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Phenotypic and genotypic detection of Enterobacter spp isolated from clinical cases

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***Abstract*---**Enterobacter Spp an opportunistic bacteria that causes a variety of infections in hospitalized patients around the world, can resist -lactams primarily by generating the CTX-M enzymes and AmpC -lactamase enzyme. The spread of ESBL is a serious public health concern, and infections with ESBL-producing organisms have been linked to negative outcomes. We looked at the prevalence and screening criteria for extended-spectrum-lactamases (ESBLs) in Enterobacter spp. that produce AmpC. The 20 isolates (18 Enterobacter Cloacae Complex,2 Enterobacter Aerogenes) isolated from clinical cases . We performed polymerase chain reaction for Ampc and blaCTX-M. The Ampc was detected in 12 of 20 isolated, and the blaCTX-M was detected in 11 of 20 isolated.

***Keywords*---**ESBL, β –lactamases enzymes, Ampc, blaCTX-M.

Introduction

Enterobacter spp. are common nosocomial pathogens in hospitals, causing a wide range of infection symptoms.(Davin-Regli, 2015). Resistance is typically caused by a combination of mechanisms, including decreased production of the intrinsic AmpC-lactamase, decreased outer membrane permeability, and increased efflux, with or without carbapenemase production.(Kawai, *et al* 2020). The beta-lactamases associated with the *AmpC* gene are located or mediated on chromosomes for some Enterobacteriaceae genera, such as Enterobacter. Infections caused by bacteria (with the AmpC gene) that produce beta-lactamases that cleave most penicillins are usually associated with significant mortality and morbidity. (Ahmad *et al* ., 2019). Over the last 60 years, the use of successive generations of beta-lactam drugs has selected successive generations of beta-lactamase enzymes, each more powerful than the last. Furthermore, *AmpC* and *CTX-M* beta-lactamases mediated by plasmids are currently causing concern.

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(Nachimuthu *et al* ., 2020). Most b-lactam resistance is caused by altered drug binding sites, porin deficiencies, or enzymatic degradation. (Bryskier, 2005).The increasing recognition of isolates producing so-called "newer" b-lactamases within the Enterobacteriaceae is particularly significant.The enzymes in question are as follows: (1) extended-spectrum b-lactamases [ESBLs] (e.g. *SHV, TEM*, and *CTX-M* types), (2) carbapenemases (e.g. KPC types), class B (metallo-b-lactamases [MBLs] such as VIM, IPM, and NDM types), class D oxacillinases (e.g. *OXA-48*-like).(Abbas *et al.,* 2019).The cell wall is made up of complex polymers of polysaccharides and peptidoglycan-like polypeptides that are linked together.Polysaccharide formation changed the amino sugars N-acetyl glucosamine NAG and N-acetyl muramic acid NAM. (Sauvage *et al.*, 2008). The -lactam ring is structurally similar to the NAM pentapeptide's D-alanine–Dalanine and penicillin-binding proteins. PBPs "inadvertently" use beta-lactam as a "building block" during cell wall synthesis. (Dörr, 2020). As a result, the peptidoglycan is only weakly cross-linked, leaving developing bacteria vulnerable to cell lysis and death. (Auer and Weibel,2017). *CTX-M* beta-lactamases are found throughout functional group 2. (Bush and Jacoby, 2010). CTX-M enzymes are not related to *TEM* or *SHV* ESBL enzymes. CTX-M ESBLs were created through horizontal gene transfer from other bacteria using genetic materials such as a conjugated plasmid or transposon, whereas SHV-ESBLs and TEM-ESBLs were created through amino acid substitutions of parent enzymes. (Liu *et al* ., 2019).

Methods and Materials

Sample

A total of 300 clinical case were achieved during the period from October 2021 to March 2022 from different sources in Al-Najaf city. (AL-Sadder Medical City and AL-Forat Hospital). Clinical specimens included urine , vganial swab ,wounds, burn, and diadetic foot .Burn , wound,diadetic foot and vganial swab were collected using sterile cotton swabs, while the urine collecting by sterile plastic bottle. . All samples were transferred to the laboratory and cultured on MacConky agar medium for 24 hours at 37° C.

Bacterial Isolates

The collected specimens were inoculated on three different types of culture media, including blood agar ,MacConkey agar and Chrom , and were spread on each plate with a sterile loop. Plates were incubated at 37° C for 24 hours. The Plates were then examined for bacterial growth, and a single pure, isolated colony was isolated from each plate.transferred to brain heart infusion agar for maintenance and morphological evaluation by Other biochemical tests and the vitek-2 compact system were used to confirm the identification isolating agents.

Identification of *Enterobacter spp*.

A single colony was taken from positive culture and it was identified according to the methods of Biochemical tests (MacFaddin, 2000 ;Brown and Smith, 2015).and Vitek – 2.

Extraction and Isolation of DNA

DNA of *Enterobacter spp.* isolates was prepared by boiling method. In brief, colonies were suspended in 100 microliters of sterile distilled water, boiled at 100C in a water bath for 15 minutes, then rapidly cooled at -20C for one hour, centrifuged, and the supernatant was saved for use in the amplification processes (Shah *et al.*, 2017).

β-lactamase gene identification

PCR amplification was used to identify the presence of blaCTX-M and Ampc gene.The primer used in this study was

Primer	Sequence	Reference
AmpC	F 5- TGCTCGGCATCTCTTGCTCT -3 R: 5- CAGCTTGAGCGGCTTAAGGA -3	Barnaud *et al.*(2001)
CTX-M	F:AAC CGT CAC GCT GTT GTT AG R:TTG AGG CGT GGT GAA GTA AG	(Newire *et al.* 2013).

Each 25 µl of PCR reaction mixture for PCR contained 2.5µl of upstream primer, 2.5µl of downstream primer, 2.5µl of free nuclease water, 5 µl of DNA and 12.5µl of master mix thin walled PCR tube. The Thermal cycler conditions were as follow

PCR gene	Temperature (c) / Time					Cycle number
	Initial denaturation	Cycling condition			Final extension	
		Denaturation	Annealing	Extension		
Amp C	94 Cº/5min	94 Cº/1 min.	56 Cº/1 min.	72 Cº/ 1min	72 Cº/5 min	35
CTX-M	94 Cº/5min	94 Cº/45sec	57Cº /45sec	72 Cº/ 1min	72 Cº/5 min	35

Results and Discussion

Entrobacter spp. were isolated from different clinical specimens using specific media such as MacConkey agar and CHROM™ .

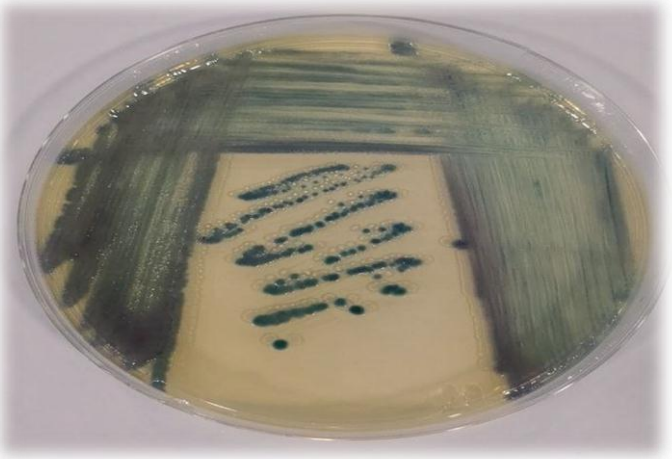

Figure 1. Colony growth of Enterobacter cloacae on Chromagar TM orientation medium

Antibacterial agent's profile of *Enterobacter spp*. isolates using disc diffusion method

The antibacterial agent susceptibility of 20 clinical isolates of Enterobacter spp. to traditional drugs suggested by CLSI (2020) was estimated. The results in a Table (1). Table (1) Antimicrobials sensitivity test of Enterobacter spp. isolates using disc diffusion method

Antibacterial agent	Resistance(%)	Intermediate(%)	Sensitive(%)
Ampicillin	20(100%)	0(0%)	0(0%)
Piperacillin	15(75 %)	2(10%)	3(15%)
Ceftazidime	20(100%)	0(0%)	0(0%)
Cefotaxime	19(95%)	1(5%)	0(0%)
Ceftriaxone	13(65%)	2(10%)	5(25%)
Cefepime	20(100%)	0(0%)	0(0%)
Cefoxitin	20(100%)	0(0%)	0(0%)
Aztreonam	9(45 %)	4(20 %)	7(35%)
Imipenem	3(15%)	0(0%)	17(85%)
Amoxicilllin – clavulanic acid	20(100%)	0(0%)	0(0%)
Ciprofloxacin	2(10 %)	3(15 %)	15(75%)

Molecular screening of *CTX-M* Genes in Clinical cases Isolates of *Enterobacter spp*

A total of 20 different Enterobacter isolates collected from clinical cases were molecularly examined for the presence and distribution of *CTX-M*genes . Molecular data of this work was show of *CTX-M* gene among 20 isolates of *Enterobacter* spp reach 11/20(55 %) (Figure;2).

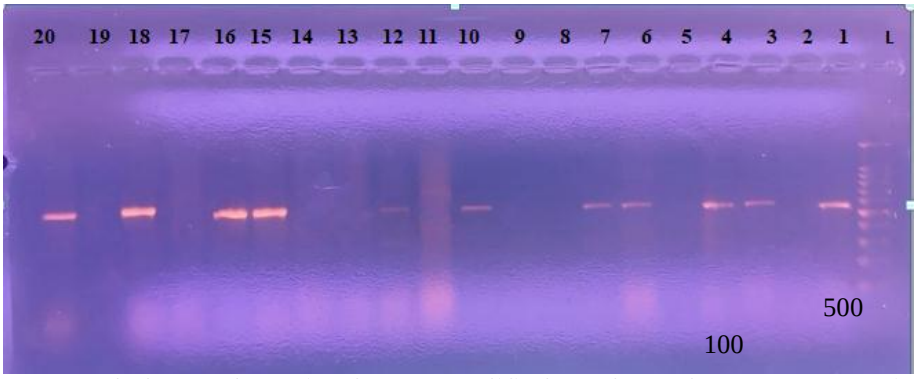

Figure 2. Gel electrophoresis of PCR amplified product of *CTX-M* gene primers with product 512 bp of Enterobacter spp. isolates. Lanes (1, 3 ,4, 6, 7, 10, 12, 15,16,18,20) positive result of Enterobacter spp (L), DNA molecular size marker (100-2000 bp ladder)

Several studies, both local and global, have found that the *bla/CTX-M* type (one type of ESBL) is emerging in a number of countries around the world. Similarly, several global studies have been conducted on the abilities of ESBL-producing Enterobacter. (Sophie *et al.*, 2006). The *CTX-M* gene encodes enzymes from the Ambler class A beta-lactamase family. These enzymes are becoming increasingly common in Enterobacteriaceae all over the world. This study discovered a high prevalence of *CTX-M* ESBL-producing *Enterobacter spp*. As a result, tracking and monitoring the global spread of *Enterobacter spp*. that produce *CTX-M* ESBLs in community settings is critical from a public health standpoint.

Detection of Ampc gene

Of the 20 isolates 12/20 (60%) show *AmpC* β- lactamase , AmpC-lactamase – producing Enterobcter isolates identified using the PCR technique. (Fig. 3) . This figure show, the bands (581bp) of sequences of DNA for *Enterobcter spp.*

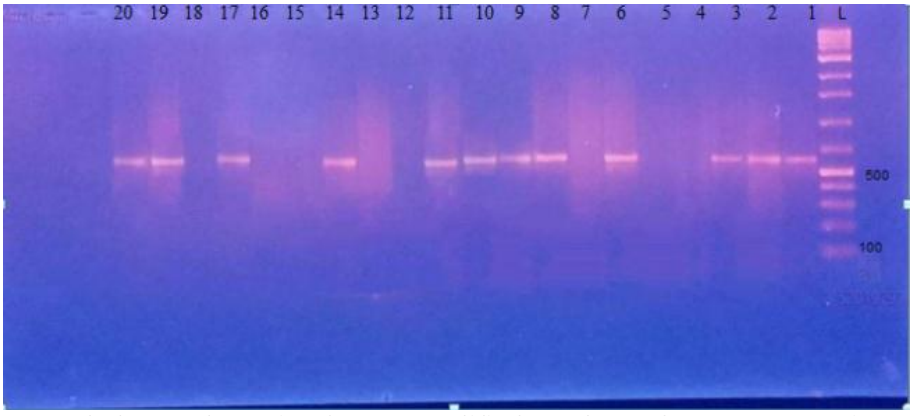

Figure 3. Gel electrophoresis of PCR amplified product of *Ampc* gene primers with product 558 bp of *Enterobacter spp.* isolates. Lanes (1,2, 3 , 6, 8,9, 10, 11, 14,17,19,20) positive result of *Enterobacter spp* (L), DNA molecular size marker (100-2000 bp ladder)

the enzymes of ESBLs produced by some pathogenic bacteria that typically play a role in the cleavage and breakage activity of drugs of third generation cephalosporins (particularly cefotaxime, ceftriaxone, and ceftazidime) as well as monobactam drugs (aztreonam). In the present study, we found that the resistance of *Enterobacter spp.* clinical isolates to ceftazidime, Ampicillin, Piperacillin were principally due to AmpD-dependent constitutive expression of *AmpC.*(Kaneko *et al* ;2005).

References

Abbas, Hisham A., et al(2019). "Impact of specific inhibitors on metallo-β-carbapenemases detected in Escherichia coli and Klebsiella pneumoniae isolates." Microbial pathogenesis 132: 266-274.

Ahmad, K. M.; Alamen, A. A. M.; Shamsi, S. A. M. S. and Elzen, A. A. (2019). Emergence of AmpC *β*-Lactamase and Metallo-*β*-Lactamase producing. M J I W AS. ;27(1): 9-16.

Auer, George K., and Douglas B. Weibel. (2017)"Bacterial cell mechanics." Biochemistry 56.29: 3710-3724.

Barnaud, G., Labia, R., Raskine, L., Sanson-Le Pors, M.J., Philippon, A., and

Arlet, G.(2001). Extension of resistance to cefepime and cefpirome associated to a six amino acid deletion in the H-10 helix of the cephalosporinase of an *Enterobacter cloacae* clinical isolate. FEMS Microbiol. Lett. 195: 185–190.

Brown, A. and Smith, K.,(2015). Benson's Microbiological Applications, Laboratory Manual in General Microbiology. 13th Ed. pp. 277

Bryskier, A. (2005)"Antibiotics and Antibacterial Agents: Classifications and Structure- Activity Relationship." Antimicrobial agents: antibacterials and antifungals : 13-38.

Bush, K. and Jacoby ,G.A. (2010): Updated functional classification of beta - lactamases. A AC.; 54(3): 969–976 .

Davin-Regli, A. (2015). *E. aerogenes* and *E. cloacae;* versatile bacterial pathogens confronting antibiotic treatment. FM.; *6*(392):1-10.

Dörr, T. (2020). Understanding tolerance to cell wall–active antibiotics. Annals of the New York Academy of Sciences.;2020:1-23.

Kaneko, Kenichi, et al. "Gene mutations responsible for overexpression of AmpC β-lactamase in some clinical isolates of *Enterobacter cloacae*." Journal of Clinical Microbiology 43.6 (2005): 2955-2958.

Kawai, Akito, et al. (2020) "Structural basis of reduced susceptibility to ceftazidime-avibactam and cefiderocol in *Enterobacter cloacae* due to AmpC R2 loop deletion." Antimicrobial agents and chemotherapy 64.7: e00198-20.

Liu, G.; Bogaj, K.; Bortolaia, V.; Olsen, J. E. and Thomsen, L. E. (2019). Antibiotic-Induced, increased conjugative transfer is common to diverse naturally occurring ESBL plasmids in *Escherichia coli.* FM.; 10:1-12.

MacFaddin, J.F.(2000). Biochemical tests for identification of medical bacteria .(3rd edition).The Williams and Wilkins-Baltimor. USA.J A M.;6(1) .

Nachimuthu, R.; Kannan, V. R.; Bozdogan, B.; Krishnakumar, V. and Manohar, P. (2020). CTX-M-type ESBL-mediated resistance to third-generation cephalosporins and conjugative transfer of resistance in Gram-negative bacteria isolated from hospitals in Tamil Nadu.A M.;3(3):141-167.

Newire, E.A.; Ahmed, S.F.; House, B.; Valiente, E.; Pimentel, G. (2013) Detection of new SHV-12, SHV-5 and SHV-2a variants of extended spectrum Beta-lactamase in *K. pneumoniae* in Egypt. A C M and A.: 12:16.1-12.

Sauvage, E.; Kerff, F. ; Terrak, M. ; Ayala, J. A. and P. Charlier. (2008). The penicillin-binding proteins: Structure and role in peptidoglycan biosynthesis. FEMS M R. ;32(2): 234–258.

Sophie, B.; Francois-Xavier ,W.; Axel ,C.; Martine; V.; Eric M and Karine P.; (2006).Clonal Emergence of Extended-Spectrum *β*-Lactamase (*CTX-M-2*)-Producing *Salmonella* enterica Serovar Virchow Isolates with reduced susceptibilities to ciproß oxacin among poultry and humans in Belgium and France (2000 to 2003) J C M .;44: -903- 2897.