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Original Research Article

Detection of CTX-M Gene β-Lactamase in Gram Negative Bacteria Isolate from Shendi Health Facilities

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Abstract: ***Background:*** The emergence of antibiotic resistance pathogen is an important health risk, usually gram-negative bacteria acquire resistance to antibiotics by the production of beta-lactamase enzyme, CTX-M-type enzymes are a group of class A extended-spectrum B-lactamases (ESBLs) that are rapidly Spreading among gram-negative worldwide. CTX-M-type ESBLs exhibit powerful activity against cefotaxime and ceftriaxone antibiotics but generally not against ceftazidime, in some geographical areas, CTX-M is now the most prevalent ESBLs in Enterobacteriaceae. There are 172 known variants of CTX-M genes identified until August 25, 2016. ***Objectives:*** This present study aims to identify the gene, namely: CTX-M, Responsible for extended-spectrum beta-lactamase (ESBL) among Enterobacteriaceae isolated from different samples of Sudanese patients in Shendi city (urine, pus, and wound). ***Methodology:*** 200 samples were identified as gram-negative bacteria. Identification of the isolates was done by using conventional biochemical methods, and ESBLs were screened according to (CLSI) guidelines. ESBLs Positive strains were tested for the presence of ESBL encoding CTX-M, Gene by using PCR with specific primers for the detection of CTX-M. **Results:** ESBL found to be higher in *P. aeruginosa* n=18(78.3%) *Klebsiella spp* n=45 (64.2%). *Escherichia coli* n=51(57.3%) *Proteus* n=8(53.3%) the total of ESBL from all gram-negative isolate represent n=122 (61%). The presence of the CTX-M, the gene was confirmed in *n*=57(46.8%) in all isolates. The CTX-M gene were detected in *n= 17 (37.8%) Klebsiella spp, n=28(55%) of Escherichia coli, n=3(37.5%) in Proteus* and *n=9 (50%) in P. aeruginosa isolates.* ***Conclusion:*** *P. aeruginosa* isolates represent a high percentage of ESBL producers followed by *Klebsiella spp*, then *Escherichia coli* and *proteus spp* CTX-M gen detected commonly in *Escherichia coli* followed by *P. aeruginosa* then *Klebsiella spp* and *Proteus spp.* The study concludes that the ESBL production within gram-negative bacteria was high and the CTX-M gene was spread in Shendi city.

Keywords: Extended spectrum β-lactamase (ESBL), CTX-M gene, Gram negative bacteria, PCR.

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INTRODUCTION

Gram-negative bacilli are responsible for numerous diseases some are commensal organisms present among normal intestine flora these commensal organisms plus others from environmental reservoirs may cause several urinary tract infections diarrhea peritonitis and bloodstream infections and typhoid fever are commonly caused by gram-negative bacilli **[1]**. Extended-spectrum beta-lactamase (ESBL) are bacterial enzyme that confers resistance to broad-spectrum cephalosporins including oxymino beta-lactam antibiotics, importantly ESBL producer organisms are associated with infections that result in poor clinical outcomes, delayed initiation of appropriate antibacterial therapy, longer hospitals stay, ESBL classification into three main groups ESBL A, ESBL M, ESBL GARBA, ESBL A are divided to three subgroups including TEM,

SHV and CTX-M they have been rapid and widespread dissemination of Escherichia coli and *Klebsiella*, as a result, these organisms have become important pathogen of both community and hospitals, ESBL mediated resistance in Enterobacteriaceae is leading to cause serious infections **[2]**. The CTX-M type beta-lactamase was first recognized in 1989 as a new ESPL family member. The origin of CTX-M is completely different from that of TEM and SHV type ESBL **[3]**. The CTX-M family of enzymes is thought to have derived from the initial transfer of chromosomal beta-lactamase gene from Kluyvera species to a conjunctive plasmid that readily disseminated among different members of Enterobacteriaceae **[4]**. To date, there are over 160 CTX-M varian, recorded and they are divided into five different groups according to their amino acid sequence CTX-M, CTX-M-2, CTX-M 8, CTX-M 9 and CTX-M 25 **[4]**. Antibiotics have always been considered one of the wounded discoveries of the 20th century, but the real wonder is the rise of antibiotic resistance in hospitals, communities, and the environment concomitant with their use. The extraordinary genetic capacities of microbes have benefitted from a man's overuse of antibiotics to exploit every source of resistance gene and every means of horizontal gene transmission to develop multiple mechanisms of resistance for every antibiotic introduced. To achieve complete restitution of therapeutic applications of antibiotics, there is a need for more information on the role of microbiomes in the rise of antibiotic resistance **[5].**

MATERIAL AND METHODS

Study design

A descriptive cross-sectional study was conduc ted at Shendi clinics and hospitals in River Nile state, S udan, between August to November 2019.

Sample size

From 300 different samples, 200 Gram-negativ e Enterobacteriaceae isolates were collected in Shendi c ity.

Ethical considerations

This study was approved by the ethical commit tee of Shendi University Faculty of Medical Laboratory Sciences, Department of Medical Microbiology. And ve rbal consent was obtained from all subjects enrolled in t he study.

Methods

Sample collection

For urine sample elimination of surface conta minants before collecting urine specimens, only mid-str eam urine was collected in sterile containers for culture. An adequate amount of urine was taken by sterile dry P asteur pipette for wet preparation. The wound swab spe cimens were collected aseptically before the wound was cleaned with antiseptic or normal saline. Using sterile c otton swabs were obtained from the wound site without contaminating with skin commensals and the swabs wer e then placed immediately into screw-capped Amie's tra nsport media bottle. In pus samples, two sterile swab sti cks were used to collect the pus samples. The first swab was used for gram staining and the second swab was us ed for culture.

Culture of urine specimen

Urine sample culture in CLED agar wound swab and pus culture into blood agar and MacConkey agar incubated at 37c for 24-48 hrs.

Interpretation of culture growth

The plates were observed for any bacterial colonies to grow significantly. The bacteria were well isolated and then identified by colonial morphology, Gram stain, and biochemical tests.

ESBL-detection

ESBLs were screened by detection of reduced zones of inhibition around third-generation disks in Muller Hinton agar. Such as Cefotaxime (CTX 30ug), Ceftazidime (CAZ 30µg), and Ceftriaxone (CRO 30µg).according to CLSI guidelines *[6]*. A double-disk synergy test (DDST) was performed to confirm ESBL production as described by ***[7]***. Isolates positive for ESBL production were screened by PCR using primers specific for the detection of, bla CTX-M gene.

DNA Extraction

Briefly, a few colonies were taken from a fresh culture medium and transferred to phosphate-buffered saline (pH 7.3). The suspension was heated at 100C for 30 min. The boiled suspension was transferred directly on ice. The suspension was then centrifuged at 12000 rpm for 30 minutes and the supernatant containing DNA was transferred to new Eppendorf tubes, the clear supernatant was used as template DNA in the PCR method.

Polymerase Chain Reaction (PCR)

PCR was performed and the test was carried o ut in a total volume of 25 µl, PCR reaction containing 5 µl of the extracted DNA, 2 µl from the primers forward (5'ACGCTGTTGTTAGGAAGTG-3') and reverse (5'-TTGAGGCTGGGTGAAGT-3'), 13 µl of distilled wate r was added to the Dream Taq Green PCR Master Mix DNA marker "Gene Ruler" was provided by Thermo Sc ientific (Lithuania). The amplification was done by 35 c ycles of PCR reaction (Initial denaturation at 94 ºC for 3 minutes, denaturation at 94°C for 1 minute, annealing at 58°C for 30 seconds, and extension at 72°C for 1 min ute. A final extension was performed at 72°C for 5 min utes) **[8]**.

Preparation of Agarose Gel

Amount of 2 gm. of agarose powder dissolved by boiling in 100 ml 1X TBE buffer, then was cooled to 55°C in a water bath, then, 5 µl of (10mg\ml) Ethidium

bromides were added, mixed well, and poured onto the casting tray that has been taped up appropriately and wa s equipped with a suitable comb to form well in place. Any bubbles were removed and the gel was allowed to s et at room temperature. After solidification, the comb w as gently removed and the spacer from the opened sides was removed.

RESULTS

ESBL was detected in (61) % of the bacterial isolates. A high rate of ESBL was seen in *P. aeruginosa* (78.3) %, followed by *Klebsiella spp* (64.2) %, *E. coli* (57.3) %, and *Proteus spp* (53.3) % shown in (Table1). CTX-M gene was positive in (46.8)% of the tested isolates.17 of these strains were K. pneumonia while 28 were *Escherichia coli*,9 were *P. aeruginosa*, and 3 *Proteus spp* shown in (Table 2). In figure Bla CTX-M DNA Bla CTX-M DNA results (857bp) on 1.2% agarose gel. Lane M shows a 100 bp DNA Marker, lane 1 shows negative control, lanes 2, 3, 4,5,7,9, and 10 show positive CTX-M, and lanes 6 and 8 show negative CTX-M.

Table-1: Show frequency and percentage of production of ESBL

Microorganisms Isolated	*Total*	*ESBL – production*	
		Positive (%)	*Negative (%)*
E. coli	89	51 (57.3)	38 (42.7)
Klebsiella spp	70	45 (64.2)	25 (35.8)
Proteus spp	15	8 (53.3)	7 (46.7)
S. typhi	3	0 (0)	3 (100)
P. aeruginosa	23	18 (78.3)	5 (21.7)
Total	200	122 (61)	78 (39)

Table-2: Show frequency and percentage of CTX-M gene in positive ESBL organism

Microorganism isolated	*Total*	*BlaCTX positive*	*BlaCTX negative*
E. coli	51	28(55)	23(45)
Klebsiella spp	45	17(37.8)	28(62.2)
Proteus spp	8	3(37.5)	5(62.5)
S. typhi	0	0(0)	0(0)
P. aeruginosa	18	9(50)	9(50)
Total	122	57 (46.8)	65 (53.2)

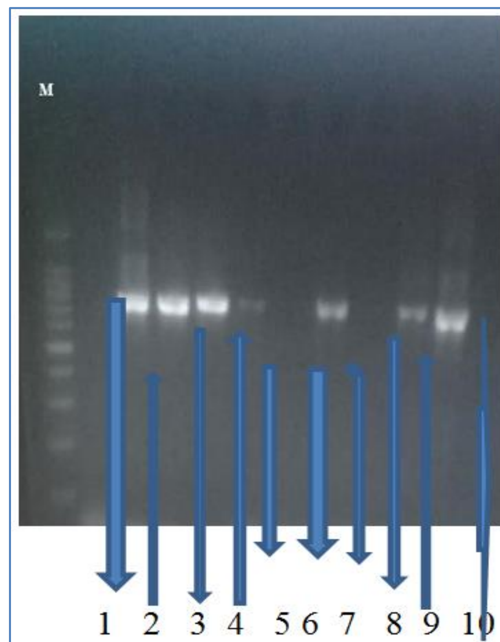

Fig-1: Bla CTX-M DNA

DISCUSSION

The production of ESBLs is one of the most important mechanisms of antimicrobial resistance in gram-negative bacteria the recently increasing number of ESBLs producing Enterobacteriaceae especially is attributed to the emergence of CTX-M gene Beta-lactamase The CTX-M gene beta-lactamase ESBLs are the most prevalent worldwide. This study detected resistance to cefotaxime among microorganisms isolated (87%), Aztreonam (72%) Ciprofloxacin

(63%), Ceftriaxone (58%), Ceftazidime (46%), this sensitivity result agrees with result done in France showed the isolates were more resistant to cefotaxime and aztreonam than to ceftazidime**.** Sudan concluded that even (81%) of ESBL Producing isolates are resistant to Ciprofloxacin compared to (63%) reported in the current study, with high resistance in Ibrahim's study due to the study done only in *E. coli*. In the current study, ESBL was found to be positive in 122 isolates (61%) similar to other studies done by Ahmed, Omar B., *et al.* ESBL was found positive in 130 isolates (59.6%) [11]. agree with the current study. In current study showed a high prevalence of ESBL in *P. aeruginosa* (78.3%) followed by *K. pneumonia* (64.2%) then *E. coli* (57.3%) [12]. Lim *et al*. have reported ESBL production in *P. aeruginosa* to be very low, (3.7%) and (4.2%), respectively [13], Disagree with this study, other study done by Mane, Vijay, *et al.,*, Wadekar, and Mita D., *et al.,* detected (57%), (42.3%) and (40%) respectively ESBL production in *P. aeruginosa* agrees with the current result. In Sudan study done by Ahmed, Omar B., *et al* shows only (10%) ESBL producers in *P. aeruginosa* which is too low compared to this study and this indicates new organisms have become high in ESBL production [14, 15]. Also in Sudan study done by Abdurrahman *et al.,* in different hospitals in Khartoum state showed the frequency of ESBL genes among *P. aeruginosa* isolates was (53.8%) and the CTX-M gene represent (23.3%) which disagrees with a current study where CTX-M gene represent (50%) In *P. aeruginosa* [16]. Another study was done among *P. aeruginosa* where ESBL producers were isolated in Khartoum military hospital reported (17.6%) of the *P. aeruginosa* [17]. The high prevalence of *P. aeruginosa* ESBLS reported in this study is due to the extreme empirical use of third-generation cephalosporins in clinical settings. The differences in the ESBL rates may be attributable to the geographic difference. In 2010 Mekki and his colleagues reported ESBL production among gram-negative bacteria isolated from Sudan, *E. coli* and *K. pneumonia* was (57.4%) close to the current study, and (71.7%) for *K. pneumonia* which is high compared to this study [18]. A study done by Iroha in Nigeria reported a higher prevalence of ESBL production in *E. coli* isolates (56.6%) [19]. Agreeing with the current study showed a result (of 57.3%). The percentage of ESBL in *Proteus* strains in this study was (53.3%). This percentage is higher than in previous studies in Sudan, where the detected percentage was (29.6%) in Hassan, *et al.,* 2016 and (33.3%) in 2013, Plasmids with Multidrug-resistant genes are common among the family of Enterobacteriaceae. Historically, *Proteus* species were known to be free of the beta-lactamase genes, However, *Proteus*, as a member of the family Enterobacteriaceae, can acquire the plasmids from other members of the family**.** Also, it was a bit higher in comparison with a study in Turkey, where the percentage of ESPL producers was (48.5%) in *Proteus spp* [20]. This study found that the isolated ESBL producer organisms had CTX-M target gene as (55%) bla CTX-Positive in *E. coli* organisms and its high percentage of CTX-M compared to other ESBL producer organisms. Followed by *P. aeruginosa* (50%), *Klebsiella spp* represent (37.8%) bla CTX-M positive, *Proteus spp* (37.5%) and, no CTX-M gene detected in *Salmonella spp,* In another study done In Khartoum [11]. CTX-M gene was found in (71.4%) of *Escherichia coli* strains and (68.4%) of *Klebsiella* species disagrees with the current study these differences might be due to the differences in the study time and population sample size. Another study was done to identify the CTX-M, gene, among *Escherichia coli* and *Klebsiella spp* isolated from Sudanese patients infected with urinary tract infection (UTI), in Khartoum State show the *Klebsiella spp* (68.8%) were ESBL producer and followed (65%) of *Escherichia coli* disagree with the current study [11]. ESBL producers which were negative for bla CTX-M genes 65/122(53.2%) in this study may harbor other ESBLs genes which confirms the need for further research in Shendi city. This study highlighted the alarming moderate spread of bla CTX-M producing Enterobacteriaceae in Shendi city compared to other studies that showed a high prevalence of bla CTX-M producing bacteria in different Sudanese regions, there Was reported that Bla CTX-M genes were detected in 96/128 (75%) of the ESBLs producers [11].

CONCLUSION

CTX-M extended-spectrum beta-lactamase (ESBL) has emerged as the most common type of ESBL globally, in this study *P. aeruginosa* was the most ESBL production followed by *Klebsiella spp*, *E. coli*, and *Proteus spp*. In positive ESBL isolated organisms, the CTX-M gene is most common in *E. coli,* followed by *P. aeruginosa*, *Klebsiella spp*, and *Proteus spp* CTX-M gene in all ESBL positive organisms represented (64.8%). The study concludes that the ESBL production in gram-negative bacteria was high, and the CTX-M gene was prevalent in Shendi city.

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