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ORIGINAL ARTICLE

PCR Detection of Spreading TEM and CTX-M gene in *Klebsiella oxytoca* isolate from Urinary Tract Infection

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Key words:
Klebsiella oxytoca, Urinary tract infection (UTI), CTX-M, and TEM

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ABSTRACT

Background: *urine is usually free of bacterial infection occurs by ascending or descending infection pathway. CTX-M enzymes are a group of class A extended-spectrum β-lactamases (ESBLs) that are rapidly spreading among Enterobacteriaceae, Most of the ESBLs are mutants of the TEM and the SHV enzymes and developed from point mutation of genes that code production of primordial TEM-1, TEM-2, or SHV-1 β lactamase enzymes.* ***Objectives:*** *This study aimed to detect K. oxytoca bacteria in urine samples obtained from individuals with urinary tract infections.and, we aimed to the existence of blaCTX-M and blaTEM genes in Klebsiella oxytoca in Najaf governorate from November 2022 to June 2023.* ***Methodology:*** *The study included 540 urine specimens. Based on culture and biochemical features, K. oxytoca isolates were identified. The Vitek-2 compact system provides a confirmatory test, PCR was used for detecting the blaCTX-M and blaTEM genes.* ***Result:*** *seventeen isolates were identified as K. oxytoca. Vitek further confirmed this, PCR technique targeting the blaCTX-M gene detected in 14 isolates, while 4 from the 17 isolates were positive for the blaTEM gene.****Conclusion:*** *The molecular study revealed that blaCTX-M and blaTEM gene were detected in (82.3%) and (23.5%) of K. oxytoca isolates carrying blaCTX-M and blaTEM genes respectively.*

INTRODUCTION

Urinary tract is usually colonized by microorganisms. Colonization implies the persistence and growth of bacteria at the infected site, but the infection may or may not produce clinical signs. Bacterial urinary tract infection (UTI) is the third most prevalent illness in humans, behind respiratory and gastrointestinal infections. It affects individuals of all age groups and has a greater prevalence in females compared to men, UTIs are affecting 150 million people worldwide each year[1] Urinary tract infections may be managed and prognosed differently depending on the location of the illness and any variables that increase the risk of infection.[2]

Urinary tract infections (UTIs) are categorized into two types: simple and difficult. Uncomplicated urinary tract infections (UTIs) often occur in persons who are in good health and do not have any structural or neurological abnormalities in their urinary system. A lower urinary tract infection (cystitis) or an upper urinary tract infection (pyelonephritis) can cause these infections.[3]

Although gram-positive and gram-negative bacteria can both cause UTIs, the majority of simple UTIs are caused by *E. coli* (75%-95% of cases), *Proteus mirabilis*, *Enterococcus faecalis*, group B *streptococci*, *Staphylococcus saprophyticus*, and *Klebsiella pneumoniae*. Less than 5% of UTIs are multifactorial.[4]

One of the most common pathogens in UTI is *Klebsiella oxytoca*, fimbriae play a pivotal role in nosocomial infections in hospitals, which may result in septicemia, and are essential pathogenic factors by adhering to host tissues[5] *Klebsiella oxytoca* is colonized in the intestine, urinary tract, and on the skin surface of 2–10% of the general population and is connected with antibiotic-associated hemorrhagic colitis (AAHC).[6]

Bacteremia caused by *Klebsiella oxytoca* may be obtained in the community or a healthcare facility. It is often seen in individuals with hepatobiliary or pancreatic illnesses, neoplastic disorders, diabetes mellitus, and other underlying conditions.[7] It is crucial to recognize that *K. oxytoca* may also lead to a range of problems, such as wound infections and urinary tract infections (UTIs). Moreover, the substantial ESBL-mediated resistance shown by *K. oxytoca* isolates is recognized as a serious issue for public health. The illness is getting more resistant to treatment due to the failure of therapeutic interventions and the limitations of antimicrobial drugs, which are being caused by the rising levels of these enzymes.[8]

Thus far, the chromosomal β-lactamase responsible for *K. oxytoca's* resistance to amino and carboxypenicillins, as well as the amoxicillin-clavulanate combination. This enzyme is classified as a

member of class A β-lactamases. Which its synthesis is not controlled, and cause it to be overproduced. This overproduction leads to bacterial resistance to certain third-generation cephalosporins (cefotaxime and ceftriaxone) and aztreonam[9].

The aims of this investigation is to detect the presence of the *blaCTX-M* and *blaTEM* genes in *K.oxytoca* isolates isolated from patients with urinary tract infections (UTIs). *K. oxytoca*, isolated from UTI patients, has these two genes which serve as a constituent of the antibacterial resistance mechanism.

METHODOLOGY

Ethical Consideration

The Scientific Research Committee in the Najaf Health Department and the Scientific Research Ethics Committees in the University of Kufa's College of Medicine both gave their approval.

Patients and Clinical Specimens' Collection

This research delete enrollment of 540 patients, of various ages ranging from 1 to 60 years, who were diagnosed as urinary tract infections (UTIs). The participants were selected from both genders and were recruited throughout a set period from November 2022 to June 2023. Participants were enlisted from AlSader Medical City, Al-Hakeem General Hospital, Al-Furate Al-Awsat Hospital, and Al-Zahra Maternity and Children in Najaf City, Iraq. Specimens were obtained from the midstream portion of the urine into a clean, sterile container.

Bacterial Isolation and Identification

Urine samples, were transported to the laboratory for culture on MacConkey's agar and blood agar plates. The primary isolates were identified by colony morphology, grams stain, biochemical reactions and The Vitek2 system. Furthermore, the final identification of bacteria isolates was biochemically verified through the utilization of the VITEK2-automated system. antibacterial sensitivity pattern were evaluated of K. oxytoca isolates using the disc diffusion method by CLSI 2023 (Fig. 1). Susceptibility profiles were performed with 20 antibiotics, including Amoxiclav (20/10) with Ampicillin (10), Ticarcillin /Clavulanic acid (75/10), Ampicillin /sulbactam (10/10), Cefotaxime (30), Cefepime (10), Ceftazidime (30), Aztreonam (30), Co- Trimoxazol (23.75/1.25), Gentamycin (10), Amikacin (30), Tobramycin (10), Ciprofloxacin (5), Cefoxitin (30), Ceftriaxone (30), Meropenem (10), Kanamycin (30), Nalidixic acid (30), Norfloxacin (5), and Chloramphenicol (30).

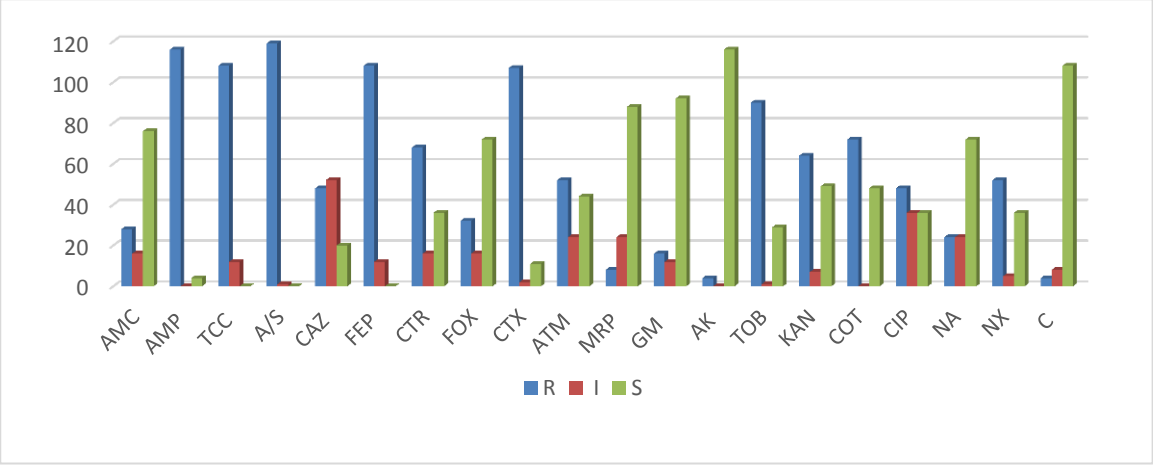

Fig. 1: Susceptibility of isolates K. oxytoca to antibiotics

DNA Extraction

The DNA extraction technique included the following steps: a) A volume of one milliliter of the bacterial growth in brain heart infusion (BHI) broth was transferred into a microcentrifuge tube with a capacity of 1.5 milliliters. Subsequently, it underwent centrifugation at a rotational speed of 10000 r.p.m for 1 minute. b) the supernatant, was discarded. Extraction of genomic DNA was carried out on the bacterial pellets. c) The genomic DNA was obtained by using a commercially available extraction kit (Genomic DNA Promega Kit), following the directions supplied by the manufacturer. The UV spectroscopy was used to assess DNA yield and purity from bacterial cultures. A Shimadzu ultraviolet (UV)-1800 double-beam UV/Visible Scanning Spectrophotometer was used to measure the Optical Density (OD) for evaluating the purity of DNA at 260/280 nm.

PCR amplifucation

As mentioned earlier, DNA samples (5 µL) were amplified using the PCR equipment in a 25 µL reaction mixture using the Sure Cycler 8800 (Agilent Technologies, Inc. USA). The *blaCtxm* gene was amplified using the following precise thermal cycling pattern: The PCR protocol done according to manufacture instruction. Gen of *blaTEM* was amplified per the prescribed requirements: The procedure. Alpha DNA Company (Canada) used the sequence primers specified in table 1. The examination of DNA fragments included the use of electrophoresis according to manufacture instruction. A 100-base pair DNA ladder manufactured by Pioneer, a company based in Korea, was employed as a benchmark for evaluating the DNA fragments' size. The solo band was rigorously detected at the suitable location using an ultraviolet light trans illuminator (Cleaver, UK). A gel documentation device manufactured by Cleaver in the United Kingdom was used to capture the bands.

Table 1:Primers utilized in the present study

Gene	Primary Denaturation	Denaturation	Annealing	Stretching	Last Stretching	Cycle
blaCTX-M	94˚C/ 5min	94˚C / 1min	57˚C/1min	72˚C/1min	72˚C/10min	40
blaTEM	94˚C/ 5min	94˚C/ 30sec	56˚C/30sec	72˚C/1.5min	72˚C/7min	35

RESULTS

Distribution and Characteristics of Patients

Patient data are seen in Table 2. Many of the participants were from the Najaf Provincial Medical Center known as AL-Sadar Medical City. It was determined that 365 specimens (67.6% of the total) were collected from females and 175 specimens (32.4% of the total) were collected from males. Based on their residence, 48.5% of the specimens were collected from patients in urban areas and 51.5% from patients in rural areas. The gender and residence distribution of the patients are shown in Table 3. Ages 1–20 made up the largest demographic, while those between 21 and 40 made up the smallest (both sexes combined).

Table 2: gender and position of their Residence distribution of the patients

Hospital	Male	Female	Rural	Urban
Al-Sadr Medical City	64	128	78	81
Al-Hakeem General Hospital	58	102	69	76
Al-Furate Al-Awsat Hospital	32	56	52	45
Al-Zahra for Maternity and Children	21	79	63	76
Total	175	365	262	278
	540		540	

Table 3: The distribution of patients by age and gender

Age(year)	Male	Female	Total %
1-20	68	118	186(34.4%)
21-40	25	64	89(16.5%)
41-60	38	78	116(21.5%)
>60	44	105	149(27.6%)
Total	175(32.4%)	365(67.6%)	540 (100%)

Detection of the blaCTX-M Gene

In Figure 2, 14 out of the 16 *K. oxytoca* isolates were positive for the *blaCTX-M* gene, indicating that the PCR approach was successful in detecting the *CTX-M* gene of this the size of amplified genom.

Detection of the blaTEM gene

Four *K. oxytoca* isolates (or 23.5% of the total) were positive for the *blaTEM* resistance gene, as shown in Figure 3.

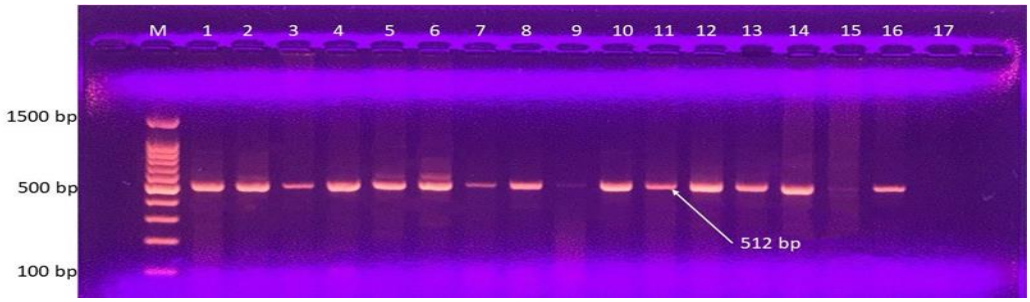

Fig. 2: Products of PCR amplification of K oxytoca isolates amplified with blaCTX-M primers. Lane M , DNA molecular size marker (1500-bp ladder), and Lanes (1,2,3,4,5,6,7,8,10,11,12,13,14,16) show positive results with blaCTX-M gene.

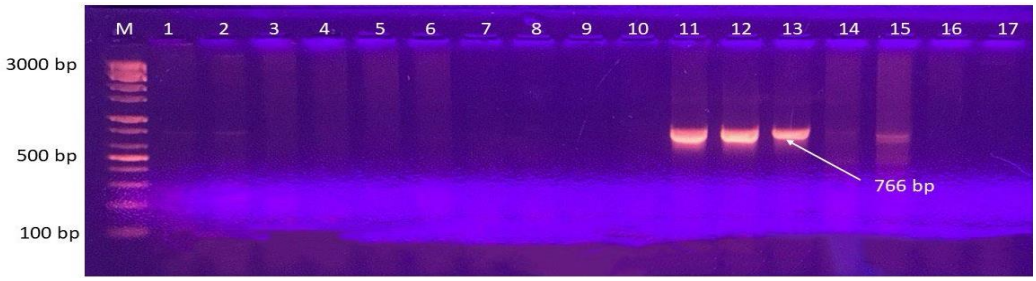

Fig. 3: PCR amplification products of *K. oxytoca* isolates that amplified with blaTEM gene primers with product 766 bp. Lane (L), DNA molecular size marker (3000-bp ladder), and Lanes (11,12,13,15) show positive results with blaTEM gene.

DISCUSSION

Klebsiella species, which are emerging as important pathogens across most populations. Infections caused by *Klebsiella* species are more likely to occur in individuals with weakened immune systems and those who have been exposed to several drugs. These infections are particularly common in hospital settings and may lead to extended hospital admissions. Additionally, the risk of developing multidrug-resistant (MDR) infections is increased under these circumstances.[10]

All the *K. oxytoca* isolates showed 100% resistance to Amoxiclav, Norfloxacin, and Chloramphenicol. The resistance to aminoglycoside antibiotics varied where tobramycin and Gentamicin with a percentage of (5.9%), Amikacin with a percentage of (58.8%), but *K. oxytoca* isolates showed no resistance to Ampicillin, Ceftazidime, Cefepime, Cefotaxime, Kanamycin. Therefore, the detection of *blaCTX-M* gene in clinical isolates of *K. oxytoca* is crucial since these bacteria exhibit resistance to extended-spectrum β-lactam antibiotics. Consequently, selecting an effective antimicrobial drug for treating condition triggered by these organisms becomes challenging.[11]

The resistance of *Klebsiella spp* to the antibiotics is primarily caused by enzymatic modification or degradation. This is facilitated by various mechanisms, with a significant one being the synthesis of enzymes encoded by multiple genes present on certain bacterial plasmids. The enzymes involved in this process include β-lactamase and extended-spectrum β-lactamase.[12]

Extended-spectrum β-lactamases (ESBLs) are enzymes that are carried on plasmids and can break down and render ineffective a broad range of β-lactam antibiotics, including various types of penicillins and cephalosporins. These enzymes can cause resistance to Piperacillin or first-generation Cephalosporins by overexpressing Beta-lactamases or producing both SHV-1 and TEM-1. Clavulanic acid has activity against the SHV-1 strain[13].

It is challenging to select an appropriate antimicrobial drug for treating infections induced by *K. oxytoca* that are resistant to extended-spectrum β-lactam antibiotics[14].

Klebsiella oxytoca consistently develops chromosomal β-lactamases that provide resistance to ampicillin and amoxicillin. bacteria that exhibit excessive production of these B-lactamases or newly emerged bacteria that develop extended-spectrum Beta-lactamases exhibit resistance to other beta-lactam antibiotics[15].

The widespread dissemination of *K. oxytoca* strains that are resistant to many drugs, including those that

produce extended-spectrum β-lactamases (ESBLs), carbapenemases, and other enzymes that inactivate aminoglycosides, has sparked fresh worldwide concern about these diseases[16].

Klebsiella oxytoca inherently demonstrates resistance to amino- and carboxypenicillins, however, it is vulnerable to the amalgamation of amoxicillin and clavulanate. So far, the particular chromosomal β-lactamase that causes this β-lactam resistance has only been found in *K. oxytoca*. This β-lactamase is classified as a class A β-lactamase. While its synthesis is not controlled, up-mutations may stimulate its overproduction. Consequently, some strains develop resistance to third-generation cephalosporins (ceftriaxone and cefotaxime) and aztreonam[17].

It is important to take into account several restrictions in the current research when evaluating the results. Initially, we were unable to investigate the correlation between additional ESBL genes and other antibiotic resistance. Nevertheless, a significant number of the isolates exhibited resistance to some drugs. Subsequent investigations should only focus on resistant microorganisms. In addition, implementing effective infection control measures along with sensible antimicrobial policies can ensure a restriction in the transmission and proliferation of multidrug-resistant (MDR) and extensively drug-resistant (XDR) isolates. To assess the spread of β-lactamases in *K. oxytoca* clinical isolates across the country, it is recommended to employ real-time polymerase chain reaction (RT-PCR) to evaluate if these genes are being expressed and producing proteins. A PCR test was used to identify the *blaCTX-M* gene in K. *oxytoca* isolates. Out of the *K. oxytoca* isolates tested, 14 (82.3%) showed positive findings for the *blaCTX-M* gene[18].

A study examined the presence of *bla TEM* and *bla CTX-M* genes in 21 *K. oxytoca* isolates. It was discovered that 66.6% of the isolates carried *bla TEM*, and 73.3% contained *bla CTX-M* genes, which are responsible for β-lactamase resistance. Another study conducted by Reyam & Al-Muhanna, A. S.(2021) showed that *bla TEM* and *bla CTX-M* were present in 9.72% and 8.33% of the isolates, respectively. *Klebsiella oxytoca* strains obtained from people exhibiting multidrug resistance had previously been shown to have four separate *blaCTX-M* genes, namely *blaCTX-M*-3, *blaCTX-M*-9, *blaCTX-M-15*, and *blaCTX-M-35*, which were isolated from various nations [19].

CONCLUSIONS

Our study indicates that *K. oxytoca* is a prevalent source of urinary tract infections in Iraqi individuals. The levels of multidrug resistance (MDR) shown by *K. oxytoca* isolates in hospitals in Najaf were unquestionably elevated. The bulk of bacterial isolates exhibit the occurrence of the CTX-M gene and TEM. The existence of these genes is a significant contributor to the ability of the organism to cause disease and likely contributes to its ability to withstand the effects of antibiotics.

Recommendations:

Further study to detected another species of *Klebsiella* that involved in urinary tract infections and β-lactamase examination should be performed for all isolates diagnosed in Iraqi hospitals, and make it a routine examination for all bacterial isolates that cause urinary tract infections.

Ethical approval

The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki). Confidentiality of data, the authors declare that they have followed the protocols of their work center on the publication of patient data. Right to privacy and informed consent, the authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author is in possession of this document. Use of artificial intelligence for generating text, the authors declare that they have not used any type of generative artificial intelligence for the writing of this manuscript, nor for the creation of images, graphics, tables, or their corresponding captions.

Declarations:

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