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PREVALENCE OF *AMPC* β-LACTAMASE GENES AMONG CLINICAL ISOLATES OF *ENTEROBACTER* SPP. FROM SOME IRAQI HOSPITALS.

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ABSTRACT

Background: Resistance to broad spectrum β-lactams mediated ESBLs and AmpC β-lactamase enzymes is a rising problem worldwide. Determination of their prevalence is essential to the creation of an effective antibiotics policy and hospital infection control measures. **Method:** A total of 35 clinical isolates of *Enterobacter* spp. that were collected from different clinical specimens during the period between October 2015 to January 2016 were studied. Antibiogram profile was done by disc diffusion test (DDT), along with recommended tests for phenotypic detection of ESBLs and AmpC production. Isolates were also screened for their possessions of AmpC β-lactamase-encoding genes by using polymerase chain reaction (PCR) technique. **Results:** Antibiogram revealed that most *Enterobacter* spp. isolates were multi-drug resistant with a high resistance to cephalosporins; cephalexin(100%), cefazolin and cefotaxime(94.28%), while Susceptibility to imipenem. was highest(20%) followed by amikacin, pipracilli/tazobactam (22.85%).The phenotypic detection of ESBL and AmpC β-lactamases revealed that 25(71.42%), 29(82.85%) were ESBLs and AmpC β-lactamase producers respectively, in addition to 22(62.85%) of isolates were gave positive results for both ESBL and AmpC β-lactamases production. Detection of *AmpC* encoding genes revealed that a total of 28(96.55%) of 29 *Enterobacter* spp. were possessed *AmpC* genes that encoding for AmpC β-lactamases, with predominance of *blaCIT*, *blaEBC* and *blaDHA* genes, 16(57.14%), 11(39.28%), 8(28.57%) respectively. **Conclusions:** Phenotypic and genotypic methods for detection of AmpC-β-lactamese-producing *Enterobacter* spp. revealed that most of these isolates were AmpC β-lactamase producers;29(82.85%) and 28(96.55%) respectively, with predominance of CIT 16(45.71%) followed by 10(28.57%) , 8(22.85%) for EBC and DHA respectively. furthermore, 22(62.85%) of isolates were gave positive results for both ESBL and AmpC β-lactamases production.

KEYWORDS: *Enterobacter* spp., Amp C β-lactamases, ESBLs, PCR.

INTRODUCTION

In the preantibiotic era, species in the *Enterobacter* genus were not encountered in surveys of nosocomial infections. However, by the 1970s, they were becoming more and more important hospital-acquired pathogens[1,2],*Enterobacter* spp. can cause many infections, including: bacteremia, eye and skin infections, pneumonia, urinary tract infections, meningitis, wound and intestinal infections.[3] The predominant cause of resistance to β-lactam antibiotics in gram negative bacteria is the production of β-lactamase, which are encoded chromosomally or plasmids and deactivate β-lactams by hydrolyzing the four membered of β-lactam ring.[4] Some of these β-lactamses enzymes include Extended spectrum β-lactamase, AmpC β-lactamase, and carbapenemase [5].AmpC lactamase are clinically important because they may confer resistance to pencillins, cephalosporins, oxyamino-cephalosporins, cephamycins and monobactams and are not inhibited by β-lactam/β-lactamase inhibitors combinations.[6,7] In many bacteria AmpC enzymes are inducible and can be expressed at high levels via mutation. Over expression confers resistance to broad spectrum cephalosporins including: ceftazidime, cefotaxime, and ceftriaxone and this a problem especially in infections due to *Enterobacter aerogenes* and *Enterobacter cloacae*, where an isolates initially susceptible to this agents may become resistant during therapy.[8] Commonly reported AmpC β-lactamase genes are *CIT*,*MOX*, *FOX*, ACC, *DHA* and EBC genes.[9]

Detection of AmpC-producing organisms is important to ensure effective therapeutic intervention and optimal clinical outcomes. Many clinical laboratories currently test *Enterobacter* spp. for ESBLs production but do not try to detect AmpC β-lactamases. Many infectious disease personnel remain unaware of the clinical important of AmpC β-lactamases. Furthermore, AmpC β-lactamases lead to false *in vitro* susceptibility to

cephalosporins. In Iraq Amp C detection is not routinely done in most laboratories. So this study was aimed to detect of the ability of *Enterobacter* spp. to produce AmpC β-lactamase enzymes phenotypically, as well as genotypic detection of β-lactamase genes that responsible for encoding of these enzymes.

MATERIALS AND METHODS

A total of 35 clinical isolates of *Enterobacter* spp. were isolated from various clinical specimens such as: blood, urine, wounds and sputum, and skin swab, these specimens were collected over a period of three months (October 2015 to January 2016) from some hospitals in Baghdad city/Iraq. The isolates were identified using VITEK® 2 Compact system.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing (Antibiogram) were performed by the disc diffusion method on Mueller-Hinton agar based on the CLSI guidelines.The antibiotic disks used were: amoxicillin(25 µg), cefazolin (30µg), cephalexin (30µg), cefoxitin (30 µg), ceftriaxone(30 µg), ceftazidime(30 µg), cefotaxime (30µg), cefepime (30µg), amoxicillin/clavulanic acid(20 µg /10 µg),ampicillin\sulbactam(10µg /10µg), pipracilli/tazobactam (100 µg/10µg), aztreonam(10µg), imipenem (10µg), trimethoprime\ sulfamethoxazole (1.25µg /23.75µg), ciprofloxacin (5µg), tetracycline (30µg), amikacin (30µg), chloramphenicol (30µg), gentamicin (10µg).

Results were interpreted as sensitive, intermediate, resistant according to CLSI guidelines.[10]

Phenotypic testing for ESBLs

Isolates were tested for ESBLs production via the combination disk method by using cefepime disks (30 µg) with or without clavulanic acid(10 µg). A ≥5mm increase in diameter of the inhibition zone of the cefepime with clavulanic acid,when compared to the cefepime alone,was interpreted as phenotypic evidence of ESBL production.[10]

Phenotypic testing for AmpC

The detection set comprises three disks: disk A (cefpodoxime 10 µg and AmpC inducer), disk B (cefpodoxime 10 µg, AmpC inducer and ESBL inhibitor) and disk C (cefpodoxime 10 µg, AmpC inducer, ESBL inhibitor and AmpC inhibitor).The test was performed and interpreted in accordance to the manufacturer's instructions (MASTDISCS™ID, UK).

Molecular analyses of *AmpC* β-lactamase genes

All *Enterobacter* spp. isolates were analyzed by PCR for molecular detection of genes that encoding for AmpC β-lactamase enzymes. Bacterial DNA was extracted using presto™ Mini genomic DNA purification Kit supplemented by (Geneaid, Thiland), and an initial multiplex screening PCR was performed in which, four micro liters of the DNA were mixed with PCR mixture that composed of 25 µl of GoTaq®Green Master Mix, 1.5 µl primer of MOXF,MOXR,CITF,CITR, DHAF,DHAR and 1.25 µl primers of ACCF,ACCR,EBCF,EBCR and 1 µl primer of FOXF, FOXR genes and 5 µl of nuclease free water was added to get final volume 50µl.[11] The primers used for PCR are listed in **Table 1**. The cycling conditions of amplification were applied according to Manoharan *et al*. (2012) which consist of an initial denaturation at 95 for 2 min, followed by 30 cycles of DNA denaturation at 94 for 45 sec, primer annealing at 62 for 45 sec, and primer extention at 72 for 1 min. After the last cycle, a final extention step at 72 for 5 min was added.

Table: 1 Primers used for amplification of *AmpC* β-lactamase genes.[12]

Primers name	primers sequence 5′----------→3′	Accession number	Product size (bps)
MOXMF	GCT GCT CAA GGA GCA CAG GAT GATGAT	D13304	520
MOXMR	CAC ATT GAC ATA GGT GTG GTG C		
CITMF	TGG CCA GAA CTG ACA GGC AAA	X78117	462
CITMR	TTT CTC CTG AAC GTG GCT GGC		
DHAMF	AAC TTT CAC AGG TGT GCT GGG T	Y16410	402
DHAMR	CCG TAC GCA TAC TGG CTT TGC		
ACCMF	AAC AGC CTC AGC AGC CGG TTA	AJ133121	346
ACCMR	TTC GCC GCA ATC ATC CCT AGC		
EBCMF	TCG GTA AAG CCG ATG TTG CGG	M37839	302
EBCMR	CTT CCA CTG CGG CTG		

	CCA GTT		
FOXMF	AAC ATG GGG TAT CAG GGA GAT G	X77455	190
FOXMR	CAA AGC GCG TAA CCG GAT TGG		

RESULTS

Thirty five isolates of *Enterobacter* spp. were collected from different specimens including: blood (24) isolates, urine (6) isolates, (2) isolates for each of wounds and sputum and one isolate from skin swab, which distributed as follows: 29, 4, and 2 isolates belong to *E. cloacae*, *E. aerogene*, and *E. sakazakii*, respectively.

Antimicrobial susceptibility test

Antibiogram showed high percentage of resistance to cephalexin (100%), cefazolin and cefotaxime (94.28%), amoxicillin (91.42%), amoxicillin/clavulanic acid and cefoxitin(82.85%), ceftriaxone (77.14%), aztreonam(68.57%), trimethoprime\ sulfamethoxazole (62.85%), ampicillin\sulbactam (60%), gentamicin (57.14%), ceftazidime(48.57%), cefepime(40%), tetracycline (37.14%), chloramphenicol (31.42%), ciprofloxacin(28.57%). While Susceptibility to imipenem was highest (20%) followed by amikacin, pipracilli/tazobactam(22.85%).

Phenotypic detection of ESBLs and AmpC β-lactamases production.

Out of 35 isolates, 25(71.42%) isolates were ESBLs producers and 29(82.85%) isolates were gave positive result for production of AmpC β-lactamase enzymes. Moreover 22(62.85%) of *Enterobacter* spp. isolates were gave positive for both ESBL and AmpC β-lactamase production tests **(Figure 1, 2)**

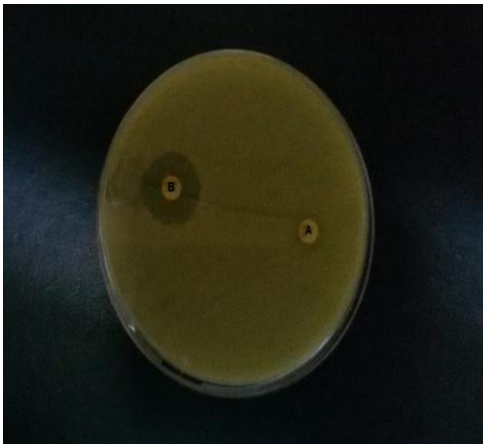

Figure 1: Positive ESBLs producing *Enterobacter* spp.: (A)Cefepime, (B) Cefepime +Clavulanic acid

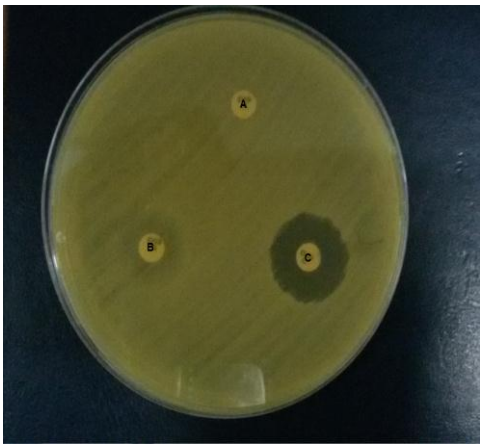

Figure 2: Positive AmpC producing *Enterobacter* spp.(A) cefpodoxime 10 µg and AmpC inducer (B) cefpodoxime 10 µg, AmpC inducer and ESBL inhibitor (C)cefpodoxime 10 µg, AmpC inducer, ESBL inhibitor and AmpC inhibitor.

3-5 Detection of AmpC genes by PCR

A total of 28(96.55%) of 29 *Enterobacter* spp. positive for phenotypic AmpC test, were possessed AmpC genes that encoding for AmpC β-lactamases,with predominance of *blaCIT*, *blaEBC* and *blaDHA* genes. The prevalence of these genes was as follows: 16(57.14%) of isolates with 462 bp amplified product, 11(39.28%) of isolates with 302 bp amplified product, and 8(28.57%)of isolates with 405 bp amplified product respectively. In addition to some isolates were carried more than one gene; bla*CIT-blaEBC-blaDHA* together were reported in one isolates(2.85%), *blaCIT – blaEBC* and *blaCIT - blaDHA* in 2 isolates (5.71%) and *blaEBC-blaDHA* in one isolates (2.85%). While 31.42%, 17.14%, 11.42 % of isolates were possesses only *blaCIT*, *blaEBC*, *blaDHA* gene respectively.

DISCUSSION

Antibiogram profile revealed that the local isolates of *Enterobacter* spp. were possess highly resistance towards most antimicrobial under study, in other sense were multi-drug resistant pathogens, especially towards cephalosporins, wherein the resistance patterns of isolates were have the following patterns:100% for cephalexin ,94.28% for cefazoline and cefotaxime, 82.85% for Cefoxitin, and77%for ceftriaxone.on other hand, the most effective antimicrobials were imipenem, amikacin and piperacillin\tazobactam, while susceptibility of isolates towards other antimicrobials showed obvious differences in their patterns of resistance.

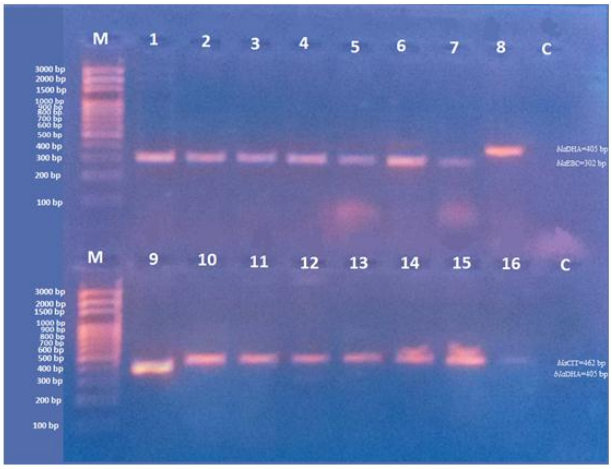

Figure 3: Gel electrophoresis for amplification of *bla*$_{CIT,EBC,DHA}$ gene of *Enterobacter spp.* electrophoresis was performed on 1.5 % agarose gel and run with a 7 Volt/cm for one hour, visualized under U.V after staining with ethidium bromide. M= DNA ladder 100-3000 bp ; 1-7= *bla*$_{EBC}$ (302bp) ; 8,9= *bla* DHA(405bp) ; 10-16=*bla*CIT(462bp) ; C=Negative control (contain all precursors of PCR mixture without DNA to be amplified)..

In Iraq several studies showed high resistance patterns among *Enterobacter* spp., AL-Atby[13] showed that *Enterobacter* spp isolated from different sample in *Baqubah* city were have the following resistance patterns:100 % for imipenem,80% for ceftazidime and aztreonam,70% for cefotaxime, 50% for augmentin, 30% for gentamicin, 10% for amikacin and ciprofloxacin, while in Erbile Saeed *et al*.[14] noticed that resistance patterns among five *Enterobacter aerogenes* isolated from urine as follows: 100% for cefotaxime, 80% for trimethoprim-sulfamethoxazole and ceftriaxone, 60% for augmentin and cephalexin, 40% for chloramphenicol and ciprofloxacin, 20% for gentamicin, 0% for amikacin, Abid *et al*.[15] showed that resistance among 15 *Enterobacter cloacae* collected from a variety of clinical sources were 100% to ampicillin and cepholothin , 66 % to cefotaxime, 60% to cefoxitin, 53%, ceftazidime , 26% to aztreonam, whereas cefepime, imipenem ,aminoglycoside and gentamicin were 6% and the resistance rate of isolates to the remaining antibiotics: trimethoprim-sulfamethoxazole, chloramphenicol and naldixic acid were (100%,60% ,26%) respectively.

In Turkey noticed that susceptibility profile of *Enterobacter* spp. isolates were as follows: 100% for augmentin, amikacin, ceftazidime and ciprofloxacin, 92.31 % for cefepime, 61.54% for ceftriaxone ,cefoxitin and imipenem, 30.77% for gentamicin, 7.96% for trimethoprim-sulfamethoxazole.[16] While in Iran the resistance patterns among *Enterobacter* spp. isolated from blood and urine were as follows: 40% for ciprofloxacin and gentamicin, 20% for ceftazidime, amikacin and tetracycline, 0% for imipenem[17]

Enterobacter spp. readily develop resistance to second and third generation cephalosporins owing to an inducible β-lactamase, and this is associated with previous use of extended-spectrum cephalosporins. *Enterobacter* isolates may initially test as being susceptible to cephalosporines *in vitro*, but the emergence of resistance occurs during therapy because of increased β-lactamase production. The emergence of antibiotic resistance in *Enterobacter* has impacted the clinical outcome of affected patients in some studies which showed that infection with multi-resistant *Enterobacter* spp. was associated with a higher mortality rate.[18]

Despite the relative variation in the pattern of resistance of *Enterobacter* spp. towards the antimicrobials in the studies mentioned previously, which is due to many reasons like geographical differences, size of studied samples, site of infection, source of specimens,but obviously from the general context of the antibiogram that the *Enterobacter* spp. isolates were multi drug-resistant pathogens and they have a relatively high resistance to cephalosporins as well as the more effective antimicrobials were imipenem and amikacin and this is consistent to some extent with this results. It is worthy to note that the results of Antibiogram have indicator that the local isolates may possess AmpC encoding genes.

ESBLs are plasmid-mediated bacterial enzymes derived from older, narrow spectrum β-lactamases (e.g. TEM-1, TEM-2, SHV-1).[19] Wide spread use of third generation cephalosporins and aztreonam is believed to be the major cause of the mutations in these enzymes that has led to the emergence of the ESBLs.[20] ESBLs have evolved remarkably during the last 20 years and the organisms producing these genes are responsible for increasing in nosocomial infections, morbidity and mortality especially amongst patients on intensive care and high dependency units.[21]

Results showed that there is a high percentage of isolates under study were ESBLs producers, which is an indication of possess of those isolates to a high resistance to many of antibiotics and therefore difficult to treat infections caused by these bacteria that causing a threat to the patients and increase the morbidity and mortality. Many reports pointed to the apparent variance in the rate of production of the ESBLs enzymes among *Enterobacter* spp. isolates. In Iraq: Jarjees[22]; AL-Haidary[23] from Erbile reported that (60% ,100%) of *Enterobacter spp.* were ESBLs producers respectively. On the other hand, AL-Dahmoshi[24]; Abid *et al*. [25] in Al-Hilla noticed that(71.42%, 73%) of *Enterobacter cloacae* were ESBL producers. In Baqubah city, AL-Atby[13] noticed that 10% of *Enterobacter cloacae* isolates appeared positive to ESBLs and Salih *et al*. [26] didn't show any positive ESBL isolate among 120 isolates of *Enterobacter cloacae*.

In korea Pai *et al.* [27] have been reported that of 72 isolates of *Enterobacter* spp. isolated from blood stream patients in a tertiary hospital, 31(43%) isolates were detected as ESBLs producers, and in Taiwan Chen and Huang[28] have been reported that 28.57% of *Enterobacter* spp. isolated from patients with blood stream infections were identified as producing ESBLs. It is worth noting that some of the ESBLs-producing *Enterobacter* spp. in this study were resist to β-lactam\β-lactamase inhibitors (84.85% for amoxicillin\clavulanic acid, 60% for ampicillin\sulbactam, 22.85% for piperacillin\tazobactam,), to cephamycin (82.85% for cefoxitin), and to carbapenems (20% for imipenem).
These findings are not in line with phenotypic characteristics of common ESBLs, this discrepancy in the resistance pattern of ESBLs producers may be due to different reasons such as: possession of these isolates to AmpC β-lactamases that found concomitant with ESBLs enzymes. AmpC β-lactamases preferentially hydrolyze narrow- broad-, and expanded-spectrum cephalosporins and cephamycins and resist inhibition by β-lactamase inhibitors. Another reason may be as a result of production of another type of ESBLs enzymes that revealed resistance to β-lactamase inhibitors.

Finally, in regard to resistance to imipenem in seven studied isolates may be due to their ability to produce another type of β-lactamases called carbapenemase.

Enterobacter spp. are common pathogens frequently associated with infections in the intensive care units. Infection caused by AmpC β-lactamase producing bacteria usually associated with adverse clinical outcomes because this enzyme shows resistance to all β-lactam antibiotics except cefepime and carbapenems. The rampant use of broad-spectrum antibiotics can cause colonization with resistant strains with an increase in morbidity, mortality and significant economic loss.[29]

Results of AmpC detection showed the predominance of AmpC producers among studied isolates of *Enterobacter* spp. The spread of these organisms reduces the antibiotic alternatives for the treatment of infections by these pathogens to mainly carbapenems; which are often reserved for life threatening infections. So this study emphasizes need for routine detection and reporting of AmpC producers in medical facilities of Baghdad, so that measures are taken to avoid their uncontrolled spread and possible therapeutic failures. An antibiotic use policy is also imperative to limit the dissemination of these organisms.

In this study, the Antibiogram patterns of studied AmpC producing *Enterobacter* spp. isolates were in keeping with characteristics of AmpC producers, that is their resistantce to most β-lactam antibiotics like pencillins, cephalosporins, in particular of oxyimino cephalosporins, cephamycins and monobactam except for carbapenem and fourth generation cephalosporins (cefepime) and their resistance to β- lactamase inhibitors. In Iraq, Mohammed[29] has reported a higher frequency 13\13(100%) of AmpC β- lactamase Producers among *Enterobacter* spp., while Lafi[29] showed a low percentage 2/17(11.8%) of isolates were producing AmpC β-lactamases.

In Iran Shahandeh *et al*. (2016) noticed that (71.4%) of *Enterobacter* spp. were AmpC producing[31] A study in India have been reported that 53.84% of *Enterobacter* isolates were produced of AmpC β-lactamase[32], whereas another study in India revealed that 33.3% of *Enterobacter cloacae* were positive for AmpC production.[33] Apfalter *et al*.[34] have been showed that 197(95%) of 208 *Enterobacter* spp. isolated from different sample were AmpC producers. While study carried out in Malaysia revealed that 40 (34.2%) of 117 *Enterobacter* isolates were positive for AmpC gene [35]. Generally, AmpC β-lactamases are associated with multiple antimicrobial resistance, limiting the therapeutic regimens.[36]

In conclusion it is important for clinical microbiology laboratories to be able to detect the AmpC β-lactamase producing isolates because these enzymes have a risk for developing resistance during treatment of infected patients with broad spectrum pencillins and cephalosporines. AmpC β-lactamase have been associated with false *in vitro* suscebtibility to cephalosporins. Thus the type of β-lactamase produced by the organism should be detected along with the antibiogram before administering the β- lactam drug to the patient. The potential benefits would include better patient outcomes in term of avoiding inappropriate therapy, hence production of these enzymes in clinically significant Enterobacteriaceae represents an increasing problem resulting in higher morbidity and mortality, also failure to identity AmpC β-lactamase producers may lead to inappropriate antimicrobial treatment and may result in increased mortality, this is alarming and requires urgent action from both a therapeutic and infection control perspective.

The co-existent phenotypes could be due to the transfer of plasmids (encoding both AmpC and ESBL enzyme-producing genes) between members of the family *Enterobacteriaceae*. Multi-drug resistant strains are expected to be more common among organisms harboring genes for ESBL and AmpC β-lactamases.[36] Resistance to broad spectrum β-lactams, mediated by ESBL and AmpC β-lactamase enzymes among *Enterobacteriaceae* is a rising problem worldwide [37]. Extended-spectrum β -lactamases (ESBLs) have become increasingly prevalent in species characterized by inducible class C cephalosporinase (AmpC), such as *Enterobacter* spp., *Citrobacter freundii*, or *Serratia marcescens* [38].Although ESBLs have been reported at a much lower frequency among these chromosomal AmpC β-lactamase-producers,the occurrence of ESBL in these species is increasing[24] and represent a problem of serious concern due to the ability of spread resistance to

other bacterial species and because ESBLs are typically encoded by plasmids that also harbor genes for resistance to non β-lactam antibiotics.[38] Infections caused by such resistant organisms can result in intensive care unit (ICU) admission and prolong hospital stay.[39] Detection of ESBL and AmpC β-lactamase is not routinely carried out in many microbiology units of service laboratories. This could be attributed to lack of awareness or lack of resources and facilities to conduct ESBL identification.[40] Presence of isolates that harbor these enzymes in clinical infections making the selection of an effective antibiotic difficult[27],and can result in treatment failure if one of the β-lactam drugs, including extending Spectrum β- lactamase, is used.[37] Also inappropriate treatment of these complex infections can increase mortality and morbidity[39], and the co-existence of ESBLs and AmpCs in the same strain may lead to false negative tests for the detection of ESBLs by the current CLSI criteria[37]. So, fast detection of these enzymes allows for describe the more appropriate therapy and it is also an essential to formulate of hospital infection control measures. The emergence of plasmid-mediated AmpC and ESBL β-lactamase producing *Enterobacter* spp. may pose potential risk to the spread of antibiotic resistance in the clinical settings.
Phenotyping method is mainly used for identification of Amp C production in Gram-negative strains. Further genotypic experiments are essential to determine the mechanisms involved in the phenotypic expression of AmpC β-lactamase production in these isolates. PCR is a valuable tool for characterization of AmpC in medical and search settings and it is the gold standard method for detection of AmpC β-lactamase. Detection of *AmpC*-encoding genes by molecular techniques in producer bacteria may give benefit data about their epidemiology and risk factors related with these infections. Up to date, there are few reports to related incidence of *AmpC* β-lactamase genes in *Enterobacter* spp. worldwide and to the best of our knowledge, this is the first report of prevalence of *AmpC* β-lactamase genes in clinical isolates of *Enterobacter* spp. in Iraq.

The finding revealed presence of *blaCIT,blaEBC* and *blaDHA* genes among *Enterobacter* spp. with predominance of *blaCIT* gene. Although many studies have reported the variation in prevalence rates or the predominance of one of the AmpaC β-lactamase-encoding genes, however, there are some reports were fairly consistent with our findings in the presence or predominance of one or more of these three genes that referred above.

Kiratisin *et al*. [41] have mentioned the prevalence of *blaCIT,blaACC* genes in Thailand. A study in Taiwan reported presence of blaDHA in one *Enterobacter cloacae* isolates and *blaEBC* in three isolates [42]. Adwan *et al*. [43] noticed that only *blaDHA* gene was detected in clinical isolates of *Enterobacter cloacae* in Palestine with prevalence 4.9%.

CONCLUSION

Antibiogram profile showed that *Enterobacter* spp. isolates were having high resistance towards betalactam antibiotics with high rate of isolates showing multi-drug resistance. Also findings of phenotypic detection of ESBLs and AmpC enzymes revealed prevalence of extended-spectrum β-lactamases (71.42%) and AmpC(82.85%) among these isolates, in addition to more than half of isolates were possess the capacity to produce both ESBLs and AmpC concurrently 22(62.85%), which would confer to them resistant to a variety of antibiotics. PCR showed that blaCIT genes were the most common AmpC-encoding genes (57.14%) followed by blaEBC (39.28%) and blaDHA (28.57%) genes respectively, so detection of these enzymes among isolates in the laboratory is very important to select appropriate therapy and prevent treatment failure which cause higher morbidity and mortality.

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