Molecular Detection of *bla* $_{TEM}$ and *bla* $_{CTX-M}$ encoding genes from *Klebsiella oxytoca* Isolates from Tonsillitis

Abstract

Tonsillitis is inflammation of the tonsils, a common clinical state caused by bacterial or viral infections. There are several types of tonsillitis including acute, sub-acute, chronic, and recurrent. Chronic tonsillitis is referred to enlargement of the tonsils along with recurrent infective attacks. It is the most common throat disease which is mainly observed in younger individuals. The aim of this study was the isolation and identification *Klebsiella oxytoca* (*K. oxytoca*) from tonsillitis based on diagnostic procedures. Polymerase chain reaction was performed to detect bla_{CTXM} and bla_{TEM} genes. A total of thirty-five specimens were recovered from tonsillitis using swab sampling, represented by 35 specimens which contained 30 bacterial growth. The 21 of bacteria were lactose fermentative onto the MacConkey agar. Twenty of 30 suspected isolates were confirmed as *K. oxytoca* using VITEK-2 compact system. In genotypic test, *K. oxytoca* isolates contained 11 (55%) bla_{CTXM} 10 (50%) bla_{TEM} being responsible for cephalosporines resistance.

Keywords: *Klebsiella oxytoca*, bla_{TEM} , bla_{CTXM} , Tonsillitis

Introduction

Tonsillitis is the inflammation of the pharyngeal tonsils. The inflammation usually extends to the adenoid and the lingual tonsils; therefore, the term pharyngitis may also be used. Symptoms mostly include sore throat, fever, enlargement of the tonsils, trouble swallowing, and large lymph nodes around the neck. Most cases of bacterial tonsillitis are caused by group A beta-hemolytic *Streptococcus pyogenes*, while *Klebsiella* species are also important pathogens of humans, implicating in increasing morbidity amongst the patients population [1]. Normally found in bowel of men and animals, water and soil, infections with these bacteria lead to prolonged residence in hospitals. Multiple comorbidities and compromised immune status along with exposure to multiple antibiotics are main factors which increase the risks for infections and drug resistance. *Klebsiella* species are often a cause of bronchopneumonia, urinary tract infections and septicaemia in admitted patients. 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 [2]. *Klebsiella pneumoniae* (*K. pneumoniae*) is the single, predominant species gaining more significance by developing multidrug resistance (MDR) at tertiary care centres similar to *Pseudomonas* and *Acinetobacter* spp. Among the *Klebsiella* spp, *Klebsiella oxytoca* (*K. oxytoca*) has been isolated more frequently. *K. oxytoca* is a rod-shaped, nonmotile, Gram-negative bacterium with a prominent polysaccharide capsule, which provides a resistance against host defense mechanisms. *K. oxytoca* has developed the enzymes extended spectrum β-lactamases (ESBLs) and carbapenemases causing bacterial resistance to β-lactam antibiotics. Therefore, *K. oxytoca* is usually resistant to some antibiotics like cefotaxime, ceftazidime and aztreonam. Recently, biofield treatment is reported for the alteration in sensitivity of antimicrobials against the tested microorganism [3]. The conversion of mass into energy is well known. This was earlier named *Bacterium oxytocum* by Flugge in 1886 and is an organism that forms indole, has a positive Voges–Proskauer reaction and liquefies gelatine along with other features of *Klebsiella* species. *K. oxytoca* is normally acquired from environmental sources. *K. oxytoca* is a rod-shaped, nonmotile, Gram-negative bacterium with a prominent polysaccharide capsule, which provides a resistance against host defense mechanisms. *K. oxytoca* has developed the ESBLs and carbapenemases which lead to bacterial resistance to β-lactam antibiotics. Therefore, *K. oxytoca* is usually resistant to some antibiotics like cefotaxime, ceftazidime and aztreonam [4] . Recently, biofield treatment is reported for the alteration in sensitivity of antimicrobials against the tested microorganism. Plasmid encoded blaCTX-M enzymes represent an important sub-group of class-A β-lactamases which hydrolyse broad-spectrum β-lactam antibiotics causing an ESBL phenotype, which is 8, blaCTX-M-9, blaCTX-M-25 , respectively. More recently, it has been suggested that blaCTX-M-45 forms a new, separate cluster CTX-M is considered as a family of plasmidmediated ESBLs, called CTX-M which is hydrolyzes oxy-amino cephalosporins, especially third and fourth generation, and monobactam. It also hydrolyzes cefotaxime more rapidly than ceftazidime but not cephamycins such as cefoxitin and carbapenems including imipenem, ertapenem, meropenem, or doripenem in addition. They are generally susceptible to β-lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam [5].

The first clinical isolate with a cefotaxim due to constitutive expression of a chromosomal class A β-lactamases, *K. oxytoca* was shown to have a high level resistance to ceftriaxone and cefotaxime [6].

Materials and Methods

Isolation and Identification of bacterial isolates

Thirty five clinical specimens were collected from patients suffering from tonsillitis, taken by swab from infection area and also after tonsillectomy, after the sample was taken after the surgery, the sample surface was sterilized and opened with a sterile scalpel and by using swab taken from the fibrosis found in the tissue. Specimens were inoculated on three types from culture media which included mannitol salt agar and MacConkey agar (Merk, Germany) which considered as predominant, selective and differential media for the isolation, purification and identification of many types from bacteria. 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 and VITECK system that confirmed the identification of isolates.

DNA Extraction

Genomic DNA was extracted using a commercial extraction system (Favorgen/Taiwan) as per the instructions of manufacturer.

Molecular Identification

The PCR assay was performed to detect the *bla* TEM and *bla* CTX-M genes primers specific for *K. oxytoca* as shown in table 2. These primers were designed by Alpha DNA Company, Canada as in table 1. Amplified products were confirmed using 0.8% agarose gel electrophoresis to estimate the PCR products size. The gel was stained with 4 µL of 10mg/mL ethidium bromide (Sigma, USA) and it run at 70v for 1.5h. A single band was observed at the desired position on ultraviolet light transillumintor (Cleaver, UK); bands were photographed using gel documentation system (Cleaver, UK). A 100bp ladder (Bioneer, Korea) was used to measure the molecular weights of amplified products. Gel electrophoresis was used for the detection of DNA by UV transilluminator.

Table 1. Primers used in this study

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

Table (2): PCR thermal program of *bla*TEM and *bla*CTXM genes 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 genes	95˚C for 3min	50	95˚C for 30sec	51˚C for 30sec	72˚C for 40 sec	72˚C for 5min

Results and Discussion

Isolation and identification of *K. oxytoca*

A total of 35 clinical specimens were collected from various clinical samples. The initial identification of gram negative rod was depended on the colonial morphology by produce lactose fermented colony on Maconky agar, production of mucoid appearance and giving indole, Methyl-red negative result [7]. Among 35 specimens, 30 had bacterial isolates growth. Onto the MacConkey agar, 21 isolates were lactose fermentative isolates. The final identification was performed using the automated VITEK-2 compact system using GN-ID cards which contained 47 biochemical tests and one negative control well. The results demonstrated that only thirteen from one hundred fifty suspect isolates were confirmed as *K. oxytoca* with ID message confidence level ranging between very good to excellent (Probability percentage from 95 to 99).

Molecular Detection of bla TEM and bla CTXM encoding genes from *K. oxytoca*

The existence of bla TEM and bla CTXM genes was investigated among 21 isolates of *K. oxytoca* which contained 10 (50%) bla TEM and 11 (55%) bla CTXM genes being responsible for β-lactamases. As shown, bla TEM and bla CTXM PCR products included 766 and 510 bp, respectively as shown in Figures (1) and (2). This was in agreement with Phetburom et al.,2022 [8] *bla*TEM with *bla*CTX-M (7/72, 9.72%) and *bla*TEM (6/72, 8.33%). Our study revealed that the majority of β-lactamase- carrying *K. oxytoca* isolates possessed blaCTX-M, especially blaCTX-M-1. In the current study, the dissemination of blaCTX-M-2 among *K. oxytoca* isolates collected from pigs has been reported [9] (Tsuka et al.,2021) and blaCTX-M-15 and blaTEM-1 were detected among *K. oxytoca* isolates collected from six provinces in China. *K. oxytoca* isolates harboring blaCTX-M were highly susceptible to cefotaxime, ceftazidime, and cefepime (93.1-98.3%) and 100% susceptible to ceftriaxone. Combination disk assay revealed that they were non-ESBL producers; however, ESBL NDP assay demonstrated that 43 (74.1%) of these isolates exhibited β-lactamase activity on cefotaxime. Another study observed that *K. oxytoca* showed ostensibly resistance to cefotaxime and cefepime and rare resistance to ceftazidime, with only borderline resistance to these cephalosporins (MIC 2–8 mg/L), suggesting that there was hyperproduction of K1 (KOXY)

chromosomal β-lactamase rather than ESBL production. The positive result for ESBL NDP assay but the negative result for combination disk assay was observed in our study suggesting the presence of K1 β-lactamase in this organism. Their study indicated that *K. oxytoca* isolated from slaughtered pigs was highly susceptible to cefoxitin (96.72%), ceftazidime (98.36%), and cefepime (93.44%) [10] (Hossain et al.,2020) and reported that *K. oxytoca* strains isolated from pet turtles in Korea were highly susceptible to cefoxitin (81.25%), ceftazidime (80.00%), and cefepime (80.77%), and based on antimicrobial susceptibility testing results, 98.3% of the *K. oxytoca* strains were resistant to ampicillin. *Klebsiella* spp., especially *K. pneumoniae* and *K. oxytoca*, produce different chromosomal β-lactamases, leading to natural resistance to penicillin [11].

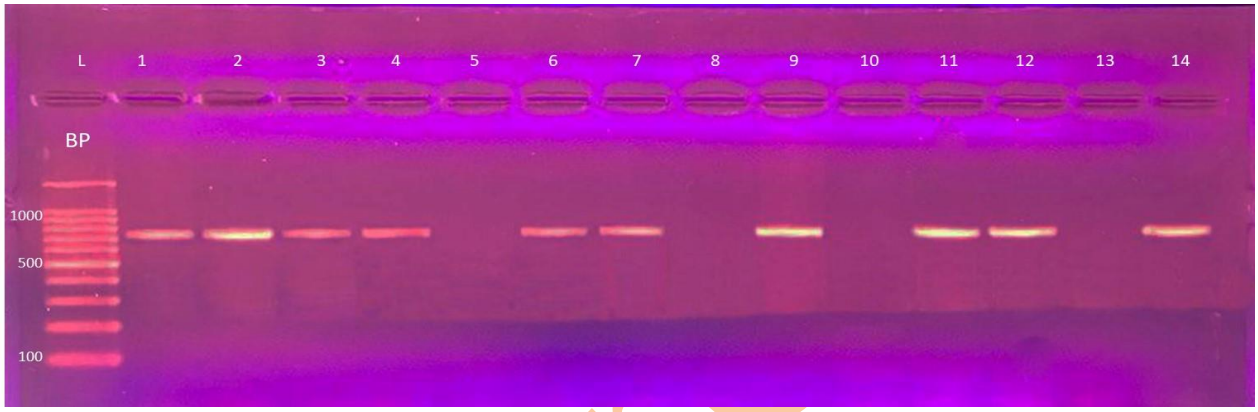

Figure 1. PCR amplification products of *K. oxytoca* isolates amplifying the bla TEM gene product with 722 bp. Lane (L): DNA molecular size marker (1000-bp ladder), Lanes (isolates no.1, 2,3,4 ,6,7,9,11,12,14,15) indicate positive results for the bla TEM gene

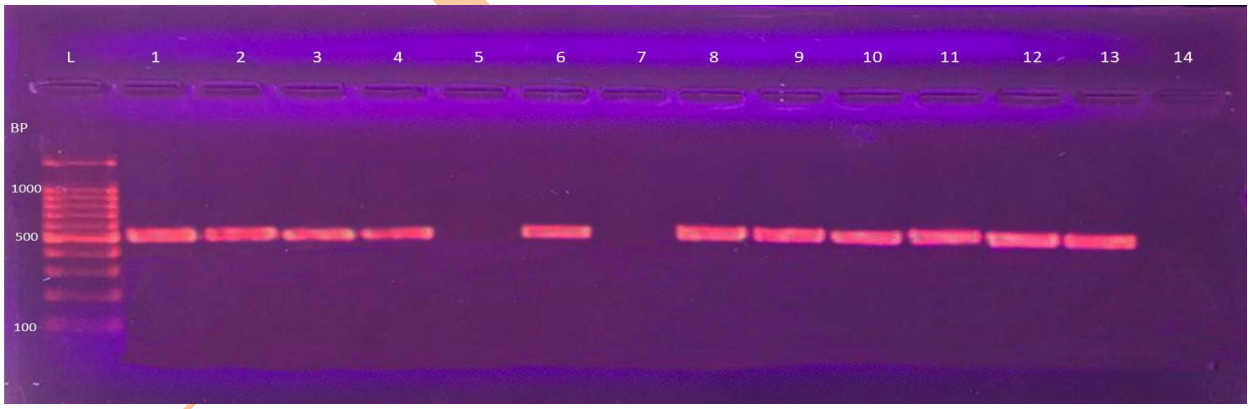

Figure 2. PCR amplification products of *K. oxytoca* isolates which amplified the bla CTXm gene product with 510 bp. Lane (L), DNA molecular size marker (1000-bp ladder), Lanes (isolates no.1, 2,3,4 ,6,8,9,10,11,12,13,15) indicate positive results for the bla CTXm gene

Conclusions

The use of Vitek-2 system, is necessary to confirm precise identification of *K. oxytoca* nosocomial pathogens from tonsillitis. The existence of bla TEM and bla CTXm gene in half of *K. oxytoca* isolates is a concern which needs control strategies.

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