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# Molecular Detection of bla$_{TEM}$ and bla$_{CTX-M}$ Encoding Genes from *Klebsiella oxytoca* Isolates from Tonsillitis

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**ABSTRACT**

**Aims** Tonsillitis is inflammation of the tonsils, a common clinical state caused by bacterial or viral infections. There are different types of tonsillitis; acute, sub-acute, chronic, and recurrent. The aim of this study was the isolation and identification of *Klebsiella oxytoca* isolated from tonsillitis based on conventional standard bacteriological methods and confirmed by VITEK-2 compact system.

**Materials & Methods** A polymerase chain reaction was performed to detect bla$_{CTX-M}$ and bla$_{TEM}$ genes.

**Findings** A total of 50 specimens were recovered from tonsillitis using swab sampling, which contained 35 bacterial growths. Onto the MacConkey agar, 15 isolates were confirmed as *K. oxytoca* using IMVIC test and VITEK-2 compact system. In the genotypic test, *K. oxytoca* isolates contained 11 (73.3%) bla$_{CTX-M}$ and 10 (66.6%) bla$_{TEM}$ genes.

**Conclusion** The use of the VITEK-2 system is necessary to confirm the precise identification of *K. oxytoca* nosocomial pathogens from tonsillitis. The existence of bla$_{TEM}$ and bla$_{CTX-M}$ gene in half of *K. oxytoca* isolates is a concern that needs control strategies.

**Keywords** *Klebsiella oxytoca*, bla$_{TEM}$, bla$_{CTX-M}$, Tonsillitis

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## Introduction

Inflammation of the tonsils in the back of the throat is known as tonsillitis. The tonsils, adenoids, and lingual tonsils are all commonly inflamed during a case of pharyngitis. A painful throat, fever, swollen tonsils, difficulty swallowing, and enlarged lymph nodes in the neck are the most common symptoms. Group A beta-hemolytic Streptococcus pyogenes is the most common bacterial cause of tonsillitis, however Klebsiella species are also important human pathogens that have been linked to rising morbidity rates [1]. These bacteria are prevalent in the environment and can be found in the intestines of humans and animals, as well as water and soil. Infections and medication resistance are more likely to occur in patients who already have a damaged immune system, have been exposed to several antibiotics, or have multiple chronic conditions. In hospitalized patients, Klebsiella species frequently cause bronchopneumonia, UTIs, and septicemia [2]. They have also the ability to cause outbreaks of nosocomial infections as they often share plasmid-mediated resistance with other bacteria, which are more common at tertiary and specialized centers [3].

Among the Klebsiella spp, *Klebsiella oxytoca* has been isolated more frequently. *K. oxytoca* is a rod-shaped, nonmotile, Gram-negative bacterium with a prominent polysaccharide capsule [4-6]. Recently, *K. oxytoca* has emerged as one of the most antibiotic-resistant organisms responsible for outbreaks in both community and clinical settings, causing infections in patients receiving medical care. It can colonize the gastrointestinal tract, nasopharynx, and the skin, and it can cause a wide variety of infections, from relatively mild ones like a sore throat or a rash to life-threatening ones like septicemia or pneumonia [7].

Antimicrobial therapy is largely successful against infections caused by this bacteria. Antibiotics are given to people with infections to counteract the body's natural defenses. Bacteria are now resistant to -lactam antibiotics because *K. oxytoca* has evolved the enzymes extended spectrum -lactamases (ESBLs) and carbapenemases [8-10]. As with other Klebsiella species, *K. oxytoca* (formerly known as Bacterium oxytocum) may produce indole, has a positive Voges-Proskauer reaction, and liquifies gelatine. *K. oxytoca* is commonly picked up in the wild. Cefotaxime, ceftazidime, and aztreonam are three antibiotics that *K. oxytoca* is notoriously resistant to [11].

Antimicrobial sensitivity changes due to biofield treatment have been documented recently. CTX-M enzymes were reported at first time in E. coli species in 1990 [12]. These enzymes hydrolyze-actam antibiotics, leading to resistance to penicillins, cephalosporins, and aztreonam, and are encoded on plasmids, making them more horizontally transmissible [13]. Because of their greater effectiveness against cefotaxime than ceftazidime and their first isolation location (Munich; Germany), CTX-Ms are known by this acronym [14]. The CTX-M type enzymes belong to a group of class A ESBLs according Ambler classification that in general exhibit much higher levels of activity against cefotaxime and ceftriaxone than ceftazidime [15, 16].

Thus far, 172 variants of CTX-M were identified worldwide. Gram-negative bacteria are responsible for encoding the vast majority of TEM, while blaTEM-encoded genes account for about 90% of ampicillin resistance in gram-negative bacteria [17]. Single or multiple amino acid substitutions around the active site characterize the majority of TEM-type ESBLs, which are produced from mutations in the traditional TEM (TEM-1) and (TEM-2) genes via plasmid-mediated evolution. In 1965, the *bla*$_{TEM-1}$ gene was discovered in *Escherichia coli* that had been isolated from a patient named Temoneira (thus, TEM) in Athens, Greece [18]. Single or several changes in the amino acid sequence of the original TEM-1 enzymes allowed for the development of TEM-2, which hydrolyzes penicillin and first-generation cephalosporins like cephaloridine [19]. These enzymes become the most commonly encountered β-lactamase among gram negative bacteria [20, 21]. Clinically, TEM-24, TEM-4, and TEM-52 are the most widely spread TEM-type ESBLs among European Enterobacteriaceae, while TEM-52, TEM-106, and TEM-116 are the most prevalent among animal isolates [22].

Because antimicrobial-resistant Klebsiella species in humans and reports on *K. oxytoca* are limited in the study. The aim of this study was the isolation and identification of *Klebsiella oxytoca* isolated from tonsillitis and detection of *bla*$_{TEM}$ and *bla*$_{CTX-M}$ in these bacteria.

## Materials and Methods

### Isolation and identification of bacterial isolates

After tonsillectomy, the sample surface is sterilized and opened with a sterile scalpel, and a swab is taken from the fibrosis found in the tissue to collect the sample. Fifty clinical specimens were collected from patients suffering from tonsillitis [23, 24]. These samples were collected for the period from February to September 2022 from patients from Al-Hakim Hospital, Al-Sadr Teaching Hospital, and outpatient clinics. Specimens were inoculated on routinely culture media: MacConkey agar which considered as predominant, selective and differential media for the isolation, purification and identification of *K. oxytoca*. As well as blood and Chocolate agar. The plates were incubated at 37˚C for 24 hours and then a single pure isolated colony was transferred to trypticase soy agar (TSA) for the preservation and to carry out other biochemical tests (IMVIC) and VITEK-2 system that confirmed the identification of isolates [25].

Specimens were inoculated on three types of culture media, including mannitol salt agar and MacConkey agar (Merk; Germany), which are considered as



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predominant, selective, and differential media for the isolation, purification, and identification of many types of bacteria. The plates were incubated for 24 hours at 37°C, and then a single pure isolated colony was transferred to Trypticase Soy Agar (TSA) for preservation and other biochemical tests, and the VITEK system confirmed the identification of the isolates.

**DNA extraction**

Genomic DNA was extracted using a commercial extraction system (Favorgen; Taiwan), according to the manufacturer's instructions.

**Molecular identification**

Primers used in the study were designed by Alpha DNA Company, Canada (Table 1).

**Table 1)** Specific primers for *K. oxytoca*

| Primer | Primer sequence (5'-3') | Amplicon size (bp) |
|---|---|---|
| ***bla*TEM** | F: TCAACATTTTCGTGTCGCCC<br>R: AACTACGATACGGGAGGGCT | 766 |
| ***bla*CTX-M** | F: ATGTGCAGYACCAGTAA<br>R: CCGCTGCCGGTYTTATC | 510 |

The PCR assay was performed to detect the *bla*TEM and *bla*CTX-M gene primers specific for *K. oxytoca* (Table 2). Amplified products were confirmed using 0.8% agarose gel electrophoresis to estimate the PCR product size. The gel was stained with 4µL of 0.5mg/mL ethidium bromide (Sigma; USA) and ran at 70v for 1.5h. Bands were photographed using a gel documentation system (Cleaver; UK). A 100bp ladder (Bioneer; Korea) was used to measure the molecular weights of amplified products.

**Table 2)** Thermal PCR program for *bla*TEM and *bla*CTX-M gene amplification in the thermocycler

| Gene | Initial denaturation | No. of cycles | Denaturation | Annealing | Extension | Final extension |
|---|---|---|---|---|---|---|
| ***bla*TEM** | 95˚C for 5min | 50 | 95˚C for 30sec | 56˚C for 45sec | 72˚C for 1min | 72˚C for 5min |
| ***bla*CTX-M** | 95˚C for 3min | 50 | 95˚C for 30sec | 51˚C for 30sec | 72˚C for 40 sec | 72˚C for 5min |

## Findings

### Isolation and identification of *K. oxytoca*

The initial identification of gram negative rod was depended on the colonial morphology, biochemical tests and VITEK-2 system, 35 out of 50 of bacteria on MacConkey agar were lactose fermented produce pink colony, 15 out of 50 of isolates were lactose non fermented bacteria produce yellow or colorless colony agar, fifteen from thirty five isolates identified as *Klebsiella oxytoca*, other characteristic features include: production of mucoid appearance and giving indole, Methyl-red negative result but Vges-Poskeur, citrate positive result. The automated VITEK-2 compact system with GN-ID cards containing 47 biochemical tests and one negative control well was used to make the final identification. From a total of 35 possible *K. oxytoca* isolates, only 15 were positively identified. The confidence level of the ID message ranged from very good to excellent (probability from 95 to 99%).

### Molecular detection of *bla*TEM and *bla*CTX-M encoding genes from *K. oxytoca*

The existence of *bla*TEM and *bla*CTX-M genes was investigated among 15 isolates of *K. oxytoca*, which contained 10 (66.6%) *bla*TEM and 11 (73.3%) *bla*CTX-M genes being responsible for β-lactamases. The *bla*TEM and *bla*CTX-M PCR products included 766 and 510bp, respectively (Figures 1 and 2).

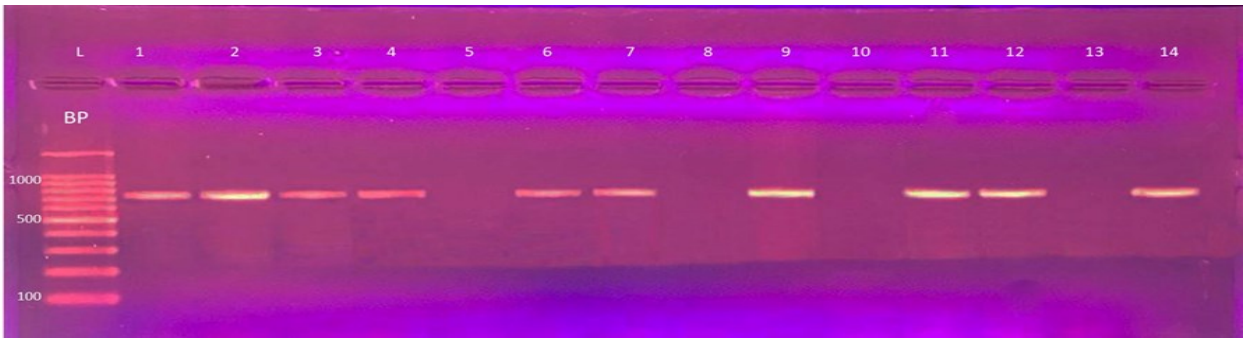


**Figure 1.** PCR amplification products of *K. oxytoca* isolates amplifying the *bla*TEM gene product with 722bp Lane L: DNA molecular size marker (100bp ladder); Lanes isolates no. 1, 2, 3, 4, 6, 7, 9, 11, 12, 14, 15 indicate positive results for the *bla*TEM gene

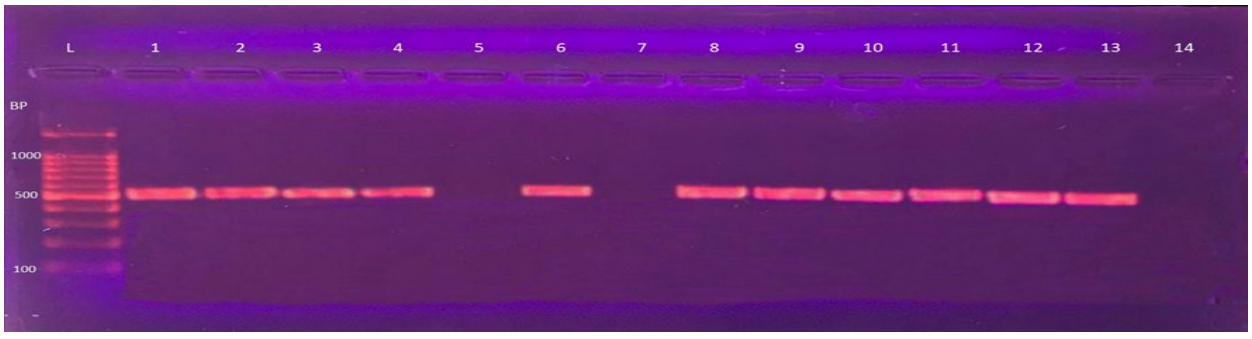


**Figure 2) PCR amplification products of K. oxytoca isolates which amplified the blaCTX-M gene product with 510 bp. Lane L: DNA molecular size marker (100bp ladder); Lanes isolates no. 1, 2, 3, 4, 6, 8, 9, 10, 11, 12, 13, 15 indicate positive results for the blaCTX-M gene**





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## Discussion

Fifteen of 35 isolates identified *Klebsiella oxytoca* were lactose fermentative on MacConkey agar, produced mucoid appearance, indole positive, MR test negative result and VP, citrate test positive result, these findings agree with [30, 31]. Klebsiella growth was distinguished by its mucoid growth appearing in pink color [32, 33]. On MacConkey agar, Klebsiella colonies were lactose fermenting colonies. They gave pink color, regular edge, round, mucoid texture with large size, and *K. oxytoca* were (3-4mm) in diameter with a weakly mucoid aspect. The number of identified strains by the VITEK-2 in this study according to the 16-digit bionumber of laboratory reports most strain for *K. oxytoca* with bionumber (6707734777564010), and the other with different bionumber, although the VITEK-2 technique of automated phenotypic identification has found widespread application in clinical and scientific laboratories, it has limited performance when it comes to distinguishing between members of the *K. oxytoca* complex at the species level [34].

The existence of *bla*$_{TEM}$ and *bla*$_{CTX-M}$ genes was investigated among 15 isolates of *K. oxytoca* which contained 10 (66.6%) *bla*$_{TEM}$ and 11 (73.3%) *bla*$_{CTX-M}$ genes being responsible for β-lactamases. As shown, *bla*$_{TEM}$ and *bla*$_{CTX-M}$ shown in Figures (1) and (2), PCR products included 766 and 510 bp, respectively; this was in agreement with [28, 29]. The current study revealed that only 66.6% of the *bla*$_{TEM}$ and 73.3% of the *bla*$_{CTX-M}$ genes were found in clinical isolates, while a study by Phetburom *et al.* [35] exhibit *bla*$_{TEM}$ with *bla*$_{CTX-M}$ (7.72, 9.72%) and *bla*$_{TEM}$ (6.72, 8.33%). The plasmid-mediated beta lactamase TEM-1 was identified in the early 1960s. TEM-type ESBLs are a subset of this enzyme. The enzyme was named after the Greek patient Temoneira, whose blood culture contained the original strain of *Escherichia coli* that led to its discovery. Bacteria have evolved resistance to several of the standard antibiotics used to treat them [36].

Numerous studies have found evidence of ESBL resistance genes generated by Gram-negative bacteria. In the Asia-Pacific region, *bla*$_{CTX-M}$ and *bla*$_{TEM}$ predominated. Among the Enterobacteriaceae found in Burkina Faso, *bla*$_{CTX-M}$ (40.1%) and *bla*$_{TEM}$ (26.2%) were shown to be the most prevalent ESBL resistance genes [37]. While in Saudi Arabia the study by Ibrahim *et al.* [38] exhibited *bla*$_{TEM}$ (84.7%) and *bla*$_{CTX-M}$ (33.3%). The study by Alag & Aziz [39] at Maysan Province, Iraq, stated that 100% of the genes from *E. coli* were *bla*$_{CTX-M}$ and *bla*$_{TEM}$. In conjunction with the present results, this shows that the prevalence of distinct ESBL gene types varies by region and even by neighborhood [40].

*K. oxytoca* strains isolated from humans with multidrug resistance have previously harbored four distinct *bla*$_{CTX-M}$ genes which *bla*$_{CTX-M-3}$, *bla*$_{CTX-M-9}$, *bla*$_{CTX-M-15}$, and *bla*$_{CTX-M-35}$ were isolated from different countries [41].

## Conclusion

The use of the VITEK-2 system is necessary to confirm the precise identification of *K. oxytoca* nosocomial pathogens from tonsillitis. The existence of *bla*$_{TEM}$ and *bla*$_{CTX-M}$ gene high frequency among of *K. oxytoca* isolates is a concern which needs control strategies.

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