resistant and intermediate isolates, AmpC phenotype was confirmed by disk-based inhibitor method in 22.7 % (n=23) (Table-4), whereas, 5 (4.9 %) isolates were identified as inducible AmpC β- lactamase producers by the ceftazidime-imipenem antagonism test (Table-5).

Table (4): Phenotypic characterization of AmpC B-lactamase producing isolates

Bacteria	No.(%) of isolates with negative disk-based inhibitor method	No.(%) of isolates with positive disk-based inhibitor method	No. of isolates were deemed intermediate and resistant to Cefoxitin
***E. coli* (n=64)**	4 (3.9 %)	12 (11.9 %)	16 (15.9 %)
***Klebsiella sp.* (n=28)**	5 (4.9 %)	4 (3.9 %)	9 (8.8 %)
***Enterobacter sp.* (n=9)**	0 (0.0 %)	7 (6.9 %)	7 (6.9 %)
Total (n=101)	9 (8.9 %)	23 (22.7%)	32 (31.6%)

Table (5): Numbers and percentage of bacterial isolates able to produce inducible AmpC B-lactamase

Bacteria	No.(%) of isolates with negative ceftazidime-imipenem antagonism	No.(%) of isolates with positive ceftazidime-imipenem antagonism	No. of isolates were deemed intermediate and resistant to Cefoxitin
***E. coli*(n=64)**	14 (13.9 %)	2 (1.98 %)	16 (15.9 %)
***Klebsiella* spp. (n=28)**	8 (7.9 %)	1 (0.99 %)	9 (8.8 %)
***Enterobacter* spp. (n=9)**	5 (4.9 %)	2 (1.98 %)	7 (6.9 %)
Total (n=101)	27 (26.7 %)	5 (4.9 %)	32 (31.6%)

The overall occurrence of AmpC genes in the our study was determined to be 18(17.8%), were found as 11 (10.9 %) of *Escherichia coli*, 2 (1.9 %) of *Klebsiella* spp. and 5 (4.9 %) of *Enterobacter* spp. when detected by conventional PCR(Table-6).

Table (6): Occurrence of AmpC β-lactamase among bacterial isolates

Bacteria	No.(%) of AmpC gene positive isolates	No.(%) of AmpC gene Negative isolates	No. of bacterial isolates
E. coli	11 (10.9 %)	53 (52.5 %)	64 (63.4 %)
***Klebsiella* spp.**	2 (1.9 %)	26 (25.7 %)	28 (27.7 %)
***Enterobacter* spp.**	5 (4.9 %)	4 (3.9 %)	9 (8.9 %)
Total	18 (17.8 %)	83 (82.2%)	101 (100 %)

All AmpC positive isolates showed diversity presence of plasmid- mediated AmpC β-lactamases. Occurrence of AmpC genes among *E. coli* were found as *FOX (6.25 %), CIT* (4.7%), *EBC* (3.1%), *ACC* (3.1%) and *MOX* (14%), but *DHA* was absent. Occurrence of *AmpC* genes were found as *FOX* (7.1%), *CIT* (3.57%) and *DHA* (3.57%), but EBC, ACC and MOX were absent among *Klebsiella* spp. While, the Occurrence of AmpC genes among *Enterobacter* spp. was found as *EBC* (55.5%), *CIT*, *DHA* and *ACC* (each 11.1%) but *FOX* and *MOX* were absent, Table -7, Figures 1-6.

Table (7): The distribution of plasmid mediated AmpC-genes among the isolate

Plasmid mediated AmpC B-lactamase gene family						No. of bacterial bacterial isolates
FOX	***EBC***	***MOX***	***CIT***	***DHA***	***ACC***	
2 (7.1%)	0 (0%)	0 (0%)	1 (3.57%)	1 (3.57%)	0 (0%)	***Klebsiella* spp. (n=28)**
0 (0%)	5 (55.5%)	0 (0%)	1 (11.1)	1 (11.1)	1 (11.1)	***Enterobacter* spp. (n=9)**
4 (6.25%)	2 (3.1%)	9 (14%)	3 (4.7%)	0 (0%)	2 (3.1%)	***Escherichia coli* (n=64)**

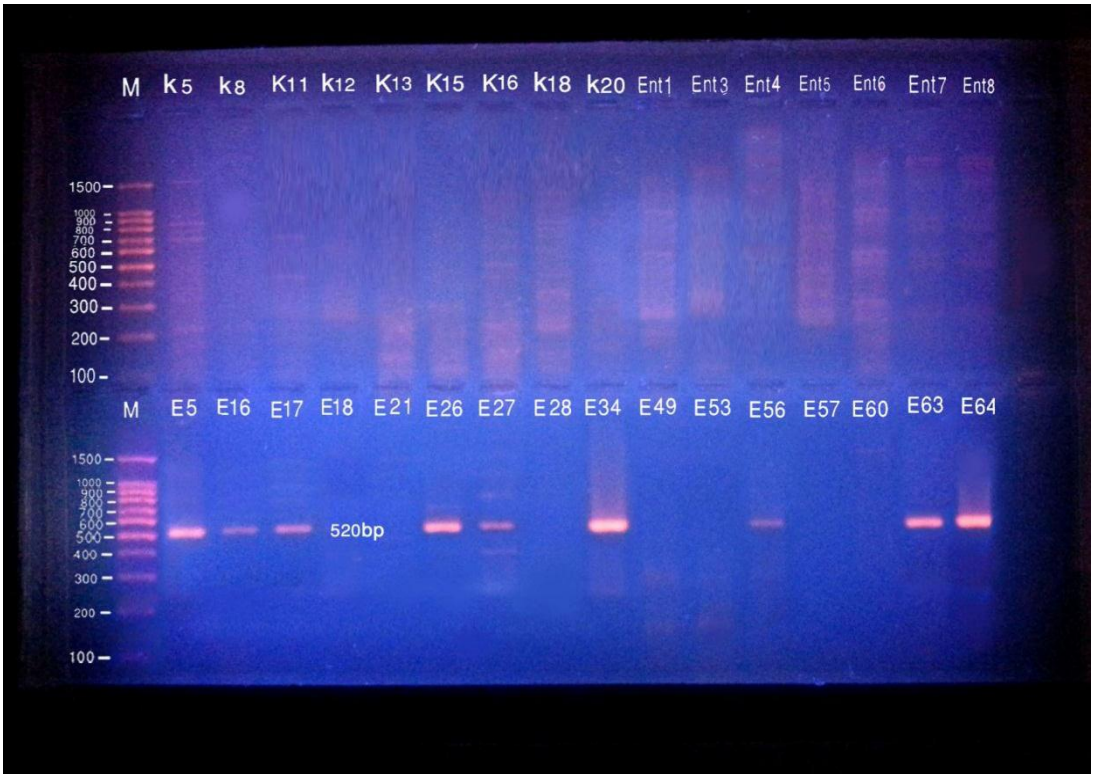

Figure (1): Extracted DNA of suspected isolates amplified with *MOX* gene primers

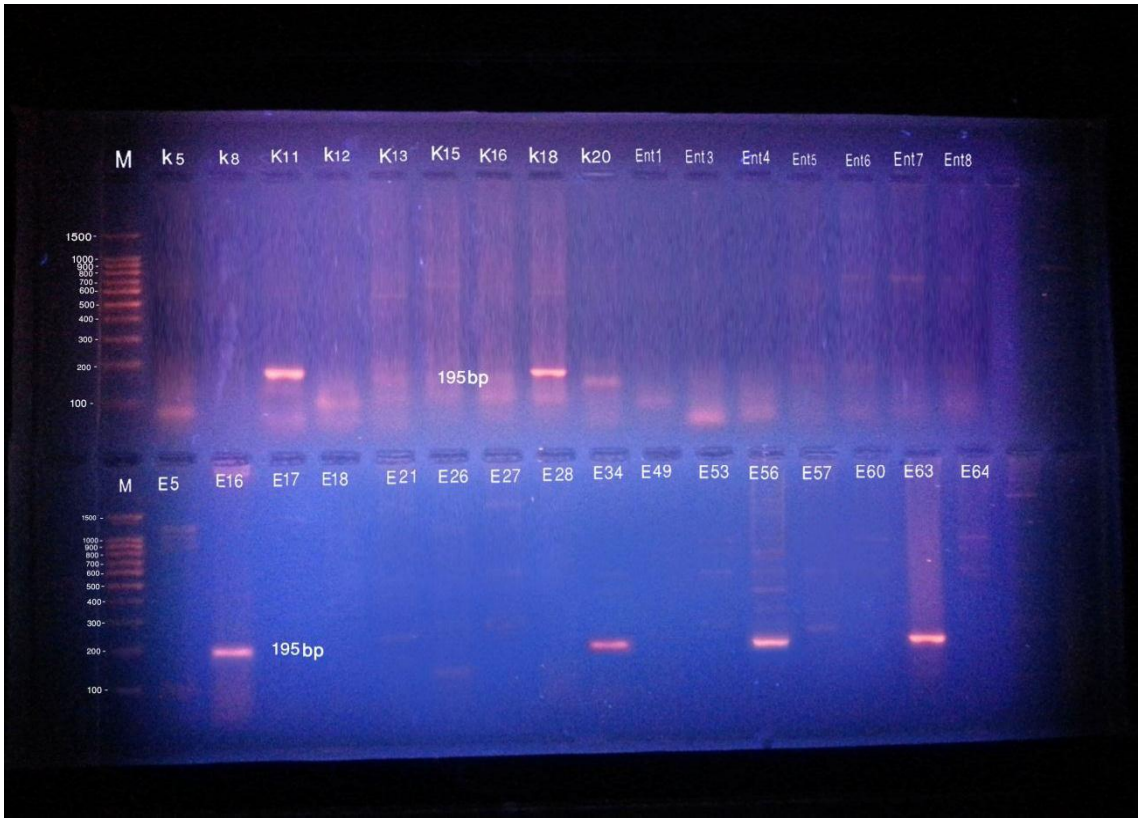

Figure (2): Extracted DNA of suspected isolates amplified with *FOX* gene primers

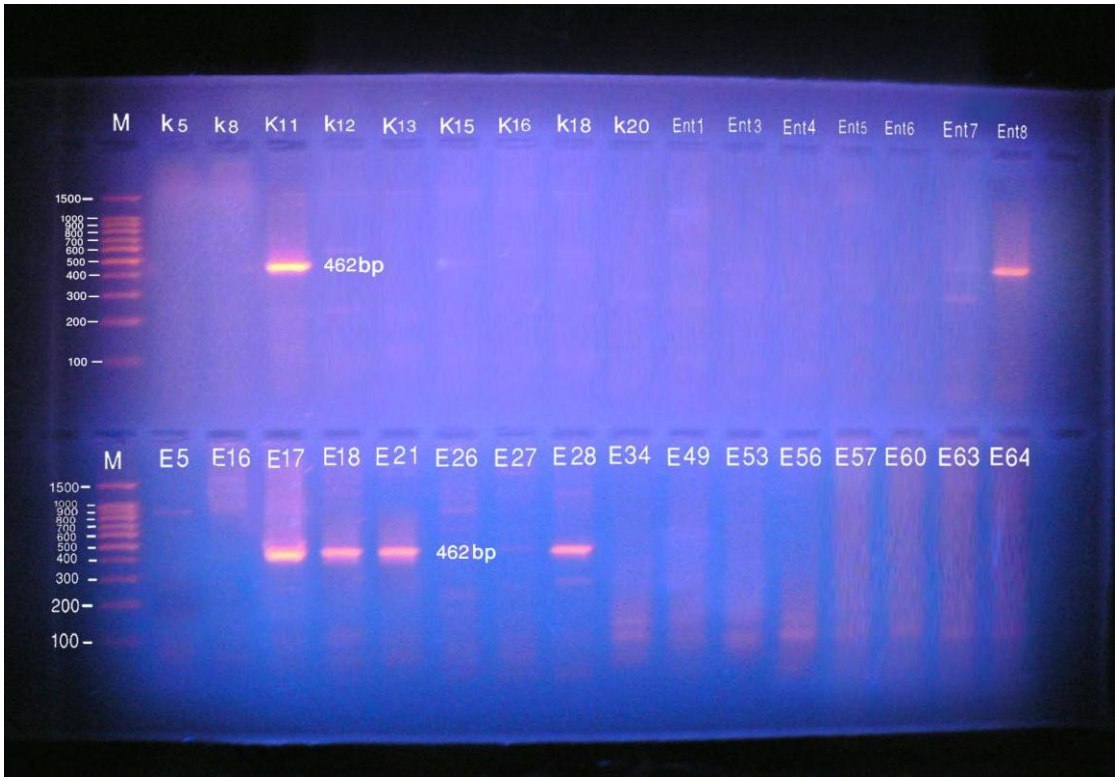

Figure (3): Extracted DNA of suspected isolates amplified with *CIT* gene primers

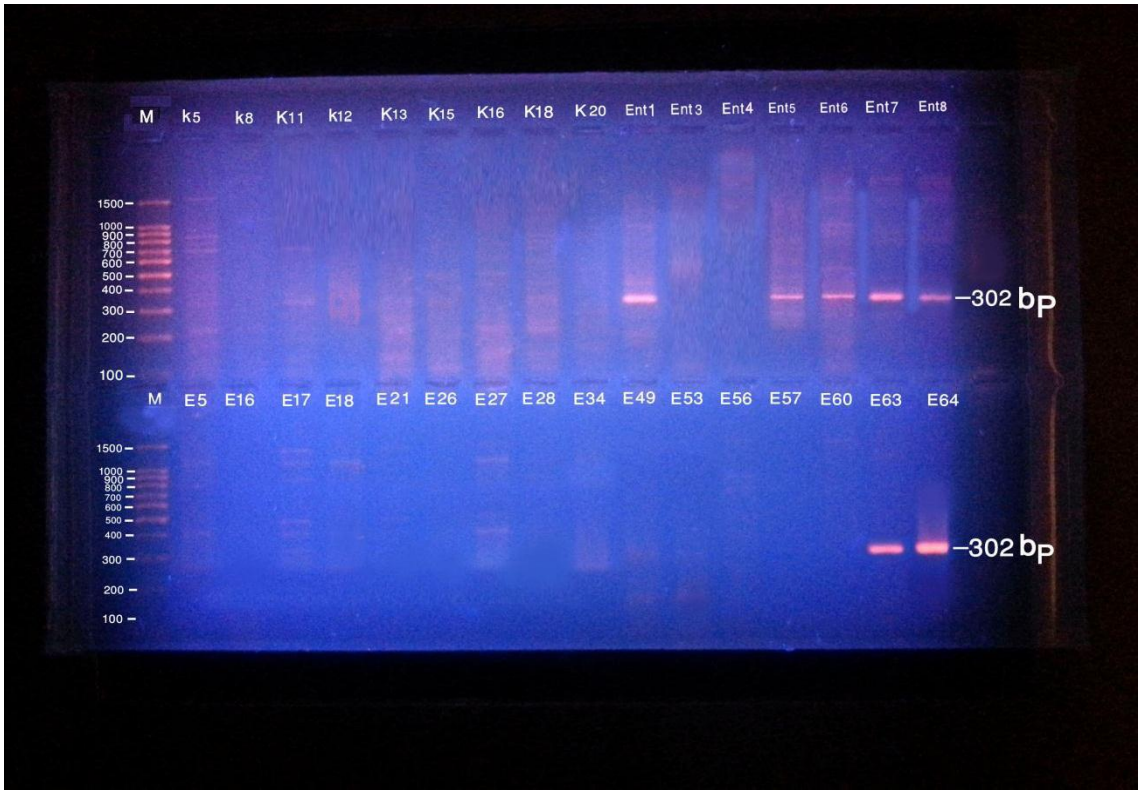

Figure (4): Extracted DNA of suspected isolates amplified with *EBC* gene primers

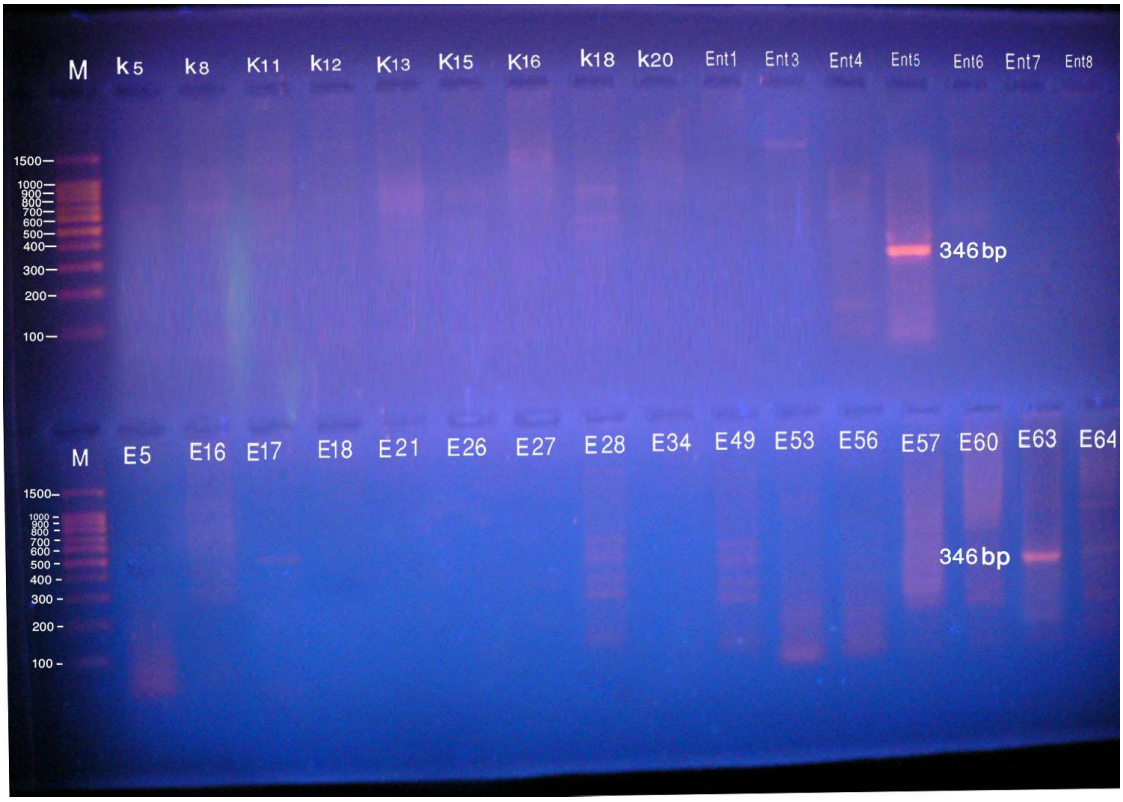

Figure (5): Extracted DNA of suspected isolates amplified with *ACC* gene primers

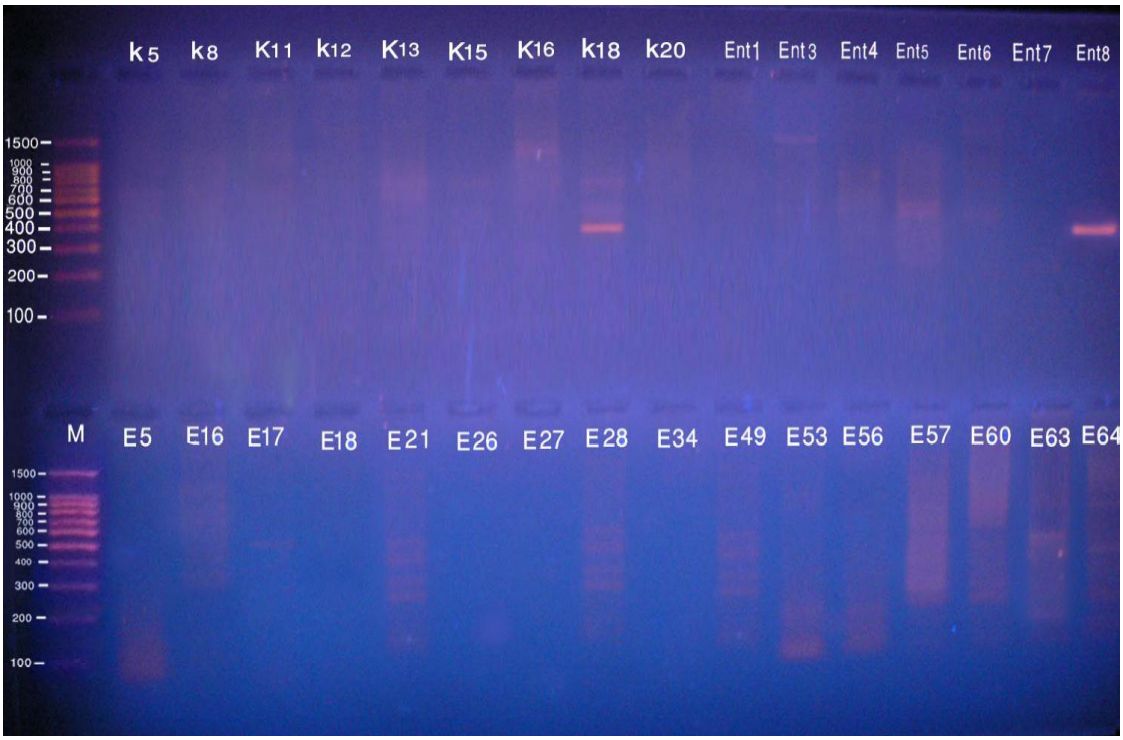

Figure (6): Extracted DNA of suspected isolates amplified with *DHA* gene primers

Among the 11 *AmpC* carrying *Escherichia coli* isolates, highest resistant (100%) to Cefotaxime, Aztreonam, Amoxiclave, Carbencillin, Pipercilin, Nalidixic acid, Ampicillin, Cotrimethoxazole, Ciprofloxacin and Levofloxacin (Table-8). In addition, a high resistance level against Ceftriaxone, Cefixime, Cefepime (90% each). While the 2 *AmpC* carrying *Klebsiella* spp. isolates had 100% resistance to Ceftriaxone, Ceftazidime, Cefotaxime, Cefixime, Aztreonam, Nitrofurantion, Carbencillin, Pipercilin, Cefepime, Nalidixic acid and Ampicillin. Resistance of the 5 *AmpC* carrying *Enterobacter* spp. isolates to the tested antibiotics revealed (100%) resistance to Ceftazidime, Cefotaxime, Carbencillin, and Ampicillin. Table (8) also reveales that Norfloxacin, Amikacin, Ciprofloxacin and Levofloxacin were considered the most potent antibiotics, which was found to be effective against 100% of the AmpC harboring *Enterobacter* spp.
Additionally, Imipenem and Meropenem were found to be effective against 60% of the *AmpC* harboring *E. coli*, 50% of *Klebsiella* spp.and 80% of *Enterobacter* spp.

Table (8): Antibiotic resistance pattern of AmpC harboring bacterial isolates

Antibiotic	*E. coli* (n=11)	*Klebsiella* spp. (n=2)	*Enterobacter* spp. (n=5)
Cefoxitin	(80%)	(100%)	(100%)
Ceftriaxone	(90%)	(100%)	(80%)
Ceftazidime	(100%)	(100%)	(100%)
Cefotaxime	(100%)	(100%)	(100%)
Cefixime	(90%)	(100%)	(80%)
Aztreonam	(100%)	(100%)	(40%)
Imipenem	(40%)	(50%)	(20%)
Meropenem	(40%)	(50%)	(20%)
Amoxiclave	(100%)	(50%)	(80%)
Gentamicin	(80%)	(0.0%)	(20%)
Norfloxacin	(80%)	(50%)	(0.0%)
Nitrofurantion	(10%)	(100%)	(60%)
Carbencillin	(100%)	(100%)	(100%)
Pipercilin	(100%)	(100%)	(60%)
Amikacin	(30%)	(50%)	(0.0%)
Cefepime	(90%)	(100%)	(40%)
Nalidixic acid	(100%)	(100%)	(20%)
Ampicillin	(100%)	(100%)	(100%)
Cotrimethoxazole	(100%)	(50%)	(80%)
Ciprofloxacin	(100%)	(50%)	(0.0%)
Levofloxacin	(100%)	(50%)	(0.0%)

Discussion :

There are numbers of surveillance studies seeking clinical strains producing AmpC β-lactamase to detect the resistance mechanism of antibiotics. The present study showed plasmid mediated AmpC β lactamases in 11 (10.9 %) of *Escherichia coli* isolates, 2 (1.9 %) of *Klebsiella* spp. and 5 (4.9 %) of *Enterobacter* spp.
Plasmid AmpC β-lactamases have differential activity on β-lactamases inhibitors, *Escherichia coli* derived enzymes have shown to exhibit resistance to inhibitor combinations with possible exception of piperacillin-tazobactam[12]. In previous Iraqi studies, cefoxitin resistant strains

were tested for the production of AmpC β lactamases by three dimensional extract methods [13, 14,15]. Indian study has recommended use of piperacillin and piperacillin-tazobactam discs for AmpC screening [16]. Cefotetan with phenyl boronic acid has also been used to detect AmpC especially *MOX, FOX, ACC* producing isolates [17].

AmpC β-lactamases also have differential activity on substrates. *Escherichia coli* with *ACC* can be resistant to ceftazidime but not to cefotaxime or cefotetan while an isolate with *DHA* may show intermediate resistance to cefoxitin but susceptible to cefotaxime or ceftazidime due to hyperproduction of chromosomal AmpC together with *OmpF* porin loss. Furthermore, not all strains with plasmid-mediated AmpC enzymes meet the NCCLS criteria for resistance to cephamycins [1].

In present study, sizeable numbers of cefoxitin resistant isolates were not positive for AmpC production by the disc potentiation test or PCR; this warrants further investigation into the other mechanisms of resistance and their laboratory detection such as mutations in some organisms that reduce influx (outer membrane porin loss) or enhance efflux (efflux pump activation). Clinical isolates rarely express more than one plasmid-mediated AmpC β-lactamases[18].Two reasons could explain this observation. First, the inability of current phenotypic tests to accurately detect the type of transferable AmpC β-lactamase does not allow for the differentiation of multiple AmpC enzymes. Second, it is possible that there is a limit to the amount of AmpC β-lactamase that a bacterial cell can accommodate and still be a viable pathogen [18]. A single type of test will not be able to accurately characterize the resistance mechanisms in these complex organisms. Although automated systems are available for susceptibility testing, the accuracy of these is inadequate for organisms expressing plasmid-mediated AmpC β-lactamases alone or in combinations with ESBLs [19, 20].

Conventional phenotypic methods used to detect isolates expressing AmpC β-lactamases have restricted the detection of this resistance mechanism to mainly organisms without an inducible chromosomal *ampC* gene, such as *K. pneumoniae* and *E. coli* [17,20]. In *K. pneumoniae* no chromosomal gene is present. Therefore, no endogenous AmpC β-lactamase can interfere with either susceptibility testing or hydrolysis assays [19]. Since *E. coli* produces its chromosomal *ampC* gene at a low constitutive level, the endogenous enzyme has little influence on susceptibility testing or β-lactamase hydrolysis assays [20]. However, molecular analysis will be required to verify the presence of transferable *ampC* genes in hyperproducing *E. coli* or gram-negative pathogens coding for inducible chromosomal AmpC β-lactamases.

The use of cefoxitin resistance as a screening agent/marker for AmpC production is quite reliable with a good negative predictive value as found in this study. The disc potentiation test reliably detected AmpC β-lactamase when compared with the PCR technique in the present study. It should be noted that AmpC β-lactamases can be encoded on chromosomal- or plasmid-mediated genes or both, as exemplified by one of our isolates. This raises questions as to how AmpC has found its way onto a plasmid. Some enteric pathogens may also be cephamycin resistant by virtue of porin mutations [18].

Clinical laboratories interested in distinguishing AmpC mediated resistance from other β-lactamase resistance mechanisms will need to use combination of phenotypic and molecular identification methods. The PCR technique described in this study will be an important tool for the detection of plasmid-mediated AmpC β-lactamases genes in Gram-negative bacteria.

In the present study, Multi Drug Resistant(MDR) among AmpC positive study isolates was high suggesting plasmid mediated spread. Current therapeutic options include use of cefepime or carbapenems[20], however, the high co-carriage of ESBL and AmpC in this study and the fact that majority of these were nosocomial in origin is a cause for concern.

This study findings indicate the necessity for continued surveillance of mechanisms of resistance among clinical and nosocomial pathogens.

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