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Molecular Detection of Extended Spectrum β-Lactamase *OXA Gene* encoding *Klebsiella species* in Khartoum State (Sudan)

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Abstract

Background: Extended-spectrum β-lactamase (ESBL) producers are Gram negative bacteria (*Klebsiella species*) producing enzymes and are widely spreading worldwide producing resistance to most β-lactam antibiotics, e.g. penicillin and cephalosporin.
Objective: To perform molecular detection of extended spectrum β-lactamase *OXA gene* encoding *Klebsiella species* in Khartoum State (Sudan).
Materials and methods: A total of 100 ESBL *Klebsiella species* were isolated from patients attending Omdurman Teaching Hospital. Full identification of species was performed using analytical profile index (API) protocol. ESBL phenotype was determined by the double disk diffusion test (DDDT) and E-test. Confirmed species were tested for existence of *OXA gene* using convential polymerase chain reaction (PCR).
Results: Out of the 100 ESBL *Klebsiella* species, none was found positive for *OXA gene*.
Conclusion: This study revealed no association between *OXA gen*e and ESBL producing *Klebsiella* species in Khartoum State (Sudan).

Keywords: *Klebsiella* species, **E**xtended spectrum β-lactamase, *OXA gene*, PCR.

Introduction

Klebsiella species is a major cause of community and healthcare associated infections. Infections caused by multidrug resistant *Klebsiella* species have been increasingly reported in many clinical settings. *Klebsiella* species is a microorganism that causes serious diseases such as sepsis, pneumonia, urinary tract infection, chronic lung disorders, and nosocomial infection. The emergence of extended-spectrum beta lactamase (ESBL) producing bacteria particularly *Klebsiella* species represents a potential danger in nosocomial settings as well as community acquired infection[1].
The ESBL producing bacteria are defined as those bacteria resistant to the β-lactam ring antibiotics, e.g. penicillin, amoxicillin, amoxyclav, and third generation cephalosporins; in addition to aminoglycosides and quinolones. Bacterial strains encoding extended spectrum lactamases were first described in 1980. The responsible genes had been detected in *Klebsiella*

species and later in *Escherichia coli, Pseudomonas aeruginosa, Serratia marcescens,* and other gram-negative bacilli. ESBLs are a group of enzymes encoded by genes located predominantly on plasmid. Thus they are rapidly transformed between bacteria. ESBLs are an increasingly important cause of transferable multidrug resistance in Gram negative bacteria throughout the world. These bacteria had spread rapidly and had become a serious threat to humans worldwide[2]. ESBLs are undergoing continuous mutation causing the development of new hetro genes which transcript into many new enzymes showing expanded substrate profiles. At present time there are more than 300 different ESBLs variants. These variants had been clustered into nine different structural and evolutionary families based on amino acid sequence. Temoniera (TEM) and Sulphydryl variable (SHV) were the major types, however, CTX-M type is more common in some countries. CTX-M-9 group, SHV-1a, TEM-116, SHV-27, SHV-5a and SHV-41 extended spectrum beta-lactamase gene of *Klebsiella pneumoniae* strains are prevalent in Taiwan[3].
Klebsiella is non-motile, Gram negative, oxidase negative, rod-shaped microorganism with a prominent polysaccharide based capsule belonging to the family of *Enterobacteriaceae* and it was named after the German microbiologist Edwin Klebs (1834–1913). The epidemiology of ESBLs is quite complicated, and had a worldwide distribution. The first ESBL to be known was detected in Germany in 1983, followed by France in 1985, and in the United States at the end of 1990. The first nosocomial outbreaks had occurred in 1990. TEM-type ESBL *Klebsiella oxytoca* was first isolated in Liverpool (England) in 1982. New TEM and SHV enzymes are still emerging in Europe, and distinct epidemic clones had been reported[4].
In Sudan there is few published data on ESBL among *Klebsiella* species, and there are a number of running projects sponsored by global funds.

Materials and methods

This was an analytical, surveillance study investigating 100 *Klebsiella* species isolated from different clinical specimens collected from patients attending Omdurman Teaching Hospital in Khartoum State (Sudan), over a period of 5 months from March to July 2017. It was conducted at the Research Laboratory, Faculty of Medical Laboratory Sciences, Data was collected by direct interviewing questionnaire. Ethical clearance and approval was taken from Omdurman Ahlia University. and Ministry of Health Ethical Board. Permission to collect the specimens was granted by authorities of Omdurman Teaching Hospital, Khartoum State (Sudan).
In this study, the identification and susceptibility tests were carried out using convential bacteriological techniques. Antibiotic susceptibility showing reduced zones of inhibition to first, second, and third generation cephalosporins and amoxyclav were adopted to screen *Klebsiella* species for ESBL production.
ESBL detection: 100 *Klebsiella* species found positive to ESBL test were further tested phenotypically for ESBL production using the double disc synergy test (DDST) and E-test as recommended by the Clinical Laboratory Standards Institute (CLSI)[5].
The test was performed by using both cefotaxime (30µg) and ceftazidime (30µg) alone, and in combination with amoxyclav-clavulanic acid. As per manufacturer's instructions. an inhibition zone greater than or equal to 5 mm diameter was considered positive for ESBL production. The

result was father confirmed by E-test.

Molecular characterization of ESBL-producing *Klebsiella* species: ESBL producing *Klebsiella* species isolates were selected for detection of β-lactamase-encoding *OXA gene*. DNA was extracted from bacterial cells using (iNtRON-KOREA) kit and as per manufacturer's instructions. PCR amplification and detection was carried out using a master-mix (4 µl) containing: PCR reaction buffer, $MgCl_2$, dNTPs, and Taq DNA polymerase (iNtRON-KOREA). Primers used were (Macrogen-Korea):

a) 1 µl OXA forward primer: AAG TTC TGC TAT GTG CGG TA (5` to 3`).

b) 1 µl OXA reveres primer: TGT TAT CAC TCA TGG TTA TGG CAG C (5`to 3`).

11 µl H_2O, and 3 µl DNA were added to a final volume of 20 µl mixture. Amplification was performed in a thermal cycler (AERIS-China) briefly, denaturation at 95°C, annealing at 59°C, followed by extension at 72°C with a total of 35 cycles. Visualization was carried out by adding 7 µl of the products to a ready-made 1.5 % agarose gel with ethidium bromide (8 µg/ml) at 150V in 0.5×TBE buffer for 40 min using electrophoresis technique (Bio Rad-USA). Bands were detected using UV transilluminater (Gel Documentation System-Bio Rad-USA). Finally the results were compared to the standard DNA ladder of 1000 kd and any band margining 281 kd were referred to the OXA primer and thus considered positive. Control positive OXA primers and control negative distilled water were included with each separate experiment (Fig. 1).

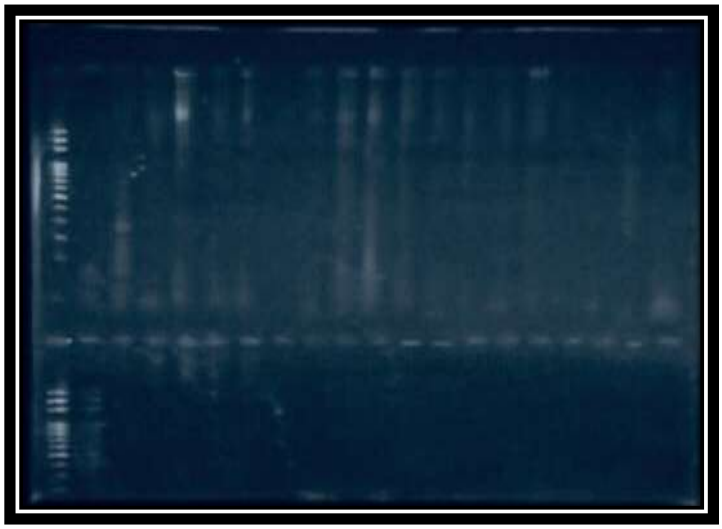

Fig. (1): Detection of *OXA* gene using electrophoresis plot

Gradient temperature initially used to determine the annealing temp. for each primer by running the sample according to a 3-step protocol (below protocol) ensuring that the annealing temp. had been defined on 59°C gradient.

Results

Out of 100 ESBL *Klebsiella* species, none was found positive for *OXA* gene.

Discussion

Antimicrobial resistance represents a danger for human well-being and the daily increase in bacterial resistance requires a huge economical and personal efforts. In the last two decades, the prevalence rate of ESBL production by *Enterobacteriaceae* had increased considerably. Among *Enterobacteriaceae, K. pneumoniae* and *E. coli* were the most important causative agents of nosocomial infections[3]. Occurrence of infection caused by the extended spectrum beta-lactamase producing *Klebsiella* species had been widely reported all over the world following the wide-spread use of the expanded spectrum cephalosporins[6].

In this study, phenotypic screening of ESBL showed that 100% of *Klebsiella* species were not positive for ESBL production. Based on these results, the prevalence rate of ESBL producing *Klebsiella* species was very high in Sudanese hospitalized patients. This high prevalence rate of ESBL-producing *Klebsiella* species had also been reported by previous studies, such as Feizabadi and his colleagues[1] who reported a prevalence rate of 44.5% ESBL-positive *Klebsiella* pneumoniae isolated from clinical specimens in Tehran, and *OXA-48* and *KPC-2* genes were detected in clinical isolates of *Klebsiella* species in some community settings.

Other workers[7] reported similar findings as found in our study, i.e. the prevalence rate of OXA gene was 0% in North of Iran (2014).

However, our study findings were different from those reported by other authors[8] who reported a prevalence rate of 89% positive OXA gene in Norway (2007 to 2014). Also, some workers reported a prevalence rate of a 55.3% positive OXA gene in Russia (2013 to 2014).

Conclusion: This study revealed no association between *OXA gen*e and ESBL producing *Klebsiella* species in Khartoum State (Sudan).

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