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# Prevalence of bla$_{CTX-M}$ and blaTEM genes in *Enterobacter cloacae* isolated from urine samples in Iraqi patients

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**KEYWORDS:**

bla$_{VIM}$ and bla$_{NDM}$, *Enterobacter cloacae*, UTIs, ESBLs

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**ABSTRACT**

A clinically relevant uropathogen and an increasing cause of opportunistic infections is Enterobacter cloacae, primarily driven by worldwide distribution of extended-spectrum β-lactamase--encoding genes. Among those, blaCTX-M and blaTEM are predominant genes responsible for the resistance towards β-lactam antibiotics and hence restricting treatment choices. The present work was designed to analyse the occurrence of blaCTX-M and blaTEM genes in E. cloacae isolated from urine samples of Iraqi patients, as well as assess their correlation with antimicrobial resistance profile. This condition-specific, cross-sectional study was performed at Al-Sadr Medical City Hospital, in Najaf in Iraq from summer season 2024 to early winter 2025. Eighty-four patients with a suspicion of UTI were included in this study. Midstream urine specimens were cultured and E. cloacae isolates were identified by routine microbiological and biochemical tests. Susceptibility to ceftriaxone, ciprofloxacin, gentamicin, imipenem and meropenem testing was determined by the Kirby–Bauer disc diffusion method according to CLSI guidelines. blaCTX-M and blaTEM genes were identified by traditional PCR. The relationship between gene carriage and antibiotic resistance was evaluated by statistical analysis. Ceftriaxone (75.0%) had the highest resistance rate, followed by ciprofloxacin (54.8%), and gentamicin (48.8%), while meropenem (16.7%) and imipenem (14.3%) were less resistive agents. Molecular investigation determined the prevalence of blaCTX-M and blaTEM genes in 69.0% and 52.4% of isolates, respectively. The presence of blaCTX-M and resistance ceftriaxone, ciprofloxacin and gentamicin ($p < 0.001$) were highly significantly associated between them while resistant to ceftriaxone and ciprofloxacin had a significant association with blaTEM ($p < 0.05$). The presence of either gene did not show a statistically significant relationship with carbapenem resistance. The high rates of blaCTX-M and blaTEM genes among urinary E. cloacae isolates drawn attention to the emerging problem of ESBL-mediated resistance in Iraq. The high prevalence of ESBL gene carriage and resistance to the commonly used antibiotics calls for ongoing molecular surveillance, judicious antibiotic use, and implementation of efficient infection control measures to curb the spread of resistant E. cloacae strains.

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## INTRODUCTION

Urinary tract infections (UTIs) are among the most common bacterial diseases worldwide and affect millions of persons annually; they also lead to substantial morbidity and health-care expenditures (Foxman, 2014). Among various pathogens, Gram-negative bacteria of the family Enterobacteriaceae predominate and members of the Escherichia coli, Klebsiella pneumoniae and Enterobacter cloacae complex frequently emerge as causative agents isolated from clinical samples (Flores-Mireles et al., 2015). Traditionally, most could be easily treated with β-lactam antibiotics because they are broad spectrum agents. Nonetheless, ESBLs (Extended-Spectrum Beta-Lactamases) especially those conferred by blaCTX-M and blaTEM genes, have appeared outside of Asia and have spread worldwide with the result that third-generation cephalosporins and other β-lactams are no longer effective (Castanheira et al., 2021).

Within the ESBL-encoding genes, blaCTX-M has now become the most prevalent genotype worldwide frequently replacing classical blaTEM and blaSHV enzymes in recent isolates (Cantón et al., 2012). Systematic reports from different countries have demonstrated that blaCTX-M is found in more than 70% of clinical isolates with CTX-M-15 variant being the most common, highlighting its high burden globally (Mazumder et al.; 2020). These genes are usually located in plasmids, which allow them to be horizontally transferred among species of bacteria and further promote the spread of MDR phenotypes (Carattoli, 2013). The presence of blaTEM with blaCTX-M further blunts the used therapeutic options as isolates co-harboring both genes are generally resistant to more than one antimicrobial classes (Rawat & Nair, 2010).

ESBL-producible bacteria in HCAIs also poses a special problem when it comes to UTIs because an adequate empirical therapy is important especially if complications like pyelonephritis, sepsis and damage to the kidney are to be avoided. A survey of Enterobacteriaceae from community-acquired UTIs in Egypt, found 81.6% and 60.7% of isolates had blaCTX-M genes and blaTEM genes (commonly detected together), respectively, which were correlated with high multidrug resistance and reduction of antibiotic efficiency. Similar trends have been witnessed in other Middle Eastern region as well revealing that ESBL is rampant across the region (Mohamed et al., 2020). A one study isolates from patients in the clinic carriage of blaTEM gene was revealed 30.4% among E. cloacae between Enterobacteriaceae, bladder isolates of riches were loaded with blaCTX-M harboring clinical strains at 61.2%. These observations emphasize the significant role that ESBL genes play in resistance among uropathogens and their negative impact on patient management abroad (Pishtiwan & Khadija, 2019).

Antimicrobial resistance (AMR) has emerged as major public health hazard in Iraq. In a recent study in Al-Basrah Province, Iraq, among UTI E. coli isolates of blaTEM was found to be present in almost all and blaCTX-M in approximately half, hence illustrating the widespread occurrence of ESBL producers under clinical settings (Mohammed et al., 2024). High detected rate of these resistance determinants in UTI and other infections causing isolates, were also reported using regional studies of multiple Enterobacteriaceae species (Hamad & Khadija, 2019). However, there is limited information of the molecular epidemiology of ESBL genes in Enterobacter cloacae from urinary isolates particularly among Iraqi patients. E. cloacae is an opportunistic pathogen, which causes nosocomial complicated UTIs and its ability to act as a reservoir for plasmid-mediated resistance determinants encoding blaCTX-M as well as blaTEM is cause of concern in the clinical practice (Mezzatesta et al., 2012).

It is important to know the distribution of ESBL genes in E. cloacae because these genes not only encode a resistant variety but also are usually accompanied by other resistance mechanisms. The detection of blaCTX-M was also linked to increased co-resistance to aminoglycosides and fluoroquinolones limiting therapeutic alternatives (Cantón & Coque, 2006). Treatment In the clinical setting, infections caused by ESBL-producing bacteria often require carbapenems, which can lead to the development of carbapenem resistance finally (Tängdén & Giske, 2015), then aggravate AMR crisis. Furthermore, the transfer of ESBL genes between bacteria and strains within the hospital setting contribute to spread of resistant clones and underscore the importance of surveillance and stewardship programs (Cave et al., 2021).

Due to the rapidly growing burden of UTIs caused by ESBL-producing Enterobacteriaceae and the importance of blaCTX-M and blaTEM genes in conferring resistance, it appears critical to provide current molecular data on phenotypic prevalence of these genetic determinants among E. cloacae isolates. The present study aims to fill this gap through exploring the frequency of blaCTX-M and blaTEM genes in E. cloacae isolates recovered from urine specimens among Iraqi patients, and also by yielding practical data which can be used as a care guide for empirical therapy, in addition to information that will aid health authorities to monitor resistance mechanism against antibiotics.

## PATIENTS AND METHODS

### Study Design and Setting

This cross-sectional study was carried out in Al-Sadr Medical City Hospital, Najaf, Iraq between summer 2024 and early winter 2025. A total of 84 patients who were clinically suspected for UTI, according to their clinical presentation and by laboratory analysis, were included. The study participants were 32 men and 52 women ranging in age from 18 to 70 years. Only cases with a clinical and microbiologic definite cause of Enterobacter cloacae infections were considered. Patients with a history of antibiotic use during the 3days before the collection of urine samples and patients with underlying chronic diseases (e.g., diabetes mellitus, chronic kidney disease, immunosuppressive disorders) were excluded from the study.

**Sample collection and bacterial isolation**

Mid-stream urine specimens were obtained aseptically from all patients in sterile containers and without delay delivered to the microbiology laboratory of Al-Sadr Medical City for processing. Urine samples were cultured on MacConkey agar, blood-agar (Oxoid Ltd., UK) and incubated aerobically at 37 °C for 24 hours. The presumptive Enterobacter cloacae colonies were chosen according to the colony morphology and the lactose-fermentation profiles. Identification was done by standard bacteriological and biochemical tests: Gram staining, Triple Sugar Iron (TSI) agar test, citrate utilization test, indole production test of urease test and motility test were conducted following the standard microbiological techniques (Cheesbrough, 2006). Positive isolates were stored in tryptic soy broth (TSB) supplemented with 20% glycerol at -80 °C for further molecular analysis. Enterobacter cloacae ATCC® 13047™ reference strain was used for quality control.

**Antimicrobial susceptibility testing**

Antimicrobial susceptibility test (AST) was conducted by disc diffusion method on Mueller–Hinton agar (Oxoid Ltd., UK) as per Clinical and Laboratory Standards Iinstitute (CLSI, 2024). The tested strains were exposed to the following antibiotics belonging to different antimicrobial classes:

Ceftriaxone (30 g) – HiMedia Laboratories, India

Ciprofloxacin (5 µg) – Mast Private Limited, UK

Gentamicin (10 µg) - Bio-Rad, France

Imipenem (10 µg) – Bioanalyse, Turkey.

Meropenem (10 µg) – Oxoid, UKAfter 18–24 h of incubation at 37 °C, the diameters of the inhibition zones were determined and read as susceptible, intermediate or resistant using CLSI standards. Isolates resistant to three or more classes of antimicrobials were defined as MDR. Escherichia coli ATCC® 25922™ and Enterobacter cloacae ATCC® 13047™ were selected as quality control strains.

**DNA extraction**

Total genomic DNA was extracted from all confirmed E. cloacae isolates according to the manufacturer's instructions with GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific, Lithuania). The DNA was kept at −20° C after extraction until analyzed with molecular techniques.

**Determination of ESBL (blaCTX-M and blaTEM) genes.**

blaCTX-M and blaTEM genes were also amplified by the conventional polymerase chain reaction (PCR) method as per the previously published report with gene-specific primers (Poirel et al., 2011; Dallenne et al., 2010). All confirmed Enterobacter cloacae isolates were tested by PCR for detection of the blaCTX-M and blaTEM genes. Plasmid was amplified with gene-specific primers following established cycling parameters. The products of the PCR were visualized on 1.5% agarose gels, stained with ethidium bromide, and photographed under UV light. The presence of target genes was inferred according to the amplicon size (Table 1).

Each PCR reaction was performed in a total reaction volume of 25 µL, which contained 12.5 µL of 2× PCR Master Mix (Promega Coompany, USA), 1 µL (10 pmol/µL) each of the forward and reverse primers, 3 µL DNA template and sufficient nuclease-free water to bring the volume up to 25 µL. PCR amplification was carried out in a thermal cycler (Eppendorf Mastercycler, Germany) as following: an initial denaturation at 94 °C for 5 min; followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 40 s and extension at 72 °C for 45 s; with a final extension at 72°C for7min.The PCR products were resolved in 1.5% agarose gel with ethidium bromide (0.5 µg/mL) and visualized under UV transilluminator on a Gel documentation system (Bio-Rad, USA). Standard ESBL-producing strain positive for blaCTX-M and blaTEM were used as positive control and nuclease free water was served as negative control.

**Ethical considerations**

The study design was approved by the Ethical Committee of Kufa University / Al-Sadr Medical City Hospital, and an informed consent was signed from all participants before taken specimen.

**Table1: List of primers applied for amplification of $bla_{CTX-M}$ and $bla_{TEM}$ genes**

| Gene | Amplicon size (bp) | Annealing temp (°C) | Primer sequence (5′–3′) |
|---|---|---|---|
| **$bla_{CTX-M}$** | 593 | 55 | F: ATGTGCAGYACCAGTAARGTKATGGC<br>R: TGGGTRAARTARGTSACCAGAAYCAGCGG |
| **$bla_{TEM}$** | 867 | 55 | F: ATGAGTATTCAACATTTCCGTG<br>R: TTACCAATGCTTAATCAGTGAG |

## RESULTS

A high resistance rate of E. cloacae isolates to third generation cephalosporins, especially ceftriaxone (75.0%) in our study revealed that ESBL-producing strains were widely prevalent as shown in Table 2. Moderate resistance to ciprofloxacin (54.8%) and gentamicin (48.8%) was noted which suggests almost no usefulness of these agents for empirical treatment. On the other hand, carbapenems such as meropenem and imipenem were highly active against isolates with susceptibility rates greater than 85%, indicating that they remain effective treatment choices for complicated infections due to MDR E. cloacae (Table 2).

**Table 2. Rates of antibiotic resistance reported in the *Enterobacter cloacae* isolates**

| Groups | Resistant Isolates No. (%) | Susceptible Isolates No. (%) |
|---|---|---|
| **Ceftriaxone** | **63 (75.0%)** | **21 (25.0%)** |
| **Gentamicin** | **41 (48.8%)** | **43 (51.2%)** |
| **Ciprofloxacin** | **46 (54.8%)** | **38 (45.2%)** |
| **Meropenem** | **12 (14.3%)** | **72 (85.7%)** |
| **Imipenem** | **10 (11.9%)** | **74 (88.1%)** |

According to table 3, the resistance mechanism in this population is dominated by CTX-M–type ESBLs; as high blaCTX-M prevalence was recorded; 69.0% among Enterobacter cloacae strains against other types of SHV and TEM ESBLs. The blaTEM gene was detected in 52.4% of the isolates indicating its significant role in β-lactam resistance. Moreover, the concomitance of these genes in a substantial proportion of isolates indicates the growing prevalence of ESBL-based resistance among urinary E. cloacae isolates and emphasizes the necessity for ongoing molecular monitoring (Table 3).

**Table 3. Presence of blaCTX-M and blaTEM genes in *Enterobacter cloacae* isolates**

| Groups | Positive No. (%) | Negative No. (%) |
|---|---|---|
| **$bla_{CTX-M}$** | **58 (69.0%)** | **26 (31.0%)** |
| **$bla_{TEM}$** | **44 (52.4%)** | **40 (47.6%)** |

Table 4 shows significant relationship between the ceftriaxone and pro-A gene carrying and $bla_{CTX-M}$; the same was shown to hold true for its sister gene $bla_{TEM}$ ($\chi^2 = 18.42$, $P< 0.001$) ($\chi^2 \geq 11.36$ $P=0.001$). This confirms that ESBL genes play a vital role in the resistance of third-generation cephalosporins associated with Enterobacter cloacae isolates. Furthermore, there was also a significant association between $bla_{CTX-M}$ and resistance to gentamicin ($\chi^2 = 4.92$, $p = 0.027$) and ciprofloxacin ($\chi^2 = 6.34$, $p = 0.012$), with a significant relationship even clearer between ciprofloxacin and $bla_{TEM}$ ($\chi^2=4.08$, $p=0.043$) On the other hand, no definitive association was identified between resistance to carbapenems (meropenem and imipenem) and the presence of either ESBL gene. Fact is, carbapenems still work well against ESBL-producing E. cloacae isolates in our setting.

**Table 4. Association between antibiotic resistance and Presence of $bla_{CTX-M}$ and $bla_{TEM}$ genes in the isolates of *Enterobacter cloacae***

| Groups | $bla_{CTX-M}$ Chi Square (P value) | $bla_{TEM}$ Chi Square (P value) |
|---|---|---|
| **Ceftriaxone** | **18.42 (0.000)**** | **11.36 (0.001)**** |
| **Gentamicin** | **4.92 (0.027)*** | **3.11 (0.078)** |
| **Ciprofloxacin** | **6.34 (0.012)*** | **4.08 (0.043)** |
| **Meropenem** | **1.02 (0.312)** | **0.84 (0.359)** |
| **Imipenem** | **0.67 (0.414)** | **0.53 (0.467)** |

Significant at $P<0.05$; ** High significant at $P<0.001$

## DISCUSSION

In the current study, we determined the frequency of blaCTX-M and blaTEM genes in E. cloacae strains isolated from urinary tract infections in Iraqi patients and their correlation with antimicrobial resistance profiles. The results showed a high prevalence of ESBL-based resistance, indicating the increased clinical treatment difficulty by MDR E. cloacae in UTI.

In the present study, resistance to ceftriaxone was very high (75.0%) that agrees well with the heavy prevalence of ESBL-producers found at molecular level. This result is consistent with the established role of ESBL in conferring resistance to third-generation cephalosporins and indicates that these agents would not be as effective for empiric therapy of UTIs caused by Enterobacter spp.

Comparable resistance rates were observed in the Middle East and other world studies; where ceftriaxone resistance in Enterobacter cloacae and its relatives-Enterobacteriaceae were 65%– > 80% (Cantón et al., 2012). The misuse and overuse of cephalosporins in the clinical practice may have played a role in the rise and maintenance of ESBL-producing strains among this population (Esfahanian et al., 2022).

The resistance rate to ciprofloxacin (54.8%) and gentamicin (48.8%) was moderate, revealing a reduced susceptibility to fluoroquinolones and aminoglycosides. These results are in agreement with that of Araújo et al. (2023), who observed upward trends in antimicrobial resistance in Enterobacter spp., frequently correlated with ESBL production. The found co-resistance could be explained by the fact that resistance genes were located in transferable plasmids with accumulation of several resistance gene determinants thereby not only propagating to several antimicrobial classes. In the clinical setting, it restricts alternatives for oral treatment and adds difficulties in managing UTIs especially as an outpatient (Carattoli, 2013).

Carbapenems, on the other hand, proved very effective against these isolates, showing susceptibility rates that were higher than 85% for imipenem and meropenem. The low rates of resistance to carbapenems in our findings are promising and in agreement with a report from Iraq as well other countries bordering Iraq, where it was found that carbapenemases were still considered the most effective agents against ESBL-producing Enterobacteriaceae (Mohammed et al., 2024; Tängdén & Giske, 2015). Lack of carbapenemase production among strains harbouring blaCTx—m, or b1aTEM additionally supports the idea that ESBL producers are not automatically carbapenemresistant. Yet, prolonged use of carbapenems may lead to the development of carbapenem-resistant organisms in the long run and usage should be coupled with antimicrobial stewardship practices.

Molecular tests found that the most common ESBL gene was blaCTX-M (69.0% of E. cloacae isolates), followed by blaTEM (52.4%). This dominance of blaCTX-M reflects worldwide situation concerning ESBL production, in which CTX-M-type enzymes have become the most commonly encountered type, having largely replaced TEM and SHV enzymes as the predominant ESBLs in clinical isolates (Cantón & Coque, 2006). A high blaCTX-M proportion detected in this study is consistent with the results of local studies conducted in Iraq and similar countries where CTX-M producers are widely traced in hospitals (Hamad & Khadija, 2019; Mohammed et al., 2024).

The association analysis has revealed a strongly significant correlation between ceftriaxone resistance and detection of blaCTX-M ($\chi^2 = 18.42$, $p < 0.001$) as well as with the presence of blaTEM ($\chi^2 = 11.36$, $p = 0.001$) gene in isolates. This is consistent with the predominance of these ESBL encoding genes in conferring resistance to third generations cephalosporins and also underpins earlier observations describing ESBL production as a major mechanism of the β-lactam resistance observed in Enterobacter spp (Paterson & Bonomo, 2005). Significant positive associations were also found between blaCTX-M and resistance to gentamicin, ciprofloxacin and blaTEM with ciprofloxacin. These results imply the intestinal carriage of ESBL genes, along with other penicillins and cephalosporin resistance determinant, which could have been co-transferred through plasmids resulting in multi-drug resistant phenotype (Rawat & Nair, 2010).

Finally and notably, no significant relationship was found between carbapenem resistance and the presence of either ESBL gene, further confirming that development of carbapenem resistance is commonly driven by other mechanisms including production of a carbapenemase, porin loss or efflux pump over-expression in addition to if not regular rather than sole production of an ESBL enzyme. This is a clinically significant difference which emphasizes the significance of carbapenems, but also to uncertainty about carbapenemase genes that are developing (Navon-Venezia et al., 2017).

## CONCLUSION

The results of this study show a high prevalence of ESBL producing E. cloacae in UTI patients in Iraq and reveal significant association between ESBL determinants and resistance to commonly applied antibiotics. Our findings underscore the immediate necessity of molecular detection of ESBL genes, rational use of antibiotics, and introduction of efficient infection control interventions. Ongoing surveillance and region-specific antimicrobial policies are necessary to help control the dissemination of MDR E. cloacae and to maintain the efficacy of last-line antibiotics.

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