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Molecular Detection of Extended Spectrum Beta Lactamase TEM Gene Produced Encoding Klebsiella Species in Khartoum State Sudan 2017

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ABSTRACT

Background: Resistance to variety of common antimicrobials has made the proliferation of extended spectrum β-lactmase (ESBL) producing strains a serious global health concern that complicated treatment strategies. Antimicrobial resistance to cephalosporin, penicillin and aztreonam are bmediated by Extended-Spectrum Beta-Lactamases (ESBL) via hydrolysis of antibiotics. The most common bacteria associated with ESBL among the *Enterobacteriaceae* are *Klebsiella species*. This study was focus on detection of *TEM* gene associated ESBL *Klebsiella species* in Khartoum stat.

Methods : A total of 100 ESBL *klebsiella species* were isolated, strains confirmation was take place using API detection protocol, and ESBL phenotype was determined by a double disk diffusion test (DDDT) and E – test, the qualified strains were examined for the existence of *TEM-1* gene using convential PCR.

Results : Out of 100 ESBL *Klebsiella species* 20 (20%) were positive for *TEM* gene. A frequent of 18 out of 20 strains isolated was causing UTI and most of the samples were collected from females.

Conclusion : Study was revealed that the prevalence of *TEM* gen among *TEM* gene associated ESBL *Klebsiella species* in Khartoum stat represent 20%, which is require farther phenotypic analysis to sub genotyping the *TEM* gene.

Keywords : ESBLs,Cephalosporin, *Enterobacteriaceae*, *TEM* Gene.

I. INTRODUCTION

Klebsiella species is a major cause of community and healthcare associated infections *(Lai CC, et al 2014)*. Infections caused by multidrug resistant *K. species* have been increasingly reported in many clinical settings *(Lai CC, et al, 2014: Li B, et al, 2012)*. *K. species* is an microorganism that causes serious diseases such as sepsis, pneumonia, urinary tract infection (UTI), chronic lung disorders and nosocomial infection*(Amraie H. et al, 2014)*. The emergence of extended-spectrum beta lactemase (ESBL) producing bacteria particularly *K. species* represent a potential danger in nosocomial settings as well as community acquired infection *(Harada Y. et al, 2013)* The ESBL producing bacteria are defined as a resistant to Beta lactem ring antibiotics (Penicillin, Amoxcline and amoxclave) and to the three generation cephalosporins in adition to aminoglycosides, and quinolones, *(Du J. et al, 2014).* Bacterial strains encoding extended spectrum lactamases (ESBLs) activities were firstly described in the 1980; these genes had been detected in *Klebsiella species* and later in *Escherichia coli, Pseudomonas*

aeruginosa, Serratia marcescens and other gram-negative bacilli *(Harada Y. et al, 2014)* ESBLs are groups of enzymes encoded by genes located predominantly on plasmid for that it's rapidly transformed between bacteria *(Harada Y. et al, 2014)* ESBL are an increasingly important cause of transferable multidrug resistance in gram negative bacteria throughout the world, these bacteria have spread rapidly and have become a serious threat to human health worldwide *(Harada Y. et al, 2014)* 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 have 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 *(Harada Y. et al, 2014; African Journal of Microbiology Research Vol. 4, 8), pp. 650-654, 18 April, 2010)* CTX-M-9 group, SHV-1a, TEM-116, SHV-27, SHV-5a and SHV-41*(Extended-spectrum beta-lactamase genes of Klebsiella pneumoniae strains in Taiwan: recharacterization of shv-27, shv-41, and tem-11 .6Lin TL, et al .Microb Drug Resist. 2006). Klebsiella* is a genus of non-motile, grams negative, oxidase negative rod shaped bacteria with a prominent polysaccharide based capsule belong to the family of Enterobacteriaceae and it is named by the German microbiologist Edwin Klebs (1834–1913). The epidemiology of ESBLs is quite complicated, worldwide distribution and there are certain levels to consider; the wider geographical area, the country, the hospital, the community, and the hostis. The first ESBL to be identified was found in Germany in 1983, followed in France in 1985 and in the United States at the end of the 1980s wail in the 1990s that the initial nosocomial outbreaks occurred *(Rice L.B et al, 1990)* TEM-type ESBL *Klebsiella oxytoca* was first isolated in Liverpool, England, in 1982 *(Du Bois S, et al, 1995),* new TEM and the SHV enzymes are still emerging in Europe, and distinct epidemic clones have been found. In Sudan there is no clear published data about ESBL in *Klebsiella Species*, but there are number of running project as that conducted by global fund.

II. MATERIAL AND METHOD

Study area and sample collection

This study was an active surveillance study included a total of 100 *klebsiella species* isolates from different clinical samples collected from Omdurman teaching hospital, Khartoum state, Sudan, over a period of 5 month from March to July 2017 and was received and processed at the research laboratory of the faculty of Medical laboratory sciences - Omdurman Ahlia University (OAU). In this study, the identification and susceptibility tests were carried out using biochemical testing containing different substrates were utilized for isolate identification, as described in standard bacteriological methods and API 20E system, antibiotics susceptibility showing reduced zone of inhibition to first, second and third generation cephalosporins and amoxclave calolonic acid in order to screened them for ESBL production.

ESBL detection: 100 isolates were found to be ESBL positive that 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). The test was done by using both cefotaxime (30µg) and ceftazidime (30µg) alone and in combination with amoxclve clavulanic acid, an inhibition zone greater than or equal to 5 mm diameter was taken as positive result for ESBL production(as manufacture of in structure)and fatherly confirmed with E - test.

Molecular Characterization of ESBL Producing *Klebsella species*:

Twenty ESBL producing isolates of *Klebsiella species* were selected for detection of β-lactamase encoding gene TEM-1. DNA was extracted from bacterial cells

by using (iNtRON – KOREA) kit according to manufacturer of instructions. PCR amplifications and detection was carried out using ready master mix (4 µl) containing PCR reaction buffer, MgCl2, dNTPs and Taq DNA polymerase (iNtRON – KOREA) and 1 µl TEM forward primer; AAG TTC TGC TAT GTG CGG TA (5` to 3`), 1 µl TEM reveres primer TGT TAT CAC TCA TGG TTA TGG CAG C (5`to 3`), (*Khorshidi A et al, 2012),* (macrogen-Korea), 11 µl H2O, 3 µl of DNA was added and final volume of 20 µl mixture. Amplification was performed in a thermal cycler (AERIS - china) briefly; denaturation at 95 °C , annealing at 63.2 °C followed by extension at 72 °C with total of 35 cycles. Visualization were carried out by adding 7 µl of the products to a readymade 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), bands were detected using UV transilluminator (gel documentation system - Bio Rad - USA) finally the results were compared to the standard DNA ladder of 1000 kd and any band margining 717 kd were referred to the TEM primer and thus considered positive., control positive TEM primers and control negative distilled water were round with each separate experiment.

III. RESULTS AND DISCUSSION

Results :

Out of 100 ESBL *Klebsiella species* 20 (20%) were positive for *TEM* gene. A frequent of 18 out of 20 strains isolated was causing UTI and 15 out of the samples were collected from females.

The majority *TEM* gene encoding ESBL isolates were found in UTI cases (18 out of 20) as showed in figure (1), all bacterial isolates were susceptible to Carbapenems. The lowest rates of resistance to other antibiotics were observed for: tazocin (66.0%), amikacin (16.2%) and gentamicin (17.80%) as indicated in table (2).

Table (1) : Prevalence of TEM gene among ESBL *Klebsiella Species*

Gene	Frequent	Present
TEM	20	20
Other	80	80

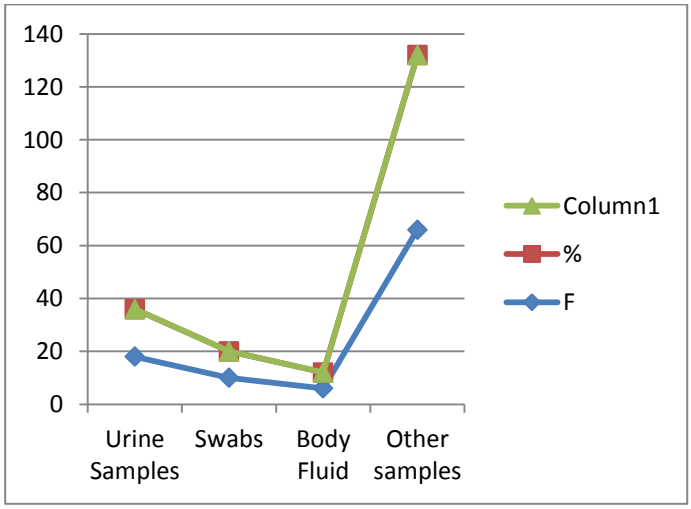

Figure 1. Distribution of TEM Gene among clinical samples

Discussion:

Antimicrobial resistances represent a danger situation for human wellbeing and the daily increase in bacterial resistance require a hard research work with huge economical and personal affords. In the last two decades, the rate of ESBL production by *Enterobacteriaceae* had increased considerably *(Clinical Laboratory Standards Institute, 2009: Mendes C et al 2004: Putman M et al, 2000).* Among *Enterobacteriaceae*, *K. pneumonia and E. coli* are the most important causative agents of nosocomial infections *(Karimi A et al, 2012).* Occurrences of infection effected by extended spectrum beta-lactamase producing *K. species* have been widely reported all over the world following the widespread use of the expanded spectrum cephalosporins (*Khorshidi A et al, 2012; Branger C, et al, 1998;*

Wang XR et al, 2012; Feizabadi MM et al, 2010). In our study, phenotypic screening of ESBL showed that 100% of *K. species* isolates were positive for ESBL production. Based on these results, the prevalence of ESBL producing *K. species* was very high in Sudanese hospitalized patients. In addition, the TEM gene encoding ESBL producing *K. species* in this study were responsible for 20% of total ESBL *K. species,* which is very relative to many previous study worlds wild, such as the study did by Jyoti Sharma in India (2013) which revealed that the prevalence of *TEM* gene encoding *Klebsiella Species* was 20% in his published article of detection of *TEM & SHV* genes in *Escherichia coli & Klebsiella Species* isolates in a tertiary care hospital. Also our findings are closely to the article of ***(De Champs).*** A 1998 Survey of Extended-Spectrum β-Lactamases in *Enterobacteriaceae* in France whose report about 22%. The high prevalence of ESBLs producing *K. species* has also been reported by a number of previous studies, as in a study of *(Feizabadi MM et al, 2010),* which showed a 44.5% ESBL positive rate among clinical *K. pneumoniae* isolated from clinical specimens in Tehran.

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