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## RESEARCH ARTICLE

### First Record of Dissemination of BLBLI-Resistant *Enterobacter cloacae* from Public Hospitals in Baghdad, Iraq

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#### Abstract:

#### Background:

*Enterobacter cloacae* are most frequently isolated from human clinical specimens.

#### Objective:

This cross-sectional study aimed to investigate the dissemination of *E. cloacae* clinical isolates resistant to  $\beta$ -lactam- $\beta$ -lactamase inhibitor (BLBLI) combinations from different clinical specimens of hospitalized patients.

#### Methods:

*E. cloacae* isolates were recovered from different clinical samples of hospitalized patients in three main hospitals in Baghdad city. *E. cloacae* isolates were identified based on their morphology and biochemical tests, and the identification was confirmed using Vitek-2 system. The antibiotic susceptibility testing of *E. cloacae* isolates to a variety of antibiotics was achieved using disc diffusion test (DDT) and Vitek-2 system.

#### Results:

Results found that among 335 culture-positive samples, 30 isolates (8.9%) belonged to *E. cloacae*. A high rate of isolation was observed in urine isolates (46.6%), followed by wounds (burns) isolates (26.6%). Out of 30 *E. cloacae* strains isolated during this study, 18 (60%) showed reduced susceptibility to BLBLI combinations. *TEM* genes (*TEM*-1 and *TEM*-2) were successfully amplified from 7/18 isolates (38.8%) and high rate of BLBLI genes was detected (*CTX*-M, *bla*-SHV, *SHV*-2, and *OXA*-1). However, no BLBLI genes of *bla*-*AmpC*, *bla*-*OXA*-2, and *bla*-*OXA*-10 were found in *E. cloacae* isolates when tested using specific primers for *bla*-*AmpC* and *bla*-*OXA* genes.

#### Conclusion:

From this study, we can conclude that the production of inhibitor-resistant  $\beta$ -lactamases by *E. cloacae* isolates could be increasingly common in nosocomial pathogens other than *E. coli* or *K. pneumoniae* in public hospitals in Baghdad, Iraq.

**Keywords:**  $\beta$ -lactamase inhibitors, BLBLI, *Enterobacter cloacae*, IRT, Hospitals, Dissemination.

#### Article History

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## 1. INTRODUCTION

*Enterobacter cloacae* are pathogens that are commonly isolated from human clinical specimens. They cause an extensive range of nosocomial infections involving skin and soft tissue, the lower respiratory tract, urinary tract, and the CNS infections. The antibiotic-resistance properties of these bacteria had been described by several authors worldwide [1].

Resistance to BLBLI combinations in Gram-negative enteric rods may be related to different mechanisms, such as *TEM*-1 penicillinase hyperproduction, chromosomal and plasmid-mediated *AmpC* production, *OXA*-type  $\beta$ -lactamase production, deficiencies in outer membrane permeability, inhibitor-resistant *TEM* (IRT)- $\beta$ -lactamase production, *CTX*-M, type- $\beta$ -lactamase production, and more recently, carbapenemase production [2].

$\beta$ -lactam- $\beta$ -lactamase inhibitor (BLBLI) is characterized as an adaptive resistance mechanism definitely established by the organism to beat off the activity of  $\beta$ -lactamase inhibitors [2].

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Inhibitor-resistant TEM (IRT)  $\beta$ -lactamases, which emerged in the 1990s, are most commonly observed in urine strains. IRT enzymes include an assembly of plasmid-mediated types of TEM-1 and TEM-2 with reduced affinities for penicillins and changed interactions with irreversible inhibitors, like clavulanic acid, sulbactam, and tazobactam [2].

BLBLI  $\beta$ -lactamases-producing isolates are frequently resistant to ampicillin-sulbactam and amoxicillin-clavulanate combinations. Inhibitor-resistant beta-lactamase enzymes have previously been described to be present in a diverse range of pathogenic bacteria, like *E. coli*, *Enterobacter cloacae*, *Proteus mirabilis*, *Klebsiella spp.*, *Pseudomonas aeruginosa* and *Citrobacter freundii* [2 - 4].

In Iraq, there is little to no information available on the prevalence of BLBLI  $\beta$ -lactamases in *E. cloacae* clinical isolates; in addition, there is no information regarding their antibiotic resistance patterns. Thus, the objective of this study was to inspect the occurrence of BLBLI  $\beta$ -lactamases in *E. cloacae* isolates recovered from different clinical specimens of hospitalized patients in three main hospitals of Baghdad city, Iraq.

## 2. METHODS

### 2.1. Study Design

This cross-sectional study was designed to examine the incidence of BLBLI  $\beta$ -lactamases in *E. cloacae* isolates recovered from different clinical specimens of hospitalized patients. In this study, *E. cloacae* isolates were recovered from 386 different clinical samples of patients hospitalized in three public hospitals of Baghdad city, Iraq, in addition to some private clinics during the period of December 2019 to April 2020. Bacteria were isolated from different clinical specimens, including blood, urine, burn, ear, skin, stool, eye, nose, and bronchial fluid. *E. cloacae* strains were isolated and identified based on their morphological characteristics and biochemical tests [5]. Identification was confirmed using Vitek-2 system (BioMérieux, France).

### 2.2. Eligibility Criteria

Selection criteria included hospitalized patients of both sexes and all ages (neonates: aged less than 28 days; infants: aged 28 days to 1 year; children: more than 1 year to 17 years of age; adults: aged 18 years or more). Exclusion criteria included clinical samples obtained from previously hospitalized individuals who had received antibiotic therapy for more than seven days.

### 2.3. Antibiotic Susceptibility Testing

The antibiotic susceptibility of *E. cloacae* bacteria to a variety of antibiotics was tested using Vitek-2 system (BioMérieux, France) and disc diffusion test (DDT). The results were interpreted based on CLSI documents [6]. The following BLBLI combinations were purchased from Oxoid,

UK, as standard antibiotic disks with an identified potency for lab use: Amoxicillin/Clavulanic acid (30  $\mu$ g), Ceftazidime/Clavulanic acid (30/10  $\mu$ g), and Ceftriaxone-Tazobactam (30/10  $\mu$ g). *E. coli* ATCC 25922 was used as the reference strain for antimicrobial susceptibility testing.

### 2.4. Detection of $\beta$ -Lactamase by Chromogenic (Nitrocefin) Disc Method

For detection of the ability of 30 *E. cloacae* isolates to produce  $\beta$ -lactamase, Chromogenic (Nitrocefin) Disc Method was used.  $\beta$ -lactamase production was noticed by the appearance of a pink color within 15 minutes.

### 2.5. Detection of AmpC and Extended Spectrum $\beta$ -Lactamases

Disk diffusion method was used for testing *E. cloacae* isolates for cefoxitin susceptibility [6]. The resistant isolates ( $\leq 18$  mm inhibition zone diameter) were considered as primarily AmpC producers. *E. cloacae* isolates were also analyzed for their ability to produce extended spectrum  $\beta$ -Lactamase (ESBL) by initial screen test. The isolate would be treated as ESBL producer, if the inhibition area of ceftazidime would be  $\leq 22$  mm [6]. ESBL producer strains positive on initial screen test were further confirmed using confirmatory ESBL production as follows: ceftazidime alone and in combination with clavulanate were established. Inhibition zone of  $\geq 5$  mm increase in diameter for ceftazidime in combination with clavulanate versus its zone alone confirmed an ESBL producing strain. The antibiotic resistance patterns (MDR, XDR, and PDR) of all *E. cloacae* isolates were detected according to the method proposed by Magiorakos *et al.* [7].

### 2.6. DNA Extraction and PCR Assay

*E. cloacae* strain was inoculated in 5 mL of Broth Heart Infusion medium and grown overnight at 37° C. DNA was extracted from bacterial cell using plasmid Mini Prep Kit DNA extraction (Bioneer, USA). The PCR reactions were performed in a thermal cycler (Clever, U.K). For quantification of the DNA (ng  $\mu$ L<sup>-1</sup>), 1  $\mu$ L of Genomic DNA was quantified with a NanoDrop™ spectrometer (NanoDrop Technologies LLC, USA), according to the manufacturer's guidelines. DNA concentration was assessed from absorbance at 260 nm.

### 2.7. PCR Protocols

The DNA purified from *E. cloacae* strains were exposed to a set of primers by PCR. A set of primers of  $\beta$ -lactamases genes [8 - 13] were used (Bioneer, USA) in an attempt to differentiate BLBLI-resistant isolates (Table 1). The PCR conditions are illustrated in Table 1. The PCR products were identified by agarose gel electrophoresis. Ethidium bromide (EtBr) at a concentration of 0.5  $\mu$ g/ml was used for visualization of the amplicons, and the gel images were acquired using a regular gel-documentation system (Clever Scientific, U.K).

Table 1. Primer sequences of plasmid-mediated  $\beta$ -Lactamases and PCR conditions with their amplicons sizes.

Primer name	Oligo Sequence (5'→3')	Product Size (Pb)	Conditions	Refs.
<i>bla<sub>AmpC</sub></i>	<i>AmpC-F</i> ATC AAA ACT GGC AGC CG	550	94°C 3 min 1x 94°C 45 sec 60°C 45 sec 35x 72°C 1 min 72°C 5 min 1x	[8]
	<i>AmpC-R</i> GAG CCC GTT TTA TGC ACC CA			
<i>TEM-1</i>	<i>TEMU1</i> ATG AGT ATT CAA CAT TTC CG	867	95°C 5 min 1x 94°C 1 min 58°C 1 min 35x 72°C 1 min 72°C 10 min 1x	[9]
	<i>TEML1</i> CTG ACA GTT ACC AAT GCT TA			
<i>TEM-2</i>	<i>TEMU2</i> ACT GCG GCC AAC TTA CTT CTG	374	95°C 5 min 1x 94°C 30 sec 62°C 30 sec 30x 72°C 30 sec 72°C 10 min 1x	[10]
	<i>TEML2</i> CGG GAG GGC TTA CCA TCT G			
<i>SHV-1</i>	<i>SHV-F</i> GGT TAT GCG TTA TAT TCG CC	867	95°C 5 min 1x 94°C 30 sec 62°C 30 sec 30x 72°C 30 sec 72°C 10 min 1x	[11]
	<i>SHV-R</i> TTA GCG TTG CCA GTG CTC			
<i>SHV-2</i>	<i>SHVU2</i> CCG CAG CCG CTT GAG CAA A	374	96°C 5 min 1x 96°C 1 min 60°C 1 min 35x 72°C 1 min 72°C 10 min 1x	[10]
	<i>SHVL2</i> GCT GGC CGG GGT AGT GGT GTC			
<i>OXA-1</i>	<i>OXA-1F</i> ACA CAA TAC ATA TCA ACT TCG C	867	96°C 5 min 1x 96°C 1 min 61°C 1 min 35x 72°C 2 min 72°C 10 min 1x	[12]
	<i>OXA-1R</i> AGT GTG TTT AGA ATG GTG ATC			
<i>OXA-2</i>	<i>OXA-2F</i> TTC AAG CCA AAG GCA CGA TAG	702	96°C 5 min 1x 96°C 1 min 65°C 1 min 35x 72°C 2 min 72°C 10 min 1x	[12]
	<i>OXA-2R</i> TCC GAG TTG ACT GCC GGG TTG			
<i>OXA-10</i>	<i>OXA-10F</i> CGT GCT TTG TAA AAG TAG CAG	651	96°C 5 min 1x 96°C 1 min 61°C 1 min 35x 72°C 2 min 72°C 10 min 1x	[12]
	<i>OXA-10R</i> CAT GAT TTT GGT GGG AAT GG			
<i>CTX-M</i>	<i>CTX-M-F</i> CGC TTT GCG ATG TGC AG	550	94°C 4.5 min 1x 94°C 50 sec 58°C 50 sec 35x 72°C 50 sec 72°C 7 min 1x	[13]
	<i>CTX-M-R</i> ACC GCG ATA TCG TTG GT			

## 2.8. Statistical Analysis

All BLBLI  $\beta$ -lactamases-producing *E. cloacae* isolates were analyzed using the Statistical Package for the Social Sciences version 26 (SPSS Inc., Chicago, IL, USA). Numerical data were evaluated using the chi-squared and Fisher's exact test, and compared with that of cefoxitin resistance among BLBLI  $\beta$ -lactamases-producing isolates; cefoxitin resistance ( $\leq 18$  mm inhibition zone) was considered an indicator of AmpC producers. P-value  $< 0.01$  was considered significant.

## 3. RESULTS

### 3.1. Isolation of *E. cloacae* Isolates:

In the present study, among a total of 386 clinical samples, 335 (86.7%) showed positive cultures, whereas no growth was observed in the remaining 51 samples (13.3%). Among 335 culture-positive samples, 30 isolates (8.9%) belonged to *E. cloacae*, of which, high rate of isolation was observed for urine strains 14/30 (46.6%), followed by wounds (burns) isolates 8/30 (26.6%), while the remaining isolates were obtained from blood, ear swab, and bronchial fluid samples. *E. cloacae* were isolated and identified based on biochemical tests, and the identification was confirmed using the Vitek-2 system. Results of the present study revealed that a high isolation rate (46.6%) of *E. cloacae* isolates was from urine samples recovered from the hospitalized patients with UTIs in three public hospitals of Baghdad.

### 3.2. Production of $\beta$ -Lactamase and Antibiotic Susceptibility to BLBLI Combinations

Results revealed that 28/30 isolates (93.3%) produced  $\beta$ -lactamase by changing the color of nitrocefin disk from yellow to reddish-orange. Regarding the resistance of *E. cloacae* isolates to BLBLI combinations, results revealed that only 18 (60%) of 30 *E. cloacae* were found to be resistant to one or more of BLBLI combination antibiotics.

Out of 18 *E. cloacae* isolates resistant to BLBLI, 17 (94.4%) isolates were resistant to ampicillin-sulbactam, 11 (61%) resistant to amoxicillin-clavulanate, and only 9 (50%) were resistant to piperacillin-tazobactam.

### 3.3. Production of ESBL and AmpC $\beta$ -Lactamase Enzymes

Results of this study revealed that out of the 18 *E. cloacae* isolates that were resistant to BLBLI, 15 (83.3%) displayed zone augmentation with clavulanate, confirming ESBL production. Vitek-2 system was also employed for recognition of ESBLs in 18 *E. cloacae* isolates. Vitek-2 system was also used to identify AmpC  $\beta$ -lactamases in 18 *E. cloacae* isolates. All isolates were susceptible to cefoxitin disks and the inhibitor area was  $\geq 18$  mm based on CLSI guidelines.

### 3.4. Antibiotics Susceptibility of *E. cloacae* Isolates

All 30 *E. cloacae* isolates showed multidrug resistance. The frequency of 18 *E. cloacae* isolates that were resistant to BLBL inhibitor combination was determined. The results revealed that all *E. cloacae* isolates were fully resistant to ampicillin and amoxicillin (100%), but they were fully susceptible to cefoxitin (100%).

Results revealed that the majority (46.6%) of *E. cloacae* isolates were recovered from patients with UTIs. All these isolates were found to be MDR,  $\beta$ -lactamases producers, and highly resistant to amoxicillin and ampicillin. The results found that most *E. cloacae* strains were resistant to at least 6 agents, which imply that a new option of antibiotic is required to eliminate *E. cloacae* related to UTI infections. It was found that out of 30 *E. cloacae* isolates, 80% of them showed reduced susceptibility to cephalexin, cephalothin, and the monobactam (aztreonam). The rate of resistance to 3<sup>rd</sup> generation cephalosporins (cefotaxime, ceftriaxone, and ceftazidime) was 76.6% while only 26.6% of isolates were resistant to cefepime (4<sup>th</sup> generation cephalosporin). Isolates were found to be fully susceptible to carbapenems and nitrofurantoin, and low rate of resistance to gentamicin was discovered (46.6%).

The greatest active quinolone towards all *E. cloacae* strains was levofloxacin (20%), followed by ciprofloxacin (30%) and nalidixic acid (53.3%). The resistance rates of *E. cloacae* strains to tobramycin, cotrimoxazole, and tetracycline were 43.3%, 73.3%, and 70.0%, respectively.

Regarding the distribution based on the type of antibiotic resistance patterns, high rate of MDR 30/30 (100%) was noticed, followed by XDR 6/30 (20%); however, no PDR type was observed among the *E. cloacae* isolates.

### 3.5. Molecular Detection of *E. cloacae* BLBLI Genes by PCR

A pair of primers of two TEM enzymes was used for detecting the existence of TEM genes in *E. cloacae* strains. TEM genes (TEM-1 and TEM-2) had been positively amplified from 7/18 isolates (38.8%) of *E. cloacae* with molecular length of 867 bp and 374 bp, respectively, indicating their antibiotic resistance to Inhibitor-resistant TEM (IRT) beta-lactamases. The amplicons were noticed in the gel and matched with the DNA ladder (Figs. 1A and 1B).

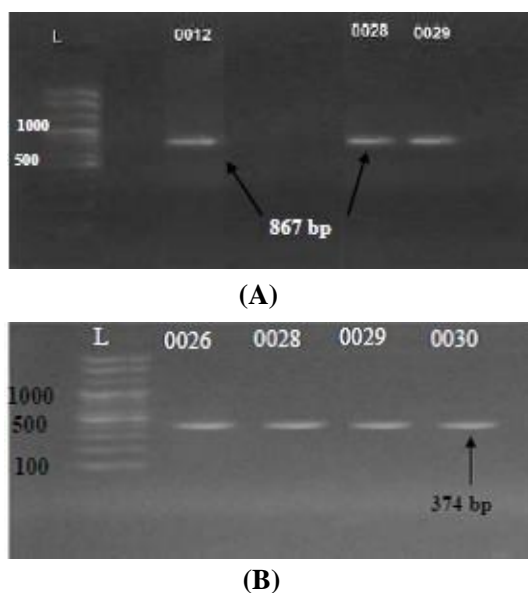
Regarding SHV enzymes, a pair of primers of two SHV enzymes was used for detecting the presence of SHV genes in *E. cloacae* isolates. SHV genes (SHV-1 and SHV-2) had been positively amplified from 6/18 isolates (33.3%) of *E. cloacae* (4 isolates had bla-SHV and 2 isolates had bla-SHV-2) with a molecular length of 867 bp and 477 bp, respectively. The amplicons were noticed in the gel and matched with the DNA ladder.

A pair of primers of bla-AmpC enzyme gene was employed for identifying the occurrence of bla-AmpC gene in *E. cloacae* isolates. The result exhibited that bla-AmpC gene was not present in any of the isolates. However, the phenotypic assays showed all 18 *E. cloacae* strains to be susceptible to cefoxitin and the zone of inhibition was  $\geq 18$  mm based on CLSI guidelines, which confirmed our molecular results regarding this resistance gene.

Regarding OXA type of BLBLI genes, a pair of primers of three OXA enzymes (OXA-1, OXA-2, and OXA-10) was employed for detecting the existence of OXA genes in *E. cloacae* strains. Only OXA-1 gene had been positively amplified from 6/18 isolates (33.3%) of *E. cloacae* with molecular length 813pb. The result showed *E. cloacae* isolates

to not be able to yield *bla-OXA-2* and *bla-OXA-10* genes. The results regarding OXA-2, and OXA-10 resistance genes revealed that these genes did not originate in any of *E. cloacae* strains when established by using OXA gene primers.

In the present study, a pair of primers of *bla-CTX-M* enzyme gene was used for detecting the occurrence of *bla-CTX-M* gene in *E. cloacae* strains. *CTX-M* gene had been amplified from 5/18 isolates (27.7%) of *E. cloacae* with molecular length 550pb.



**Fig. (1).** 2.1% Agarose gel electrophoresis at 70 volt for 1.5-2 hr. for *E. cloacae* genes. PCR products visualized under U-V light. L: 100bp ladder. **A.** *TEMU-1* 867 bp PCR products. **B.** *TEMU-2* 374 bp PCR products.

#### 4. DISCUSSION

In the present study, 86.7% of the clinical samples showed positive cultures, whereas 13.3% of them showed no bacterial growth. The negative culture may be due to fungal, viral, or fastidious bacterial infection that might be missing during sample transportation. Among 335 culture positive samples, only 30 isolates (8.9%) belonged to *E. cloacae*.

Regarding the identification of the isolates, *E. cloacae* were recognized based on biochemical tests and the identification was confirmed using Vitek-2 system. Nimer *et al.* [14] investigated the performance of the Vitek-2 system for direct rapid identification of Gram-negative rods and 74 Gram-positive cocci from inpatients, and they suggested that VITEK-2 cards inoculated directly from positive blood culture bottles are appropriate for rapid diagnosis and sensitivity testing of the pathogenic bacteria.

Saffar *et al.* [15] investigated the incidence and pattern of antimicrobial sensitivity of uropathogens in UTIs from three hospitals in Iran, and they found *E. coli* to be the prominent cause of UTI in hospitalized patients followed by *Pseudomonas* spp. (10.4%) and *Enterobacter* spp. (5.7%).

Harris *et al.* [16] investigated cases of bacteraemia caused by *Enterobacter* spp. in hospitals in Australia. They found that

only 3.4% of patients were at an overall risk of relapsed or persisting bacteraemia, and only two patients with an emergent resistance to 3GCs.

Chromogenic (Nitrocefin) disc method was used for detection of  $\beta$ -lactamase in *E. cloacae* isolates. Nitrocefin is more sensitive to recognize all  $\beta$ -lactamases. The reason may be that nitrocefin is more sensitive to recognize all  $\beta$ -lactamases formed by Gram-negative bacilli [17]. Also, this technique is suitable for the recognition of most  $\beta$ -lactamase types and sensitive for identifying low level of  $\beta$ -lactamases yielded by gram negative rods.

Results of this study regarding the antibiotic susceptibility of *E. cloacae* isolates to BLBLI combinations revealed 60% of the isolates to be resistant to one or more of BLBLI combinations antibiotics. Harris *et al.* recognized cases of bacteraemia caused by *Enterobacter* spp. in patients in Australia and they found that the condition of patients receiving complete therapy with a broad-spectrum BLBLI did not relapse; they concluded that more studies are required to determine whether BLBLIs denote an efficient option to avoid carbapenem for bacteraemia caused by *Enterobacter* spp [16].

Miro *et al.* [18] found that 7% of *E. coli* isolates from a university hospital in Spain exhibited resistance to Augmentin. Kaye and his colleagues [10] reported that 69 clinical isolates resistant to ampicillin-sulbactam were also resistant to Augmentin. Among the isolates, 12 (4.7%) were nosocomial and 57 (2.8%) were community acquired.

Leflon-Guibout *et al.* [19] studied the epidemiological features of *E. coli* isolates from French hospitals that exhibited reduced susceptibility to amoxicillin-clavulanate (MIC >16 mg/ml) over a 3- year period. The incidence of resistant isolates (15%) was higher in surgical wards than in medical wards.

Results of the study found 15 of *E. cloacae* isolates (out of 18 inhibitor-resistant isolates) to be ESBL producers. Malloy and Campos [20] reported that the recognition of ESBL-producing strains can be hard since the existence of ESBLs in these bacterial cells does not continually yield a resistance phenotype.

Manzur *et al.* [21] described the epidemiology and microbiological features of an outbreak caused by ESBL-producing *E. cloacae* in a cardiothoracic intensive care unit (CT-ICU), and they found that 7 patients with ESBL-producing *E. cloacae* were successfully recognized. Since the majority of ESBL enzymes had been found to be consistently sensitive to  $\beta$ -lactamase inhibitors, BLBLI combinations were promoted as possible alternative therapy [22].

Results of this study also found all 8 *E. cloacae* isolates recovered to be susceptible to cefoxitin disks as per CLSI guidelines [6], confirming their ability to produce AmpC  $\beta$ -lactamases. Derbyshire *et al.* [23] reported that, in spite of the fact that certain AmpC-producing Gram-negative rods are sensitive to cefoxitin, a low level of enzyme gives negative or slightly positive results. Several authors worldwide reported more than 60% of bacterial isolates to be cefoxitin resistant when they tested AmpC producers by nitrocefin disk method.

AmpC  $\beta$ -lactamases are the greatest significant  $\beta$ -lactamases in Gram-negative rods. Failure to recognize AmpC  $\beta$ -lactamase producers in hospital and other health care centers may lead to unsuitable antimicrobial therapy, and consequently, an increase in the mortality rate [24]. This result is compatible with the results of several authors worldwide [25], who have reported *E. coli* to be the main uropathogen in patients with bacteriuria, displaying high rate of resistance for both amoxicillin and ampicillin.

Manzur *et al.* [21] investigated an outbreak initiated by *E. cloacae* strains in a CT ICU, and they revealed that ESBL-producing strains exhibited reduced susceptibility to quinolones and aminoglycosides.

Regarding the molecular detection of *E. cloacae* BLBLI genes by PCR, results found that 38.8% of *E. cloacae* isolates were inhibitor-resistant TEM (IRT) beta-lactamases while only 6/18 isolates (33.3%) were inhibitor-resistant SHV (SHV-1 and SHV-2) beta-lactamases.

Kaye *et al.* [10] analyzed the  $\beta$ -lactamase inhibitors resistant *E. coli* strains isolated in the Northeastern United States and found that TEM type alone was predominant among the bacterial strains, and there was one strain that harbored both TEM and SHV types of BLBLI genes.

Miro *et al.* [18] found that 0.8% of clinical strains isolated from a Spanish hospital were TEM-1 hyperproducers and they exhibited resistance to Augmentin. Martín *et al.* [26] found that 0.5% of *E. coli* strains producing IRT enzymes were isolated mainly from urine (77.8%), and a high degree of TEM  $\beta$ -lactamase inhibitors diversity was recognized (TEM-30, -32, -33, -34, -36, -37, -40, and -54).

Miro' *et al.* [18] found that 0.15% of clinical strains isolated from a Spanish hospital were SHV-1 hyperproducers and they exhibited reduced susceptibility to amoxicillin-clavulanate. Several authors reported resistant *E. coli* strains isolated from human and animal sources to express bla-SHV type  $\beta$ -lactamase inhibitors [27].

Kaye and his colleagues [10] analyzed the amoxicillin-clavulanate resistance in *E. coli* isolates from a tertiary care hospital in United states and revealed that one isolate had both SHV type and TEM type enzyme.

The result showed that *bla-AmpC* gene was not present in any *E. cloacae* isolates recovered in this study. Regarding OXA type of BLBLI genes, only *OXA-1* gene had been positively amplified from 6/18 isolates of *E. cloacae*, while *OXA-2* and *OXA-10* resistance genes were not present in any of *E. cloacae* strains.

Davin-Regli and Pagès [1] reported that the expression of novel  $\beta$ -lactamase enzymes, like AmpC, is not common among *E. aerogenes* and *E. cloacae* strains. Coque and his colleagues [28] reported that 2 *E. coli* strains were nominated as negative for ESBL production in phenotypic tests but they expressed the TEM gene, perhaps owing to the expression of new  $\beta$ -lactamases, like AmpC. Thus, the implementation of molecular assays together with phenotypic methods is vital for final recognition of these variants of  $\beta$ -lactamases.

Abid and his colleagues revealed a majority of *AmpC*

genes (40%) among clinical isolates of *E. cloacae* from Iraqi hospitals [29]. Soltan Dallal *et al.* [27] found that reduced susceptibility to  $\beta$ -lactamase inhibitors was encoded mostly by AmpC, OXA, TEM, and SHV  $\beta$ -lactamases. Kaye *et al.* [10] reported that the resistance to amoxicillin-clavulanate in *E. coli* clinical isolates in United States was encoded by *TEM*, *SHV*, *OXA*, and *AmpC*  $\beta$ -lactamases.

The inability of *E. cloacae* isolates to yield *bla-OXA-2* and *bla-OXA-10* genes was compatible with AL-Tememy and Al-Charrakh [4], who showed *OXA-2* and *OXA-10* resistance genes to not be detected in any of *E. coli* strains from patients with UTIs in Iraq.

Abid *et al.* [29] revealed a majority of *bla-TEM* genes (60%) among *E. cloacae* strains from Iraqi hospitals, followed by the *bla-OXA* gene, which was detected only in 20% of these strains. Several authors reported amoxicillin-clavulanate resistant clinical strains of *K. pneumoniae*, *K. oxytoca* and *E. coli* to express *bla-OXA* type  $\beta$ -lactamase inhibitors [18].

Results of this study also revealed *CTX-M* gene to be positively amplified from 6/18 isolates of *E. cloacae* isolates. A study conducted by Nishida *et al.* in Japan revealed the emergence of *E. cloacae* complex co-producing IMP-1-type carbapenemase and CTX-M ESBL [30]. Baba Ahmed *et al.* [31] revealed a widespread presence of CTX-M-15 extended-spectrum  $\beta$ -lactamases among enteric bacterial isolates in ICU in an Algerian Hospital. Several authors worldwide have described the coexistence of *bla-CTX-M* and *bla-TEM* genes among ESBL producers bacterial strains [32]. Nimri and Azaizeh [32] reported that more than 80.7% of ESBL-producing isolates had at least one ESBL gene (TEM or CTX-M).

Finally, although our results have been obtained from a single center, they involve high internal validity and the sample size included with random sampling was large enough; thus, we think that our results can be safely generalized worldwide.

## CONCLUSION

From this study, we can conclude that the production of inhibitor-resistant  $\beta$ -lactamases by *E. cloacae* isolates could be increasingly common in nosocomial pathogens other than *E. coli* or *K. pneumoniae* in public hospitals in Baghdad, Iraq.

## AUTHORS' CONTRIBUTIONS

Jawad R. Alzaidi contributed to conceptualization, data curation, original draft preparation, and supervision. Ahmed S. Mohammed validated the findings and contributed to writing, reviewing and editing of the manuscript.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Written and verbal consent was obtained from each patient before obtainment of their samples. The study protocol was approved by the committee on publication ethics at Southern Technical University under the reference No. BMS/0276/018.

## HUMAN AND ANIMAL RIGHTS

No animals were used in this research. All procedures

performed involving human participants were in accordance with the ethical standards of institutional and/or research committee, and with the 1975 Declaration of Helsinki, as revised in 2013.

### CONSENT FOR PUBLICATION

Written and verbal consent was obtained from each patient before obtainment of their samples.

### STANDARDS OF REPORTING

STROBE guidelines and methodologies were followed in this study.

### AVAILABILITY OF DATA AND MATERIALS

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

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None.

### CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

### REFERENCES

- [1] Davin-Regli A, Pagès J-M. *Enterobacter aerogenes* and *Enterobacter cloacae*; versatile bacterial pathogens confronting antibiotic treatment. *Front Microbiol* 2015; 6: 392. [http://dx.doi.org/10.3389/fmicb.2015.00392] [PMID: 26042091]
- [2] Nicolas-Chanoine MH. Inhibitor-resistant  $\beta$ -lactamases. *J Antimicrob Chemother* 1997; 40(1): 1-3. [http://dx.doi.org/10.1093/jac/40.1.1] [PMID: 9249197]
- [3] Al-Zaidi JR. Antibiotic susceptibility patterns of *Pseudomonas aeruginosa* isolated from clinical and hospital environmental samples in Nasiriyah, Iraq. *Afr J Microbiol Res* 2016; 10(23): 844-9. [http://dx.doi.org/10.5897/AJMR2016.8042]
- [4] Al-Charrakh AH. Phenotypic and Genotypic Characterization of IRS-Producing *Escherichia coli* Isolated from Patients with UTI in Iraq. *Med J Babylon* 2015; 12(1): 80-95.
- [5] Forbes BA, Daniel FS, Alice SW, Baily and Scott's Diagnostic microbiology. 12th ed. USA: Mosby Elsevier Company 2007.
- [6] Clinical and laboratory standards institute (CLSI). Performance standards for antimicrobial susceptibility testing; 28th., M100-S29. informational supplement. 2019. Informational Supplement PA, USA
- [7] Magiorakos AP, Monnet DL, Struelens MJ, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert definitions for acquired resistance. *Clin Microbiol Infect* 2012; 18: 268-81. [http://dx.doi.org/10.1111/j.1469-0691.2011.03570.x] [PMID: 21793988]
- [8] Al-Sehlawi ZSR. Occurrence and characterization of AmpC  $\beta$ -lactamases in *Klebsiella pneumoniae* isolated from some medical centers in Najaf. PhD thesis. Iraq: College of Science, University of Babylon 2012.
- [9] Eckert C, Gautier V, Arlet G. DNA sequence analysis of the genetic environment of various blaCTX-M genes. *J Antimicrob Chemother* 2006; 57(1): 14-23. [http://dx.doi.org/10.1093/jac/dki398] [PMID: 16291869]
- [10] Kaye KS, Gold HS, Schwaber MJ, et al. Variety of  $\beta$ -lactamases produced by amoxicillin-clavulanate-resistant *Escherichia coli* isolated in the northeastern United States. *Antimicrob Agents Chemother* 2004; 48(5): 1520-5. [http://dx.doi.org/10.1128/AAC.48.5.1520-1525.2004] [PMID: 15105100]
- [11] Ferreira CM, Ferreira WA, Almeida NC, Naveca FG, Barbosa M. Extended-spectrum beta-lactamase-producing bacteria isolated from hematologic patients in Manaus, State of Amazonas, Brazil. *Braz J Microbiol* 2011; 42(3): 1076-84. [http://dx.doi.org/10.1590/S1517-83822011000300028] [PMID: 24031725]
- [12] Steward CD, Rasheed JK, Hubert SK, et al. Characterization of clinical isolates of *Klebsiella pneumoniae* from 19 laboratories using the National Committee for Clinical Laboratory Standards extended-spectrum  $\beta$ -lactamase detection methods. *J Clin Microbiol* 2001; 39(8): 2864-72. [http://dx.doi.org/10.1128/JCM.39.8.2864-2872.2001] [PMID: 11474005]
- [13] Ahmed AM, Motoi Y, Sato M, et al. Zoo animals as reservoirs of gram-negative bacteria harboring integrons and antimicrobial resistance genes. *Appl Environ Microbiol* 2007; 73(20): 6686-90. [http://dx.doi.org/10.1128/AEM.01054-07] [PMID: 17720829]
- [14] Nimer NA, Al-Saa'da RJ, Abuelaish O. Accuracy of the VITEK® 2 system for a rapid and direct identification and susceptibility testing of Gram negative rods and Gram-positive cocci in blood samples. *East Mediterr Health J* 2016; 22(3): 193-200. [http://dx.doi.org/10.26719/2016.22.3.193] [PMID: 27334076]
- [15] Saffar MJ, Enayti AA, Abdolla IA, Razai MS H. Antibacterial susceptibility of 415 uropathogens in 3 hospitals, Sari, Islamic Republic of Iran. 2002–2003 *La Revue de Santé de la Méditerranée orientale* 2008; 14(3): 556-63.
- [16] Harris PNA, Peri AM, Pelecanos AM, Hughes CM, Paterson DL, Ferguson JK. Risk factors for relapse or persistence of bacteraemia caused by *Enterobacter* spp.: A case-control study. *Antimicrob Resist Infect Control* 2017; 6(1): 14. [http://dx.doi.org/10.1186/s13756-017-0177-0] [PMID: 28127422]
- [17] Bebrone C, Moali C, Mahy F, et al. CENTA as a chromogenic substrate for studying beta-lactamases. *Antimicrob Agents Chemother* 2001; 45(6): 1868-71. [http://dx.doi.org/10.1128/AAC.45.6.1868-1871.2001] [PMID: 11353639]
- [18] Miró E, Navarro F, Mirelis B, et al. Prevalence of clinical isolates of *Escherichia coli* producing inhibitor-resistant  $\beta$ -lactamases at a University Hospital in Barcelona, Spain, over a 3-year period. *Antimicrob Agents Chemother* 2002; 46(12): 3991-4. [http://dx.doi.org/10.1128/AAC.46.12.3991-3994.2002] [PMID: 12435708]
- [19] Leflon-Guibout V, Speldooren V, Heym B, Nicolas-Chanoine M. Epidemiological survey of amoxicillin-clavulanate resistance and corresponding molecular mechanisms in *Escherichia coli* isolates in France: New genetic features of bla(TEM) genes. *Antimicrob Agents Chemother* 2000; 44(10): 2709-14. [http://dx.doi.org/10.1128/AAC.44.10.2709-2714.2000] [PMID: 10991849]
- [20] Malloy AMW, Campos JM. Extended-spectrum beta-lactamases: A brief clinical update. *Pediatr Infect Dis J* 2011; 30(12): 1092-3. [http://dx.doi.org/10.1097/INF.0b013e31823c0e9d] [PMID: 22105419]
- [21] Manzur A, Tubau F, Pujol M, et al. Nosocomial outbreak due to extended-spectrum-beta-lactamase-producing *Enterobacter cloacae* in a cardiothoracic intensive care unit. *J Clin Microbiol* 2007; 45(8): 2365-9. [http://dx.doi.org/10.1128/JCM.02546-06] [PMID: 17581932]
- [22] Gupta V. An update on newer  $\beta$ -lactamases. *Indian J Med Res* 2007; 126(5): 417-27. [PMID: 18160745]
- [23] Derbyshire H, Kay G, Evans K, Vaughan C, Kavuri U, Winstanley T. A simple disc diffusion method for detecting AmpC and extended-spectrum  $\beta$ -lactamases in clinical isolates of Enterobacteriaceae. *J Antimicrob Chemother* 2009; 63(3): 497-501. [http://dx.doi.org/10.1093/jac/dkn535] [PMID: 19155228]
- [24] Ruppé É, Woerther P-L, Barbier F, E, Woerther P, Barbier F. Mechanisms of antimicrobial resistance in Gram-negative bacilli. *Ann Intensive Care* 2015; 5(1): 21. [http://dx.doi.org/10.1186/s13613-015-0061-0]
- [25] Schito GC, Naber KG, Botto H, et al. The ARES study: an international survey on the antimicrobial resistance of pathogens involved in uncomplicated urinary tract infections. *Int J Antimicrob Agents* 2009; 34(5): 407-13. [http://dx.doi.org/10.1016/j.ijantimicag.2009.04.012] [PMID: 19155228]

- 19505803]
- [26] Martín O, Valverde A, Morosini MI, *et al.* Population analysis and epidemiological features of inhibitor-resistant-TEM- $\beta$ -lactamase-producing *Escherichia coli* isolates from both community and hospital settings in Madrid, Spain. *J Clin Microbiol* 2010; 48(7): 2368-72. [<http://dx.doi.org/10.1128/JCM.00608-10>] [PMID: 20444963]
- [27] Soltan Dallal MM, Sabaghi A, Mirzaei HM, *et al.* Prevalence of SHV  $\beta$ -lactamases in *Escherichia coli*. *Tehran Univ Med J* 2012; 68(6): 315-20.
- [28] Coque TM, Baquero F, Canton R. Increasing prevalence of ESBL-producing Enterobacteriaceae in Europe. *Euro Surveill* 2008; 13(47): 1-40. [<http://dx.doi.org/10.2807/ese.13.47.19044-en>] [PMID: 19021958]
- [29] Abid AJ, Al-Charrakh AH, Husien H. Phenotypic and molecular characterization of antibiotics resistance *E. cloacae* isolates. *Int J Appl Biol Pharm Technol* 2015; 6(1): 154-64.
- [30] Nishida S, Matsunaga N, Kamimura Y, Ishigaki S, Furukawa T, Ono Y. Emergence of *Enterobacter cloacae* complex co-producing IMP-10 and CTX-M, and *Klebsiella pneumoniae* producing VIM-1 in clinical isolates in japan. *Microorganisms* 2020; 8(11): 1816. [<http://dx.doi.org/10.3390/microorganisms8111816>] [PMID: 33217991]
- [31] Baba Ahmed Z, Ayad A, Mesli E, Messai Y, Bakour R, Drissi M. CTX-M-15 extended-spectrum  $\beta$ -lactamases in Enterobacteriaceae in the intensive care unit of tlemcen hospital, algeria. *Eastern Mediterranean Health Journal* 2012; 18(4)
- [32] Nimri LF, Azaizeh BA. First report of multidrug-resistant ESBL-producing urinary *Escherichia coli* in jordan. *Br Microbiol Res J* 2012; 2(2): 71-81. [<http://dx.doi.org/10.9734/BMRJ/2012/1360>]

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