



## Detection of Extended-spectrum Beta Lactamase in Bacteria isolated from urine of pregnant women

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### ABSTRACT

The aim of this study was to detect extended spectrum  $\beta$ -lactamase (ESBL) in bacteria isolated from urine collected from urinary tract of infected pregnant women. A total of 100 midstream urine samples were collected from Omdurman Military Hospital from April to May 2013. Specimens were cultured onto cysteine- lactose- electrolyte-deficient (CLED) agar to identify urinary tract infection (UTI) causative bacteria by colonial morphology, Gram reaction and conventional biochemical tests. Out of 100 urine specimen; 56(56%) of samples gave significant bacterial growth (the Gram negative bacilli were 42(75%). The most frequent Gram negative were *Klebsiella oxytoca* 15 (26.8%) and *Escherichia coli* 10(17.9%), while the most abundant Gram positive was *Staphylococcus saprophyticus*. In The susceptibility test, 5 isolates were resistant to 3<sup>rd</sup> generation cephalosporins (ceftazidime, ceftriaone and cefotaxime) as follow: 2 *K. pneumoniae*, 2 *E. coli* and 1 *Proteus vulgaris*. ESBL production test done for all bacterial resistance to 3<sup>rd</sup> generation cephalosporin and then confirmed by combination test by using Double disc synergy test 5(11.9% ) isolates gave positive result. The PCR was done for 5 isolates using TEM, SHV, and CTX-M primers. TEM gene was the most predominant one followed by CTX-M and the least one was SHV gene. From the results it could be concluded that these genes are responsible for the resistance of cephalosporins.

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### INTRODUCTION:

Extended spectrum beta lactamases(ESBL) producing organisms are emerging pathogens with greatest spread in general population, and their presence in clinical

infection can result in treatment failure when using 3<sup>rd</sup> generation cephalosporins (Paterson and Bonomo, 2005). The extended spectrum  $\beta$ -lactamases are enzymes

elaborated by some bacteria and responsible for their resistance to extended spectrum 3<sup>rd</sup> generation cephalosporins e.g. ceftazidim, cefotaxime, ceftriaxone and monobactam e.g. azteronem (Carlos, 2000). These enzymes catalyze the hydrolysis of the  $\beta$ -lactam ring of antibiotics; ESBLs have been reported worldwide in many different genera of *enterobacteriaceae*, *Pseudomonasaeruginosa*, (Friedmanetal.2008). *Klebsiellapneumonia* and *E.coli* in addition *N. gonorrhoea* (Agrawaletal., 2008). ESBL producing organisms are often resistant to several other classes of antibiotics, as the plasmids with the gene encoding ESBLs often carry other resistance determinants; initially ESBL producing organisms were isolated from community (Pitout and Laupland, 2008).The ESBLs are mediated by *bla* TEM, *bla* SHV and *bla* CTX-M genes in *Enterobacteriaceae* and other Gram-negative bacteria, numerous molecular typing methods have been developed for their identification. The multiplex PCR assay allowed the identification of *bla* TEM, *bla* SHV, and *bla* CTX-M genes in a series of clinical isolates of *Enterobacteriaceae* with previously characterized ESBL phenotype (Monstein *et al.*, 2007). The first plasmid  $\beta$ -lactamase TEM1 was described in Germany 1980, and in France 1985 (Perez *et al.*, 2007).The National Committee for Clinical Laboratory Standards (NCCLS) recommended that microbiology laboratories reported ESBL –producing isolates of *E. coli* and *Klebsiella* species are resistant to all penicillins, cephalosporins and aztreonam, irrespective of their individual in vitro test result. Urinary tract infections (UTI) that affect parts of the urinary system occur more in women than men, especially in pregnancy due to the increased risk of kidney infection. During pregnancy high progesterone level elevated the risk of decreased muscle tone of the ureters and bladder which lead to greater

likelihood of reflux where urine flows back up the ureters and towards the kidney (Dielubanzaand Schaeffer, 2011) The bacterial spectrum and antimicrobial resistance may vary temporarily and geographically, each institution must undertake its own local evaluation such an evaluation may also be useful to detect emerging trends of antimicrobial resistance (Wagenlehneret al., 2008)

The objectives of this study were to:  
Isolate and identify the important bacterial pathogens causing UTI in pregnant women.  
Determine the drug resistance patterns of the isolated bacteria with emphasis to third generation cephalosporins, and Use molecular biology tools e.g. PCR in the detection of ESBL encoding genes

## **MATERIALS and METHODS**

This work was carried out in Military Hospital in Omdurman, during the period from April to May 2013. Pregnant women showing signs of urinary tract infection (UTI) were included in this study. One hundred mid-stream urine samples were collected. Data was collected using interviewing questionnaire.

### **Collection of specimens:**

Five ml midstream urine samples were collected in wide mouth screw-capped and leak-proof sterile containers containing 0.1g/10 ml boric acid as preservative.

### **Cultivation of specimens:**

The specimens were streaked under aseptic conditions on cystosine- lactose-electrolyte deficient (CLED) agar using sterile standard bacteriological wire loop. The cultured plates were incubated aerobically at 37C° for overnight and observed for bacterial growth.

### **Identification:**

Was done according to the criteria of Cheesbrough (2000)

### Colonial morphology:

Used as first identification depending on size, colour, edges, side views and fermentation of lactose.

### ESBL screening test:

All isolates (table 2) were tested for their susceptibility to the 3<sup>rd</sup> generation cephalosporins, i.e.: ceftazidime (30µg), cefotaxime (30µg) and ceftriaxone (30µg) by the standard disc diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI) guidelines (2010). ESBL were screened by detection of reduced zones of inhibition around 3<sup>rd</sup> generation cephalosporins.

The bacterial isolate was considered as ESBL producer when the zone diameter for: ceftazidime < 22mm. cefotaxime < 27mm and ceftriaxone < 25mm. Resistant isolates to at least one of the 3<sup>rd</sup> generation

cephalosporins were checked for ESBL production.

Double disk synergy test (DDST) was performed according to Jarlier *et al.*, (1988). Isolates were inoculated on Muller –Hinton agar plates. Discs containing ceftazidime (30Mg), cefotaxime (30Mg) and ceftriaxone (30mg) were placed 20 mm (centre to centre) away from a disc containing 20 mg amoxicillin/10mg clavulanic acid (AMC). Plates were incubated for overnight at 37°C. ESBL production was considered positive if the zone of inhibition around one or more of the 3<sup>rd</sup> antibiotic discs showed clear cut increase towards the AMCA disc (Ananthakrishnan *et al.*, 2000).

### Genotypic characterization of ESBL genes

DNA extraction and PCR assay were performed according to the method of Cao *et al.*, (2002)

**Table1: Primers used for amplification of SHV, CTX-M and TEM genes**

Gene		Primer sequence (5__3)	Products size	AnnealingTemp C	References
<i>bla TEM</i>	F R	5-TCG GGG AAA TGT GCG CG-3 5-TGG TTA ATC AGT GCA CC-3	971bp	55c	(Cao <i>et al.</i> , 2002)
<i>bla SHV</i>	F R	5-GGTTATGCGTTATATTCGCC-3 5-TTAGCGTTGCCAGTGCTC-3	850bp	55c	(Cao, <i>etal.</i> ,2002)
<i>bla CTX - M</i>	F R	5-SCS ATG TGC AGY ACC AGT AA-3 5-CCG CRA TAT GRT TGG TGG TG-3	550bp	55c	(Cao <i>et al.</i> , 2002)

## RESULTS

Out of 100 urine specimens collected from the study population, bacterial growth was obtained in 56 cultures. The results of Gram stain found 42(75%) Gram negative bacilli and 14 (25%) as Gram positive bacteria. The Gram negative was *K. oxytoca* 15(26.8%), *E. coli* 10(17.9%), *P. vulgaris* 8(14.3%), *K.*

*pneumoniae* 5(8.9%), *Citrobacter freundii* 1(1.8%), *Enterobacter spp.*1 (1.8%), *Providencia spp.* 1(1.8%) and *Serratia marcescens* 1(1.8%). The Gram positive *Staphelococcus saprophyticus* 13(23.2%) and *S. aureus* 1(1.8). The results of biochemical test for Gram negatives *E.coli*,

*K. P.vulgaris, Citrobacter, Enterobacter, Providencea and Serratia* and for Gram positive cocci *S. aureus* and *S. saprophyticus*. The antibiotic susceptibility test was found to be that 2(40%) of *K. pneumoniae* was resistant to all antibiotic used (ceftazidime, cefotaxime and ceftriaxone). All *K. oxytoca* was susceptible to all antibiotic used, 8(80%) of *E. coli* isolates were susceptible. *P. vulgaris* 7(87.5%) were susceptible. All *S. saprophyticus* were susceptible. *Enterobacter spp., Providencea spp., Serratia spp., Citrobacter spp.* and *S. aureus* were sensitive to 3<sup>rd</sup> generation cephalosporins used (table, 2).

The ESBLs producer were screened by resistant to 3<sup>rd</sup> generation cephalosporins found in 5 (11.9%) isolates confirmed by the using double disc synergy test (DDST). These were found in 2 *K. pneumoniae*, 2 *E. coli* and 1 *P. vulgaris* isolates. The commonest prevalence of ESBL gene was TEM gene in all 5 organisms (Figure, 2). CTX-M was produced by 3 organisms (2 *E. coli* and 1 *K. pneumoniae*) and SHV gene was detected in 1 *K. pneumoniae* and 1 *P. vulgaris* (table, 3 and Figure, 2).

**Table2: Antimicrobial susceptibility patterns of the isolated bacteria**

Antibiotic Bacteria	Ceftazidim		Cefotaxime		Ceftriaxone	
	S	R	S	R	S	R
<i>Klebsiella Oxytoca</i>	15(100%)	0	15(100%)	0	15(100%)	0
<i>E.coli</i>	8(79.9%)	2(19.1%)	8(79.9%)	2(19.1%)	8(79.9%)	2(19.1%)
<i>Proteus vulgaris</i>	7(87.5%)	1(12.5%)	7(87.5%)	1(12.5%)	7(87.5%)	1(12.5%)
<i>Citrobacter frundii</i>	1(100%)	0	1(100%)	0	1(100%)	0
<i>Enterobacter spp</i>	1(100%)	0	1(100%)	0	1(100%)	0
<i>Providencia spp</i>	1(100%)	0	1(100%)	0	1(100%)	0
<i>Serratia marcescens</i>	1(100%)	0	1(100%)	0	1(100%)	0
<i>Klebsiella pneumoniae</i>	3(60%)	2(40%)	3(60%)	2(40%)	3(60%)	2(40%)
<i>S. aureus</i>	1(100%)	0	1(100%)	0	1(100%)	0
<i>S.saprophyticus</i>	13(100%)	0	13(100%)	0	13(100%)	0

Key of table: S = sensitive R= resistant

**Table 3: TEM, SHV and CTX-M genes detected among ESBL producers**

Gene type	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. vulgaris</i>
TEM	2(40%)	2(40%)	1(20%)
SHV	0	1(20%)	1(20%)
CTX-M	2(40%)	1(20)	0
TEM+CTX-M	2(40%)	1(20%)	0
TEM+SHV	0	1(20%)	1(20%)

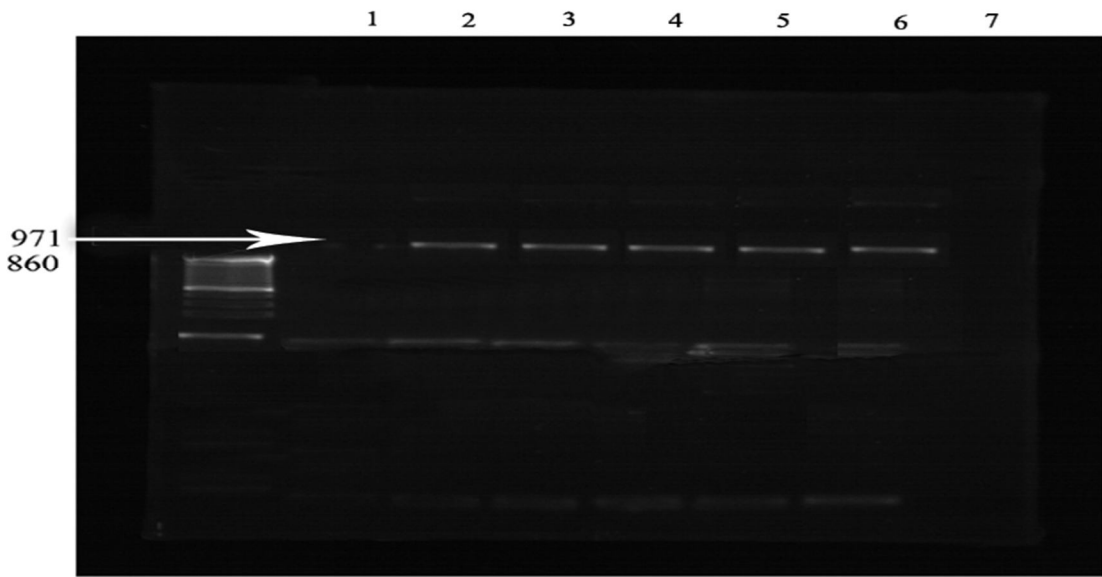


Figure 1: Agarose gel (1%) electrophoresis of TEM gene  
Lane 1: negative control  
Lanes 2,3,4,5 and 6: TEM gene positive

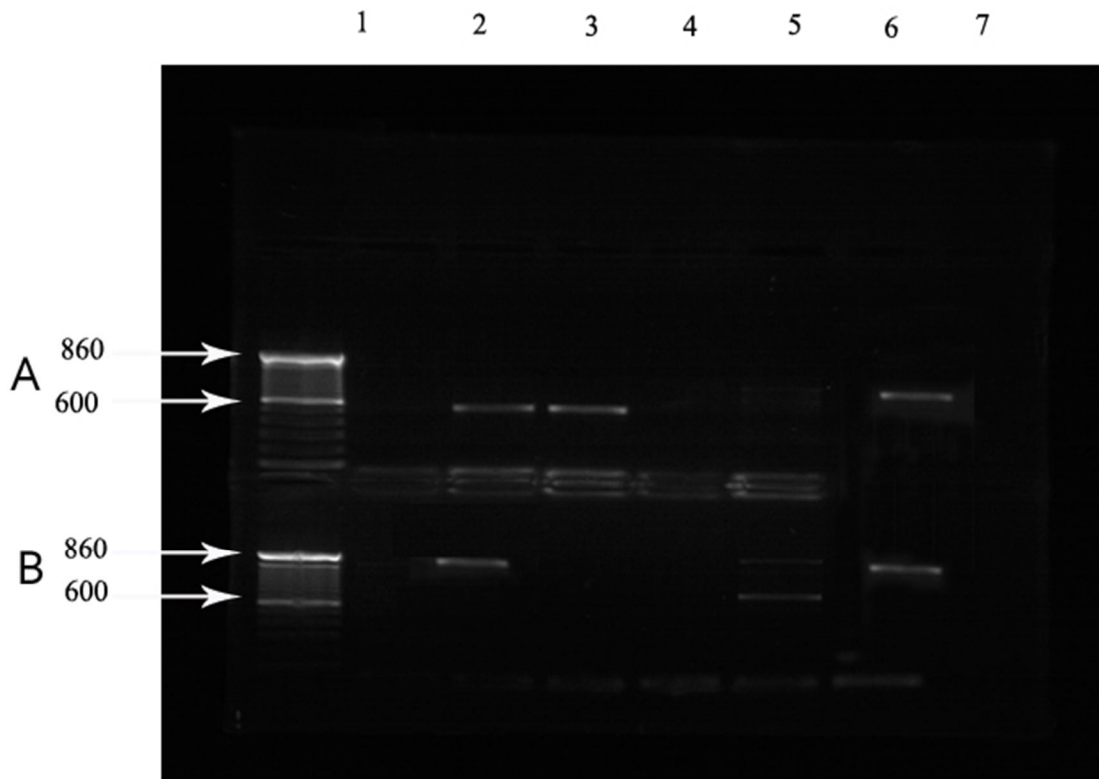


Figure2: Agarose gel (1%) electrophoresis of CTX-M and SHV genes

Lane 1, A: negative control

Lanes 2, 3, and 6, A: CTX-M gene positive

Lanes 4 and 5, A: CTX-M gene negative

Lane 1, B: negative control

Lanes 2 and 6, B: SHV gene negative

Lanes 3, 4 and 5, B: SHV gene negative

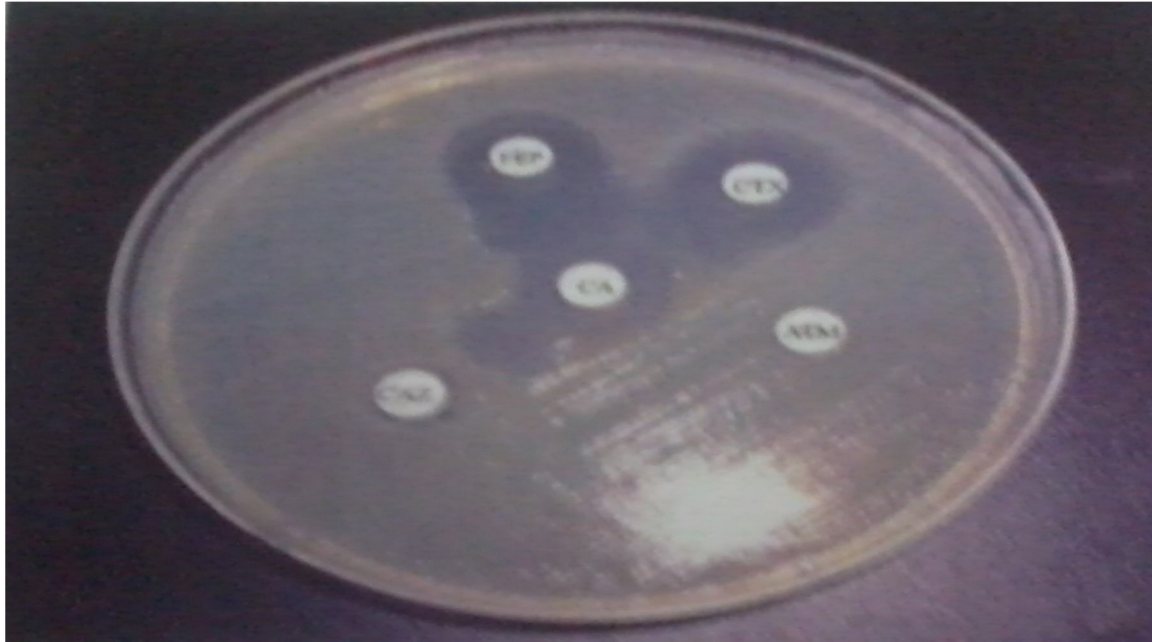


Figure 3: Double disc synergy test (DDS)

## DISCUSSION:

In this study out of 100 urine samples examined only 56 isolates gave significant bacterial growth on CLED medium. *K. oxytoca* 15(26%), *K. pneumoniae* 5(8.9%), *E. coli* 10(17.9%), *P. vulgaris* 8(14.3%), *Enterobacter spp.* 1(1.8%), *Citrobacter freundii* 1(1.8%), *Providencia spp.* 1(1.8%), *S. aureus* 1(1.8%), *S. marcescens* and *S. saprophyticus* 13(23.2%). In this study, 5(11.9) isolates producing ESBL were encountered, of which 2 (40%) *K. pneumoniae*, 2 (20%) *E. coli* and 1(12.5%) *P. Vulgaris*. These results were similar to the combined disc method currently recommended by the NCCLS (2010). For laboratories that perform susceptibility testing when using disc diffusion, when modified DDST could be easily incorporated in to an already existing system. This finding was in agreement to Omer (2013) in Sudan reported that *E. coli* and *K. pneumoniae* are most ESBL producers. Urine was the main source of ESBL producing isolates, which is in agreement with that found by Akbar

*al.*, (2007), who reported that (70.4%) of urine was the main source of ESBLs from all patient. The present showed that high prevalence of TEM gene. This was similar to that reported by Sekaret *al.*, (2009). The CTX-M was the second ESBL gene detected in this investigation. This finding that was in agreement with that reported by Feizabadi, *et al.* (2010). The present this study showed that CTX-M was most common gene among *E. coli* this was in agreement with that reported by Mirzaei *al.*, (2006). The SHV gene was the less frequent gene in this study, this result is similar with many reports around the world, such as in Thailand by Kiratisinet *al.*, (2008) and in Iran (Moosavian and Deiham, Dezfule *al.*, 2011). These results were not coinciding with those of Tasli and Bahar (2005) and Ben-Ami *et al.*, (2009), who detected SHV in 74.3% of isolates. This study is also disagreed with that reported in Sudan by Hamedelnil and Eltayeb (2012) who found CTX-M is highly prevalent (22.5%) but

agreed with SHV (2.2%) the less gene obtained.

### Conclusions

In Sudan ESBL genes were among most strains of Enterobacteriaceae, especially *E. coli* and *K. pneumoniae*. ESBL SHV, TEM and CTX-M genes are predominant in Sudan among *Enterobacteriaceae* isolates. Some strains carried one or more of these genes and this may lead to the resistance to some cephalosporins that might complicate the management of the disease problem.

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