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Molecular Detection of *SHV* Gene encoding Extended Spectrum β-lactamase-producing *Klebsiella* Species in Khartoum (Sudan)

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Abstract

Background: Resistance to a wide variety of common antimicrobials had made the proliferation of extended spectrum β-lactamase (ESBL)-producing strains a serious global health concern that has complicated treatment strategies. The high proportion of ESBL producers among the *Enterobacteriaceae* and the complex molecular epidemiology with diverse types of ESBL genes are alarming. Antimicrobial resistance to cephalosporin, penicillin and aztreonam is mediated by the extended-spectrum β-Lactamases (ESBL) via hydrolysis of antibiotics. The most common bacterium associated with ESBL among the *Enterobacteriaceae* genera is *Klebsiella* species.

Objective: To perform molecular detection of *SHV* gene encoding extended spectrum β-lactamase-producing *Klebsiella* Species in Khartoum (Sudan).

Materials and methods: A total of 100 ESBL *Klebsiella* species were isolated from patients attending Omdurman Teaching Hospital (Sudan). Isolates were fully identified using API detection protocol. The ESBL phenotype was determined by the double disk diffusion test (DDDT) and E-test techniques. The qualified *Klebsiella* strains were examined for the existence of *SHV* gene using the convential polymerase chain reaction (PCR) molecular method.

Results: Out of 100 ESBL *Klebsiella* species, 20 species (20%) were found positive for *SHV* gene. 18 species out of these 20 strains were found causing urinary tract infection (UTI); and most of the specimens were collected from females.

Conclusion: The prevalence rate of *SHV* gene encoding ESBL-producing *Klebsiella* species in Khartoum was frequent.

Key words: ESBLs, *Shv* gene, PCR, *Klebsiella s*pecies, Khartoum (Sudan).

Introduction

Klebsiella species is a major cause of community and healthcare associated infections. Infections caused by multidrug resistant *Klebsiella s*pecies had been increasingly reported in many clinical settings. *Klebsiella s*pecies (*K.* spp.) is a microorganism that causes serious diseases such

as sepsis, pneumonia, urinary tract infection (UTI), chronic lung disorders and nosocomial infection. The emergence of extended-spectrum β-lactamase (ESBL)-producing bacteria, particularly *K.* species, represent a potential danger in nosocomial settings as well as community acquired infection. The ESBL-producing bacteria are defined as resistant to β-lactam ring antibiotics (penicillin, amoxicillin, amoxyclav) and to the third-generation cephalosporins in addition to aminoglycosides, and quinolones[1].

Bacterial strains encoding extended spectrum lactamases (ESBLs) activities were firstly described in 1980; These genes had been detected in *Klebsiella* species and later in *Escherichia coli, Pseudomonas aeruginosa, Serratia marcescens* and other gram-negative bacilli. ESBLs are groups of enzymes encoded by genes located predominantly on plasmid for that it's rapidly transformed between bacteria. ESBL 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 human health worldwide. 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 had been clustered into nine different structural and evolutionary families based on amino acid sequence. TEM (and sulphydryl variable *SHV*) were the major types. However, CTX-M type is more common in some countries[2]; e.g. *CTX-M-9* group, *SHV-1a, TEM-116, SHV-27, SHV-5a* and *SHV-41* are prevalent in Taiwan.

Klebsiella is a genus: non-motile, Gram negative, oxidase negative, rod-shaped bacteria with a prominent polysaccharide based capsule belonging to the family of *Enterobacteriaceae* and it is named by the German microbiologist Edwin Klebs (1834–1913). The epidemiology of ESBLs is quite complicated, and they had a worldwide distribution in a wider geographical area, country, hospitals, and the community. The first ESBL identified was in Germany (1983), followed by France (1985). and USA (1980s). Until the 1990s that the initial nosocomial outbreaks of ESBLs had occurred. TEM-type ESBL-*Klebsiella oxytoca* was first isolated in Liverpool (England) in 1982. New TEM and the *SHV* enzymes are still emerging in Europe, and distinct epidemic clones had been reported[3].

In Sudan there is few published data on ESBL-producing *Klebsiella* species; however, there are a number of running projects supported by global funds.

Materials and methods

This study was an active surveillance study investigating 100 *Klebsiella* species isolates from different clinical samples collected from patients attending Omdurman Teaching Hospital (Sudan). Duration of the study was 5 month: from March to July 2017.

This study was approved by the Ethical Committee of Al Neelain University. Permission to collect the specimens was obtained from authorities of Omdurman Teaching Hospital. All statistical analyses were performed by the SPSS software program version.

Samples were processed at the Research Laboratory of the Faculty of Medical Laboratory Sciences (Omdurman Ahlia University).

The *Klebsiella* isolates were identified by the standard bacteriological methods and API 20E system. Antibiotics susceptibility was performed by the disc diffusion method; using the discs of

the first, second, and third generation cephalosporins and amoxyclav to screen the *Klebsiella* isolates for ESBL production.

ESBL detection: The 100 positively screened *Klebsiella* isolates for ESBL production were further tested phenotypically for ESBL production using double disc synergy test (DDST) and E-test as recommended by the Clinical Laboratory Standards Institute (CLSI)[4].

The DDST was conducted using both cefotaxime (30µg) and ceftazidime (30µg), alone and in combination with amoxyclav. An inhibition zone greater than or equal to 5 mm diameter was taken as positive for ESBL production. This was further confirmed by E-test.

Molecular characterization of ESBL-producing *Klebsiella* species: 20 ESBL-producing isolates of *Klebsiella* species were selected for detection of β-lactamase *SHV* encoding gene. DNA was extracted from bacterial cells using (iNtRON – KOREA) kit according to manufacturer instructions. PCR amplifications and detection were carried out using Master Mix (4 µl) containing: PCR reaction buffer, MgCl2, dNTPs, and Taq DNA polymerase (iNtRON – KOREA). The primers used (macrogen-Korea) were:

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

b) 1 µl *SHV* reverse primer: TGT TAT CAC TCA TGG TTA TGG CAG C (5`to 3`)[5].

Then 11 µl H_2O, and 3 µl DNA extract were added and final volume of 20 µl mixture was obtained. Amplification was performed in a thermal cycler (AERIS-China) by denaturation at 95°C, annealing at 53°C, and extension at 72°C with a total of 30 cycles. Visualization was carried out by adding 7 µl of the products to 1.5 % agarose gel with ethidium bromide (8 µg/ml) at 150 V in 0.5× TBE buffer for 40 min, using electrophoresis technique (Bio Rad, USA). The bands were detected using UV transilluminater (Gel documentation system, Bio Rad, USA). Finally the results were compared to the standard DNA ladder (1000 kd), and any band margining 717 kd primer were considered as positive *SHV* gene. Control positive *SHV* primers and control negative distilled water were included.

Results

Out of 100 ESBL-producing *Klebsiella* species, 20 species (20%) were found positive for *SHV* gene. Also, 18 out of 20 *Klebsiella* strains isolated were found causing UTI and 15 out of the 100 samples investigated were collected from females. The majority of *SHV* genes (18/20) encoding ESBL-producing *Klebsiella* species were isolated from UTI cases (Fig. 1).

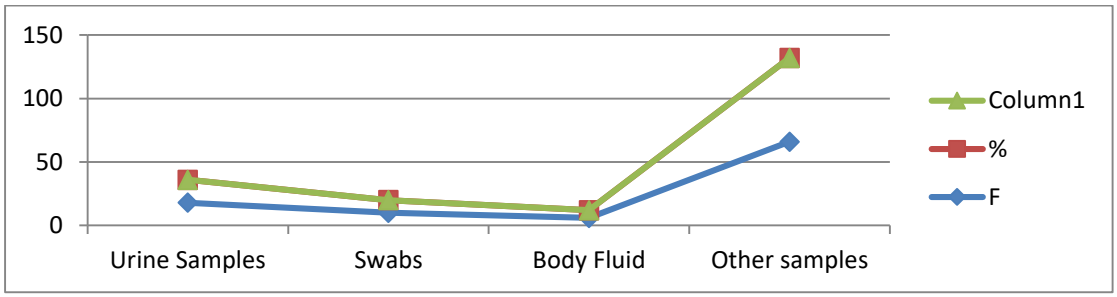

Fig. (1): Distribution of *SHV* genes among clinical samples

All *Klebsiella* isolates were found susceptible to carbapenems. The lowest frequency rates of resistance to other antibiotics observed were 66.0% for tazocin (piperacillin and tazobactam), 16.2% for amikacin, and 17.8% for gentamicin.

Discussion

Antimicrobial resistance represents a danger situation for human wellbeing and the daily increase in bacterial resistance requires a hard research work with huge economical and personal affords. During the last two decades, the rate of ESBL-production by *Enterobacteriaceae* had increased considerably (Clinical Laboratory Standards Institute, 2009)[4].

Among *Enterobacteriaceae*, *K. pneumoniae* and *E. coli* are the most important causative agents of nosocomial infections. Occurrences of infection affected by the extended spectrum β-lactamase-producing *Klebsiella* species have been widely reported all over the world following the widespread use of the expanded spectrum cephalosporins[6].

In this study, phenotypic screening of ESBL showed that 100% of *Klebsiella* species were positive for ESBL-production. Based on these results, the prevalence rate of ESBL-producing *Klebsiella* species was very high in Sudanese hospitalized patients. In addition, the *SHV* gene encoding ESBL-producing *Klebsiella* species in this study were responsible for 20% of the total ESBL-*Klebsiella* species, a finding very relative to many previous studies worldwide, e.g. the Indian study (2013), which reported that the prevalence rate of *SHV* gene encoding *Klebsiella* species was 20%[7].

The high prevalence rate of ESBL-producing *Klebsiella* species was reported by a number of studies and reported a 44.5% ESBL-positivity rate among *K. pneumoniae* isolated from clinical specimens in Tehran. Also, our findings in this study were similar to the 1998 Survey of Extended-Spectrum β-Lactamases in *Enterobacteriaceae* performed in France which reported a frequency rate of 22%[8].

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