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Prevalence of CTXM, SHV, TEM AND OXA Genes among Extended-Spectrum Beta-Lactamase Producing *Klebsiella pneumoniae* from Mukuru Slum, Kenya

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Abstract

Background: Extended spectrum beta lactamases (ESBLs) producing Enterobacteriaceae cause infections that are often reported in both hospital and community setting. These infections are on the increase and jeopardize the achievement of modern medicine because of their clinical implications. There is need for surveillance measures to be taken, both by the health care personnel and the community at large. **Methodology:** We examined 330 diarrhea stool samples from children below the age of 5 years and processed them. A total of 96 (29%) samples were identified as *Klebsiella pneumoniae* out of the bacteria isolated. Identification of ESBL was done and 42 *K. pneumoniae* isolates were tested for the occurrence of ^{bla}CTX-M, ^{bla}OXA, ^{bl}aTEM and ^{bla}SHV resistant genes by PCR, gel electrophoresis and visualized by UV illumination. **Results:** Our results revealed that ^{bla}CTXM was the most frequent ESBL type 42 (100%), followed by ^{bla}TEM in 41 (97.6%) isolates and ^{bla}SHVin38 (90.4%) of the isolates. None of the tested isolates were found to be encoding ^{bla}OXA. There was occurrence of more than one gene in most of the isolates. The double combination was detected in ^{bla}CTX-M/^{bla}TEM (9.5%) and ^{bla}CTXM/SHV (2.4%). A triple combination was noted ^{bla}TEM/^{bla}SHV/^{bla} CTX-M (88%). **Conclusion:** Our results indicate that there is Presence of Beta lactam genes associated with antimicrobial resistance among the *K. pneumoniae* isolates from Mukuru Slum, Kenya. The predominant ESBL genotype in Mukuru slums, Kenya was ^{bla}CTX-M followed by ^{bla}TEM and ^{bla}SHV respectively. There is need for surveillance measures to be taken so as to control the spread among the community.

Keywords

Klebsiella Pneumoniae, Resistant Genes, ESBL

1. Introduction

Klebsiella pneumonia commonly causes both hospital and community-acquired infections worldwide. It is a normal flora of the gastrointestinal tract and causes infections when immunity is compromised, children, neonates and the elderly being vulnerable individuals. It causes diarrhea, urinary tract infections, bacteremia, liver abscess and wound or soft tissue infections. It is one of the top three bacteria of international concern associated with nosocomial infections in WHO report on global status of antibacterial resistance [1]. Klebsiella has the ability to acquire gradually, build up and transfer multitude antimicrobial resistant determinants. It may consequentially serve as a reservoir for resistance within the gut [2]. It is well documented that *in vivo* transfer of antimicrobial resistant genes from intestinal Klebsiella to other bacterial species occur [3].

The Clinical and Laboratory Standards Institute recommends routine testing and reporting of *Klebsiella pneumoniae* because it is one of the major ESBL-producing organisms isolated worldwide. ESBL is a major concern worldwide as it alleviates treatment failure since it's responsible for antibiotic resistance. Beta-lactamases are enzymes that confer resistance to the beta-lactam antibiotics such as penicillin, cephalosporins and Carbapenem which have been used widely for the treatment of infections caused by Gram negative bacteria. Beta lactam enzymes catalyze the hydrolysis of the amide bond of beta-lactam ring rendering the antibiotic inactive against the cell wall trans peptidase which is its cellular target. This has led to bacteria resistance. Beta-lactamases are grouped into four classes on the basis of their primary structure A, B, C, and D enzymes. Enzymes of classes A, C, and D have serine at the active site, whereas the class B enzymes are zinc-metalloenzymes [4].

ESBL are often produced by Enterobacteriaceae members especially Klebsiella, and *E. coli*. These enzymes can be exchanged readily between bacteria species since they are encoded by plasmids. According to Bajpai *et al.*, 2017 [4], more than 350 different ESBL variants are known and are classified into nine evolutionary and structural families based upon their amino acid sequence such as CTXM, TEM, SHV, OXA, PER, TLA, VEB, GES and BES. In Kenya, there are limited pediatric data on the prevalence of these ESBL variants. ESBLs were first identified in Germany in 1982 and have since spread globally, becoming a significant problem in sub-Saharan Africa including Kenya [5].

Here, we examine the presence of resistant gene conferred by ESBLs in *Klebsiella pneumoniae* isolates.

2. Methodology

2.1. Study Area

The study was carried out within Mukuru Kwa Njenga slums. Mukuru slum is located on the East of Nairobi in Embakasi constituency in the period of August 2017 to August 2018 (**Figure 1**).

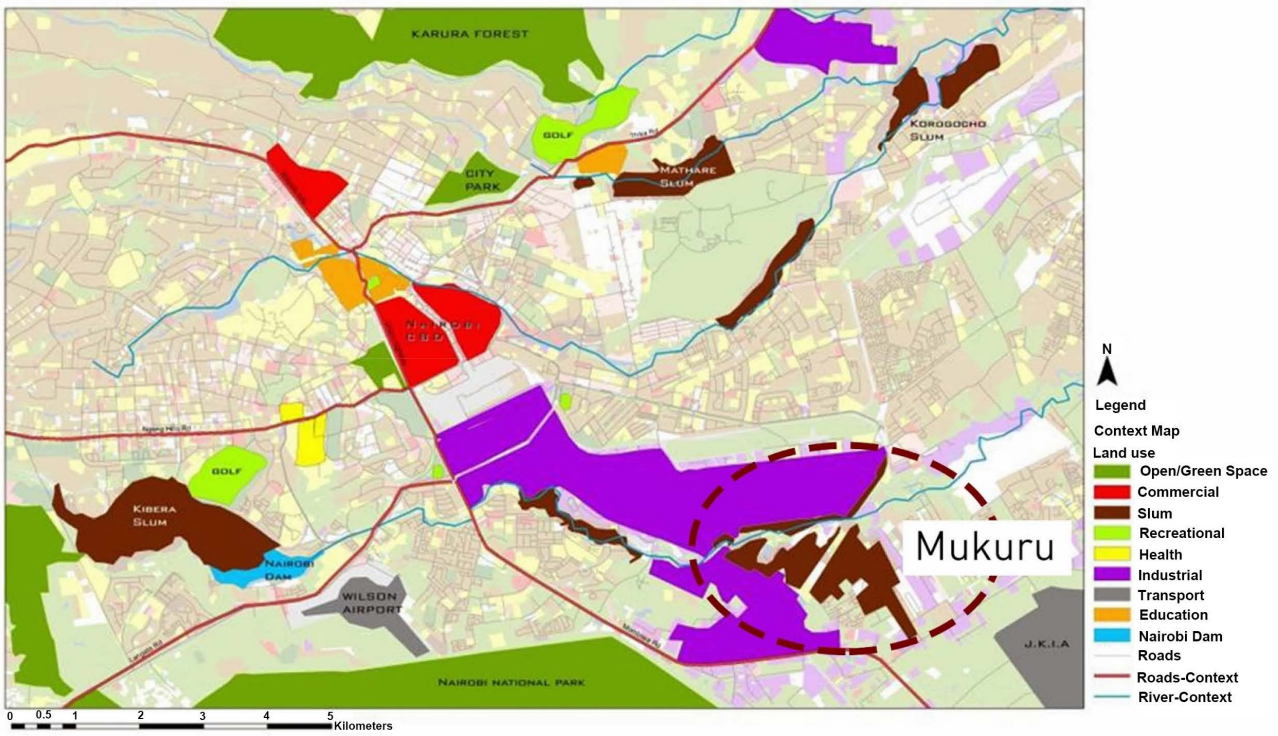

Figure 1. Map showing geographical location of study site.

2.2. Patient Recruitment

Children having diarrhoea who are 5 years of age and below. Diarrhea was taken to be three or more episodes of passing loose stool that is watery and Mucoid or bloody in a day. These children were seeking treatment at outpatient clinics in Mukuru slum. Consent was given by the parent or guardian to participate in the study by signing consent to participate having been briefed about the study. Every third patient who had not taken antibiotics before going to the hospital was recruited into the study.

2.3. Sample Collection

The patient was given a sterile stool cup to collect a single stool sample taking care not to contaminate the sample with urine, soil or water. Part of the collected stool was transported in Carry Blair transport medium to Kenya Medical Research Institute Microbiology laboratory for processing.

2.4. Bacteria Identification

On arrival at the laboratory the stool samples were examined macroscopically, recorded and immediately platted on MacConkey and incubated at 37˚C for 18 to 24 hours. Colonial morphology was done and Lactose fermenting Mucoid colonies were sub cultured on nutrient agar for purity and then biotyping was done. The criteria for *K. pneumoniae* confirmation was Tipple sugar iron: A/A, Gas+ and H_2S–. MIO: +motility, +indole, +ornithine, citrate positive and urease positive.

2.5. Antibiotic Susceptibility Testing

The Kirb-Bauer disc diffusion technique was done in antibiotic susceptibility testing on the isolates using different antibiotics such as: Beta lactams, quinolones amino glycosides and penicillins.

Zones of inhibitions were measured and the results interpreted in accordance to Clinical Laboratory Standard Institute 2015 guidelines [6]. Isolates that were resistant to third generation cephalosporins were subjected to phenotypic detection.

2.6. Phenotypic Detection of ESBL

Characterization was done on *K. pneumoniae* isolates that were resistant to cephalosporins and tested for ESBLs production using the double disk synergy test in accordance to CLSI 2015 guidelines. ESBL-producers were identified as isolates showing synergy zones between Amoxicillin/clavulanic and one or more third generation cephalosporin [7].

An inhibition zone that is distorted or enlarged forming a keyhole appearance between the cephalosporins discs and the Amoxicillin/Clavulanate disc was interpreted as an ESBL enzyme production phenotype. *K. pneumoniae* K6 ATCC 700,603 (ESBL producer) and *Escherichia coli* ATCC 25,922 (non-ESBL producer) served as the positive and negative controls respectively.

2.7. Genotypic Detection of ESBL

The boiling method was used to extract DNA of Purified colonies of ESBLs producing *K. pneumonia* suspended in TE buffer. SHV, CTXM, OXA and TEM genes were detected as described previously [7]. Specific primers for the genes, amplicon size and annealing temperatures used are shown in **Table 1**. PCR amplification for selected ESBL genes was done in 20 µl volumes containing 4 µl of 5× master mix of 0.4 µl concentrations of each primer, 12.2 µl of PCR water, BSA 1 µl and 2 µl of DNA template. A programmable thermo cycler was used with initial denaturation at 94˚C for 5 min; followed by 35 cycles at 94˚C for30 s, annealing was done between 30 second and 1 min depending on the primer temperature, then a short extension step at 72˚C for 1 min and a final extension at temperature of 72˚C for 10 min for short fragments and 20 min for longer fragments. Electrophoresis was done to the amplified PCR products, with a 1 kb DNA ladder as a standard in 1.0% agarose gel in 1× TBE buffer and stained with ethidium bromide. This was visualized under the UV trans illumination. Positive control strains were used for the different test genes and distilled water used as a negative control.

3. Results

A total of 96 (29%) *Klebsiella pneumoniae* were isolated from the 330 samples obtained from children recruited into the study. 42 ESBL *Klebsiella pneumonia* bacterial isolates were studied genotypically. PCR amplification was done to detect

Table 1. Primers used for detection of ^{bla}SHV, ^{bla}CTX-M & ^{bla}TEM genes.

Gene	Primer sequence	Amplicon size	Annealing temperature
SHV	F:5'-TCAGCGAAAAACACCTTG -3' R:5'-CCCGCAGATAAATCACCA -3'	747 bp	50
TEM	F:5'GAGTATTCAACATTTCCGTGTC-3' R:5'TAATCAGTGAGGCACCTATCTC-3'	865 bp	55
CTX-M	F:5'TTTGCGATGTGCAGTACCAGT AA-3' R:5'CGATATCGTTGGTGGTGCCAT A-3'	593 bp	60
OXA	F:5'ATGAAAAACACAATACATATCAACT-3' R:5'GTGTGTTTAGAATGGTGATCGCATT-3'	820	62

the presence of ^{bla}CTX-M, ^{bla}OXA, ^{bla}TEM and ^{bla}SHV genes by PCR, gel electrophoresis and visualized by UV illumination. ^{bla}CTX-M was identified in all 42 (100%) isolates, ^{bla}TEM in 41 (97.6%) isolates, ^{bl}aSHV in 38 (90.4%) of the isolates. None of the isolates tested were identified to be encoding ^{bla}OXA. There was occurrence of more than one gene in most of the isolates, double and triple combination was noted.

The double combination was detected in ^{bla}CTX-M/^{bla}TEM (9.5%) and ^{bla}CTXM/SHV (2.4%) while a triple combination was noted ^{bla}CTX-M/^{bla}TEM/^{bla} (88%) as shown in Table 2 (Figures 2-4).

4. Discussion

In our study, CTXM, TEM and SHV genes were detected in ESBL producing K. pneumoniae. This may be due to the fact that in Mukuru slum, factors such as poor hygiene due limited resources such as water supply, poor housing, poor drainage, overcrowding, frequent outbreaks of diseases and even malnutrition, put the inhabitants at risk of acquiring ESBL Klebsiella. Furthermore, the low social economic status and even illiteracy may contribute to the misuse of antibiotics.

CTXM (100%) was the most predominant gene, TEM (97%) and SHV (90.4%) genes responsible for ESBL production. Our results are in accordance with a study done by Maina *et al.*, (2013) [8] at Agha khan University, Kenya where ^{bla}CTX-M was the predominant genotype found in 46 (88.5%) of the 52 isolates analyzed. In other african countries, 17/17 *Klebsiella spp.* isolated in a pediatric hospital, Charles De Gaulle, Burkina Faso had CTXM [9]. In other parts of the world, like Turkey reported CTX-M (92%), followed by TEM (39%), SHV (5%). In the Philippines reported 35 (95%) ^{bla}CTX-M. In Asia, Taiwan, Japan and China, earlier reports indicated a dominance of SHV but CTXM now emerges dominance Reuland, *et al.*, 2013 [10]. Further reports from South America, Israel, Spain, New York, the United Kingdom, and several parts of Indian subcontinent revealed CTX-M a the predominant gene. These results reflect a trend not only in Africa but also in other parts of the world where ^{bla}CTXM has been reported to be the most prevalent ESBL-encoding gene and is replacing ^{bla}TEM

Table 2. Frequency of the ESBL genes detected from *K. pneumoniae.*

Gene	No	%
SHV	38	90.4%
TEM	41	97.6%
CTXM	42	100%
OXA	0	0.00%
CTXM/SHV/TEM	37	88%
CTXM/TEM	4	9.5%
TEM/SHV	0	0%
CTXM/SHV	1	2.4%

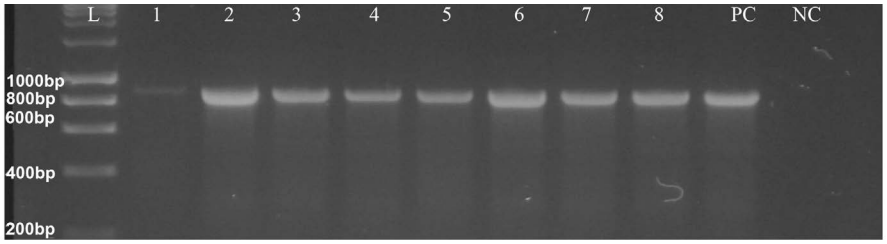

Figure 2. Electrophoresis gel for ^{bla}TEM (865 bp); L—Molecular Ladder; NC Negative Control; PC—Positive Control; bp—Base Pairs. Numbers at the top represent random DNA numbers of the isolate.

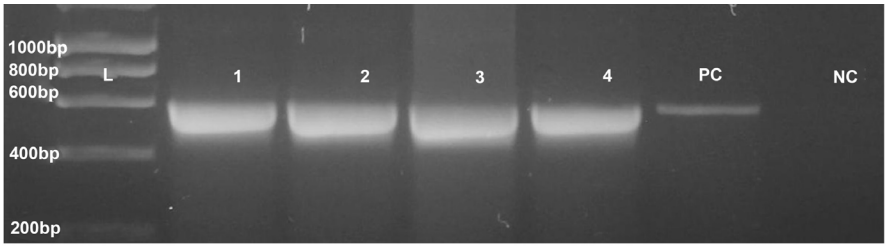

Figure 3. Electrophoresis gel for ^{bla}CTX-M (593 bp); L—Molecular Ladder; NC Negative Control; PC—Positive Control; bp—Base Pairs. Numbers at the top represent random DNA numbers of the isolates.

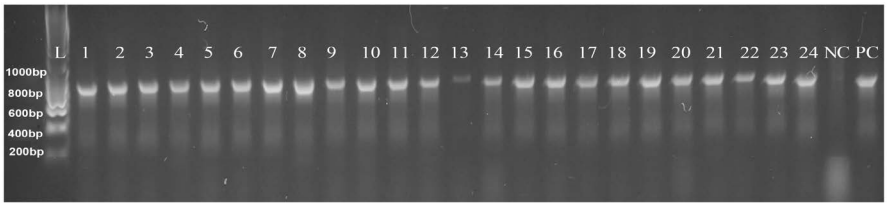

Figure 4. Electrophoresis gel for ^{bla}SHV (747 bp); L—Molecular Ladder; NC Negative Control; PC—Positive Control; bp—Base Pairs. Numbers at the top represent random DNA numbers of the isolates,

and ^{bla}SHV types as the predominant ESBL in many countries [11]. CTXM was the most dominant probably because it is located in different plasmids that belong to different incompatible groups and can be found in isolates that are not

related epidemiologically. This enzyme therefore has the ability to spread widely [12].

However, some studies performed throughout the world showed variable results. In Machakos hospital, Juma *et al.*, *(*2016) [13] found that TEM was the most predominant. A report from Canada showed SHV 22 (43.14%) as the main group of ESBLs, followed by ^{bla}TEM 18 (35.29%) and blaCTXM 16 (31.37%) [14]. The differences between our study results and those of other authors indicated that the prevalence and type of ESBL genes may vary from one geographical region to another Bajpai *et al.*, 2017 [4].

In our study, none of the 42 isolates tested was found to be encoding ^{bla}OXA. This is in accordance to a similar study conducted in Gazi University, Turkey where there were no strains harboring OXA type among the beta lactamase isolates [15]. A study done in France identified the first large outbreak of OXA among 48-positive Carbapenem-resistant *K. pneumoniae* isolates expressed both SHV and CTXM [16]. The OXA family of ESBLs has mainly been reported in *Pseudomonas aeruginosa* unlike ^{bla}TEM and ^{bla}SHV which are prevalent in Enterobacteriaceae. They belong to molecular class D and functional group 2d and have ability to hydrolyze Oxacillin. However, they are resistant to Ampicillin and Cephalothin but inhibited by clavulanic acid and they cannot breakdown the recently developed cephalosporins exceptOXA-10 that weakly destroys aztreonam, ceftriaxone and Cefotaxime. Other OXA ESBLS include OXA-11, -14, -16, -17, -19, -15, -18, -28, -31, -32, -35, and -45 [17].

Occurrence of more than one beta-lactamase within the same isolate was detected in our study. It is notable that most isolates had a double and a triple combination. Double combination was observed in CTXM/TEM, CTXM/SHV.A triple combination was noted in CTXM/TEM/SHV.

Coexistence the ESBL gene has been reported in various studies. In Africa, Tunisia has reported a Triple combination TEM/SHV/CTX-M [18]. In Tanzania, Mshana *et al.*, (2013 [19] found a combination of TEM/CTX-M, 11.96% (11/92) SHV/CTX-M and 10.87% (10/92) CTX-M alone. In Togo, combination of TEM/SHV/CTXM predominating at 61.90%, TEM/SHV 20.63%, SHV/CTXM 11.11%, TEM/SHV 4.76% and TEM 1.59 has been reported. In Bukinafaso, triple combination of TEM, SHV and CTX-M was noted. In Tunisia a triple combination TEM/SHV/CTX-M represented 44.91% (53/118), and the double combinations were SHV/CTX-M represented 28.81% (34/118) of cases, TEM/CTX-M represented 4.23% (5/118) and TEM/SHV represented 3.39% (4/118) of cases according to Diagbouga *et al.*, 2016 [20]. Similarly, in Brazil, Jaskulski *et al.*, (2013) [21] found that 16.67% (2/12) of ESBL producing *Klebsiella pneumoniae* carried TEM, CTX-M, TEM/SHV genes, while 8.33% (8/12) carried SHV alone. The double TEM/CTX-M and triple TEM/SHV/CTX-M combinations were 25% (3/12) and 16.67% (2/12) respectively.

Antibiotic policy in Kenya is not enforced. Beta lactam antibiotics are misused just like other antibiotics. Culture and sensitivity is not always done prio to the

issuance of prescription, we speculate that the misuse of antibiotics to treat infections, has selected for isolates carrying multiple bla genes such as those encountered in this study. The occurrence of double and triple bla genes carriage and combination should be further investigated.

5. Conclusion

From our report on molecular characteristics of *Klebsiella pneumoniae* isolates in Mukuru slums, Kenya, we reveal a high rate of ESBL in this area. We also identified ESBL-producing three genes of $^{bla}_{TEM}$, $^{bla}_{SHV}$, and $^{bla}_{CTX\text{-}M}$ by PCR. Combination of the three resistant genes was also noted. The most common genotype was $^{bla}_{CTX\text{-}M}$ followed by $^{bla}_{TEM}$ and $^{bla}_{SHV}$ as last. We did not detect $^{bla}_{OXA}$ in Mukuru slum. The high prevalence of these resistant genes may influence appropriate treatment of infections caused by *Klebsiella pneumoniae* among children. The data underline the need for establishment of laboratory infrastructure and protocols for continuous surveillance of resistance so as to monitor antimicrobial therapy and drug resistant isolates in order to better control the emergence and spread of ESBL producing *K. pneumoniae* strains. A high degree of awareness should be created not only among the community but also among physicians and microbiologists. Consequently, there is need for improvement of hygiene conditions in the slums.

Authors' Contribution

HS developed the concept and study design. She collected and analyzed the samples, interpreted data and drafted the manuscript. SM supervised the lab work and offered guidance. SM assisted in manuscript preparation MK corrected the proposal.

Authors' Information

HS is a master's student studying Medical Microbiology. Dr. CM and MK are lectures in Jomo Kenyatta University and Technology with great experience in proposal development. SK is an academician and Chief research scientist of international acclaim, with an extensive experience He is the head of Research and development.

Ethical Considerations

Ethical approval for the study was granted by the Kenyatta University Ethical Committee (020 8710901/12).

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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