ANTIMICROBIAL RESISTANCE PATTERNS IN EXTENDED-SPECTRUM BETA- LACTAMASE PRODUCING *Klebsiella pneumoniae* ISOLATES FROM CHILDREN UNDER FIVE YEARS IN MUKURU SLUMS, NAIROBI, KENYA

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Antimicrobial Resistance Patterns in Extended-spectrum beta-lactamase Producing *Klebsiella pneumoniae* isolates from Children in Mukuru Slums, Nairobi, Kenya

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DECLARATION

This thesis is my original work and has not been submitted for a degree in any other University.

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DEDICATION

I dedicate this piece of work to my family for their love, support, patience and encouragement in pursuit of academic excellence.

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LIST OF ABBREVIATIONS

AGNHMRC	Australian government national health and medical research council
AMC	Amoxicillin-Clavulanic acid
AMP	Ampicillin
ATM	Aztreonam
CAZ	Ceftazidime
CIP	Ciprofloxacin
CLSI	Clinical and Laboratory International Standards Institute
CMR	Centre of Medical Microbiology
CN	Gentamycin
CPD	Cefpodoxime
CRO	Ceftriaxone
CTX	Cefotaxime
DDST	Double Disc Diffusion Synergy Test
DNA	Deoxyribonucleic acid
DNDi	Drug for neglected diseases initiative
ESBL	Extended-Spectrum β-lactamase
FOX	Cefoxitin

GARDP	Global antibiotic research and development partnership
ICU	Intensive care unit
IMP	Imipenem
IVAC	International vaccine access center
KEMRI	Kenya Medical Research Institute
KNBS	Kenya national bureau of statistics
MCC	Mukuru City Council Clinic
MDR KP	Multi-drug Resistant *Klebsiella pneumoniae*
MMM	Mukuru Mission of Mary
NA	Nalidixic acid
PCR	Polymerase Chain reaction
SIM	Sulfide, Indole motility
SPSS	Statistical package for social sciences
SXT	Sulfamethoxazole
TE	Tetracycline
TSI	Triple sugar iron agar
UNICEF	United nation children fund
US	United States

UTI	Urinary Tract Infections
UV	Ultraviolet radiation
WHO	World Health Organization

ABTRACT

The multidrug-resistant rate of *K. pneumoniae* has risen rapidly and is a major challenge. Since their description, the extended-spectrum β-lactamase (ESBL) producing organisms have become recognized as a worldwide problem. Treatment of these multiple drug-resistant organisms is a therapeutic challenge yet infections caused by ESBL producing pathogens, particularly *K. pneumoniae*, are increasingly becoming common. The general objective of the study was to determine antimicrobial resistance pattern in ESBL producing *K. pneumoniae* isolates from children under five years in Mukuru slums. Antimicrobial resistance is a crucial public health problem yet in kenya there is limited padiatric data not only on the resistance pattern of ESBL producing organisms such as *K. pneumoniae,* but also the the prevalence and the association between age,gender of such infections. A cross-sectional study was conducted among 330 children.Ethical clearance was granted by the Kenyatta University Ethical Review Committee and a written informed consent obtained from parents/guardians of all participants. Diarhea stool samples were collected, cultures done and antimicrobial susceptibility testing determined using the disc diffusion test. The ESBL positive phenotypes were analyzed by PCR to detect the presence of four beta lactamase ^{bla}SHV, ^{bla}TEM, ^{bla}CTX-M and ^{bla}OXA. The prevalence of *K. pneumoniae* was 29%, with age group 13-24 months being significant to acquisition of *K. pneumoniae*.There was no statistical significant differences between *K. pneumoniae* and gender. High resistance was recorded in most of the antibiotics Ampicillin 97.9%. Cefotaxime, Aztreonam and Cefpodoxime recorded an equal resistance of 68.8%,while Ceftazidime and Ceftriaxone recorded 67.7%. Ciprofloxacin 66.7%,Sulfamethoxozole 65.6%,Gentamycin 60.4%,Amoxicillin-Clavulanic 43.8% ,Tetracycline 55.2% ,Cefoxitin 41.7% ,Nalidixic acid 32.3 % . None of the isolates exhibited resistance to Imipenem. There was no significant statistical significance difference observed between age and gender in terms of resistance.The proportion of ESBL producers was 44%. There was no statistical significance difference between age and ESBL carriage. In terms of gender, males had an elevated odds with $p<0.05$ compared to females. The ^{bla}CTXM was the most prevalent at 100%, ^{bla}TEM at 97% and ^{bla}SHV at 90%. None of the isolates analysed expressed ^{bla}OXA. In conclusion, the observed high levels of resistance among children infected with *K. pneumoniae*. ESBL genes being associated with varying degree of resistance.This poses a major therapeutic challenge among children. The study recommends patient education on proper use of antimicrobials as well as surveillance on resistance pattern of ESBL producing organisms. Screening of ESBL strains in microbiology laboratory prio to prescription will assist in revising existing empirical treatment regime.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Klebsiella pneumoniae is a Gram negative, rod shaped bacterium belonging to the family *Enterobacteriaceae*. It has in recent years become an important pathogen in nosocomial infections worldwide. (Apondi *et al*., 2016). Increasingly, many strains that are extended-spectrum-lactamase (ESBL) producing as well as Carbapenem resistant are being reported as causing outbreaks in hospitals (Shaikh *et al.,* 2015). The spread of these nosocomial infections occur from patient to patient, healthcare workers to patients and vice versa as well as contaminated hospital environment and equipment. Most of the infections occur in neonates, immunocompromised patients such as critically ill patients in ICU, patients with malignancies, patients on chemotherapy, HIV infected patients and diabetic patients. (Shaikh *et al.,* 2015) Treatment options for multi-drug resistant *K. pneumonia*e (MDR KP) are limited; more so in resource constrained settings. Most studies on MDR KP are from developed countries with scanty data from resource limited settings in developing countries (Apondi *et al*., 2016).

Study findings will not only inform policy makers but also create an understanding on the trend of ESBL production.Study findings will also fill the gap in knowledge that exists:association between age, gender and ESBL *K.pneumoniae* infected children in Mukuru slum. A slum is a contiguous settlement where the inhabitants are characterized as having inadequate housing and basic services (UN, 2015). Overcrowding, economic deprivation and substandard housing facilitate the spread of infectious diseases, such as tuberculosis, hepatitis, pneumonia, cholera and malaria. Poor sanitation and lack of access to safe food and water contribute to high prevalence of diarrhea within slums. Indoor and outdoor air pollution exacerbates risk of diseases especially respiratory diseases. Moreover, slum areas are often left out of major city networks for access to health-care services. Seasonality also influences diseases in slums (Mutisya *et al.,* 2010).

Analyzed surveillance data from Korogocho and Viwandani slums in Nairobi, found that there was a peak in mortality among the under-five in May – July, correlating with the onset of the rainy and cold period, increasing the risk of mortality with 56 –60 percent.

1.2 Statement of the Problem

Infections caused by ESBL-producing gram negative bacteria such as *K.pneumoniae* present a serious challenge to public health as they are associated with increased morbidity and mortality. Majority of ESBL associated infections are resistant to commonly used antibiotics, leaving limited therapeutic option. ESBL-producing organisms such as *K.pneumoniae* are associated with infections that result in poor clinical outcomes, delayed antibacterial therapy, longer durations of illness, longer hospital stays, greater hospital expenses and more economic burden to the patient and the country at large. Limited pediatric studies have been carried out to determine the prevalence of *K.pneumoniae* and its antimicrobial resistant profiles. Kenya is poorly investigated and the prevalence of ESBL-producers, the association between age and gender of such strains remains unknown among children below five years.

Unlike other Kenyan slums such as Kibera, data on mortality rates due to diarrhea caused by organisms such as *K.pneumoniae* among children in Mukuru slums remain very scanty. Diarrheal diseases among children aged under 5 years is the top four largest contributor of childhood mortality, in sub-Sahara Africa. In Africa it is approximated that 25% of deaths are due to diarrhea. (Pop *et al.*, 2014). According to WHO Global Burden of Disease estimates, diarrhea accounts for nearly 3 million deaths per year in developing countries (Troeger *et al*., 2018).

Mukuru slum is an informal urban settlement. This settlements possess grave threat to the health of its habitats stemming from poor quality housing, poor sanitation, poor drainage, lack of proper water supply, overcrowding due to the dense population, frequent outbreaks of diseases, malnutrition, and garbage heaps due to minimal refuse

collection, low social economic status and even illiteracy. All these are risk factors for acquiring ESBL producing bacterial infections (Omolo *et al*., 2019).

1.3 Justification

Mukuru is one of the biggest slums in Nairobi. It is estimated to be habitat of about 700,000 people in the different villages in the slums. It stretches along the Nairobi Ngong River, situated on waste lands in the industrial area of the city between the Outer Ring Road and the North Airport Road and Mombasa road. Presence of this waste land of industrial area and the heavily polluted Nairobi River predisposes the residents to infections especially respiratory and diarrheal infections.

Little research has been conducted to identify the association between age and gender to colonization with Gram-negative multidrug-resistant bacteria such as *K.pneumoniae*. This study adds new information to the knowledge-void that exists in this field of research. This is of paramount importance because it can help ascertain which patients may need empiric antimicrobial drug therapy aimed at the ESBL-producing bacteria. Infections caused by extended-spectrum beta-lactamase (ESBL)-producing *Klebsiella* species are of major concern (Tumbarello *et al*., 2011).

In developing countries mortality association with *K. pneumoniae* among children far exceeds that in developed countries. The incidence in children is estimated to be 0.29 episodes per child-year in developing and 0.05 episodes per child-year in developed countries. This translates into about 156 million new episodes each year worldwide, of which 151 million episodes are in the developing world (Rudan *et al*., 2013).

Antimicrobial resistance jeopardizes heath care gains and also puts at risk the achievement of modern medicine. Understanding on antibiotic resistance patterns and establishment of adequate infectious control programs are essential to guide therapy to avoid serious pathological conditions. This study is important as it will help in

understanding the trends of extended beta lactamase production in *K. pneumoniae* in Children from Mukuru slums hence guiding therapy in these areas.

1.4 Research Questions

1) What is the prevalence of *K. pneumoniae* from children in Mukuru slums, Nairobi, Kenya and what propotion are ESBL producers?.
2) What are the antimicrobial resistance profile of *K.pneumoniae* isolates from children in Mukuru slums, Nairobi, Kenya?
3) What ESBL genes are responsible for resistance among children in Mukuru slums Nairobi, Kenya?.
4) What is the association between age, gender, and *K.pneumoniae* infected children in Mukuru slums, Nairobi, kenya?

1.5 Objectives

1.5.1 General Objectives

To determine antimicrobial resistance pattern in ESBL producing *K. pneumoniae* isolates from children under five years in Mukuru slums, Nairobi, Kenya.

1.5.2 Specific objectives

1) To determine the prevalence of *K. pneumoniae* and the propotion that is ESBL producer from children in Mukuru slums, Nairobi, Kenya.
2) To determine antimicrobial resistance profile of *K.pneumoniae* isolates from children in Mukuru slums,Nairobi,Kenya.
3) To determine the presence of selected ESBL genes in *K. pneumoniae* isolates from children in Mukuru slums, Nairobi, Kenya.
4) To determine association between age,gender,and ESBL *K.pneumoniae* infected children in Mukuru slums,Nairobi,Kenya.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Klebsiella pneumoniae*

Klebsiella pneumoniae belongs in the family *Enterobacteriaceae*, it was named after the German microbiologist Edwin Klebs (1834–1913). The first *Klebsiella* species to be described was a capsulated bacillus from patients with rhinoscleroma. It is described as Gram negative rods shaped, facultative anaerobic, encapsulate, lactose fermenting oxidase negative, catalase positive, and non-motile bacterium (Nibogora, 2020)

It is commonly found in the human digestive tract as part of the natural micro flora but also colonizes nasopharynx and skin, *K. pneumoniae* could cause a various infection syndromes, including urinary tract infection, intra-abdominal infection, skin and soft tissue infection and pneumonia in both community and healthcare associated settings It is the cause of hospital acquired, or nosocomial infections involving the urinary and pulmonary systems, since it is able to adapt to an existence in an oxygenated or deoxygenated environment. For instance, it is thought to account for 3% to 8% of all Nosocomial bacterial infections in United States (Jondle *et al*., 2018). Immunocompromised individuals infected with *K.pneumoniae* usually develop respiratory tract infections such as pneumonia, but blood infections, wound or surgical site infections, and meningitis are also possible. In addition, pyogenic liver abscess has been associated with community-acquired *K. pneumonia* infection (Hsu *et al*., 2013).

2.2 Virulence factors for *Klebsiella pneumoniae*

This pathogen possesses many virulence factors that allow it to go undetected by the host's immune system and cause infection in a variety of ways. The most virulence factor used by the organism is the capsule. The capsule is an extracellular polysaccharide structure which offers protection from immune cells hence sustaining colonization and further infection. Research on capsule reports more than 79 types

studied as of today. It also reports association of disease severity and capsular type (Hsu *et al*., 2013). For instance, pyogenic liver abscess has been associated with strains that produce K1 and K2 capsules and are more prevalent (Qu *et al*., 2015). Lipopolysaccharides are another virulence factor used by *Klebsiella* species to cause diseases to their host. These form the outer coat of the organism and act and antigens that elicit immune reactions. They switch on inflammatory cascades within the host which are responsible for sequel in sepsis and septic shock (Tsereteli *et al*., 2018). The third virulence factor expressed by *Klebsiella* species is the fimbriae. This is mainly used for anchoring the bacteria onto the host cells thus leading into infection. Further, the organisms produce siderophore which allow propagation during infection. Specifically, they are used to acquire iron from the host an important mineral for growth and cell division (Ronning *et al*., 2019). In addition, the bacterium also produces bacteriocins, which are proteinaceous toxins that inhibit the growth of similar or closely related bacterial strains (Yang *et al*., 2014).

2.3 *Klebsiella pneumoniae* burden

Armed with an array of virulence factors, *K. pneumoniae* causes diarrhea, sepsis, urinary tract infections (UTIs) and pneumoniae in humans (Bengoechea & Pessoa, 2019). The most affected individuals include neonates, immunocompromised and the elderly. Diarrhea is a major cause of mortality among children under the age of five years in developing countries. As a matter of fact, it accounted for 0.38 million children deaths reported in 2013 (UNICEF, 2014). Globally, 9% of children deaths reported in 2015 were caused by diarrhea (UNICEF, 2018). In Kenya, 16% of deaths in children aged below five years are due to diarrhea.

In kenya According to Kenya National Bureau of Statistics (2014), diarrhea in children aged below five years is estimated at 10.4%. Yet there is limited data on the prevalence of organisms that cause diarhoea in children. According to (Akhter *et al*., 2016), diarrheal children exhibit higher *Klebsiella* bacteremia than those without diarrhea. *Klebsiella* bacteremia in children have severe outcome including fast breathing, severe

dehydration, hypotension and severe wasting. A study conducted by (Mbuthia *et al*., 2019) reported prevalence of 6.4% in feacal isolates of children aged 49-60 months from central regions of Kenya. Therefore it is necessary to conduct periodic studies that investigate the prevalence of *K. pneumoniae* in children. This will help reduce preventable children deaths from diarrhea since *Klebsiella* diarrhea usually have adverse outcome.

2.4 Prevalence of *Klebsiella pneumoniae* drug resistance

Increasing attention to *K. pneumoniae* is due to increased emergent of resistant strains associated with its diverse infections. More than 30% of the bacteria reported to the European Centre for Disease Prevention and Control(ECDPC) were resistant to at least one antimicrobial group. Combined resistance to fluoroquinolones, third-generation cephalosporins and aminoglycosides being the most common resistance phenotype (EARS-Net, 2018). The other danger is that *Klebsiella* species act as an antibiotic-resistant genes potentially transferring to other Gram-negative bacteria. This leaves narrow treatment options for the diseases it causes hence increasing human suffering,yet there is limited data on the prevalence of drug resistant *K.pneumoniae* in children under five years. The increased prevalence of this resistance further worsened as a mobile pool of virulence and antimicrobial resistance genes have shown to have access to clones of virulence and multidrug-resistant *K. pneumoniae* bacteria (Lam *et al*., 2018). The extent of this problem leaves treatment decisions undefined and probably should rely on minimum inhibitory concentration and patient-specific factors such as site of infection (Calfee, 2017). A survey in U.S analyzing data for 1998 - 2010 reported significant *K. pneumoniae* antimicrobial drug resistance increases for different drugs. Resistance of Imipenem was reported to increase to 4.3%, tigecycline at 2.6%, and aztreonam from 7.7 to 22%, ceftazidine (5.5 to 17.2%) and ciprofloxacin (5.5 to 16.8%). Notably, lower resistance prevalence change was reported for tetracycline and amikacin at 14.2 to 16.7% and 0.7 to 4.5% respectively (Sanchez *et al*., 2013).

In Iran, a study reported high resistance of *K. pneumoniae* against ampicillin, aztreonam and nitrofurantoin at 82.2%, 55.4% and 54.5% respectively (Heidary *et al*., 2018). In Kenya, multidrug resistance of *K. pneumoniae* has been reported to be over 80% for penicillins, Chloromphenical, cephalosporins, lincosamides, tetracyclines, macrolides and sulphonamides. Resistance against carbapenems was lowest at 23.2% while amikacin, meropenem, aminoglycoside and quinolone is reported at 21%, 7%, 49.2% and 41.3% respectively (Ogalo *et al*., 2016). This evidence shows that *K. pneumoniae* resistance is ever changing and thus routinely studies should be conducted to monitor it. Such effort will help keep the current treatments relevant through appropriate choices and doses as well as initiating new drug development.

2.5 Mechanism of drug resistance

Bacteria may be naturally resistant to one type of antimicrobial drugs, or they may develop resistance through de novo mutation or the acquisition of resistance genes from other organisms. Acquired resistance genes may allow a bacterium to manufacture enzymes that degrade antibacterial drugs, to express efflux systems that prevent the medication from reaching its intracellular target, and to modify the antibiotic's properties. (Elufisan *et al.*,2012)

2.5.1 Limiting uptake of drugs

There is a natural variance in bacteria's capacity to inhibit antimicrobial agent absorption. In gram-negative bacteria, the LPS layer's structure and functions operate as a barrier to particular molecules. This confers intrinsic resistance of those bacteria to certain classes of large antibacterial drugs on those bacteria.. (Blair *et al.*, 2015).

2.5.2 Modification of drug targets

Antimicrobial agents can target a variety of components in the bacterial cell, and the bacteria can modify those targets to enable resistance to those drugs. Changes in the structure and/or number of PBPs is one mechanism of resistance to –lactam antibiotics,

which are virtually exclusively employed by gram positive bacteria penicillin-binding proteins. (PBPs) (Beceiro *et al*,. 2013)

2.5.3 Drug inactivation

Bacteria inactivate antimicrobals in one of two ways: by degrading the drug itself or by t ransferring a chemical group to the drug. The lactamases are a wide set of enzymes that hydrolyze drugs. Drug inactivation by transfer of a chemical group to the drug most commonly uses transfer of acetyl, phosphoryl, and adenyl groups. A large number of transferases that have been identified. Acetylation being the most diversely used mechanism, and is known to be used against the aminoglycosides, chloramphenicol, streptogramins and fluoroquinolones. Phosphorylation and adenylation are known to be used primarily against the aminoglycosides. (Blair *et al*.,2015).

2.5.4. β-lactamases

The βlactam antimicrobial agents are the most extensively used class of antibacterial age ntsts. The basic structure of this pharmacological class is a four sided lactam ring. Resistance to -lactam antibiotic occur by three different mechanisms: (1) preventing the interaction between the target PBP and the drug, usually by modifying the ability of the drug to bind to the PBP (this is mediated by alterations to existing PBPs or acquisition of other PBPs; (2) the presence of efflux pumps that can extrude β-lactam drugs; (3) hydrolysis of the drug by β-lactamase enzymes.(Bush *et al*,.2016).

2.5.5 Drug efflux

Bacteria have genes chromosomally encoded for efflux pumps. Some of these genes are expressed constitutively, and others are induced or overexpressed (high-level resistance is usually via a mutation that modifies the transport channel) under certain environmental stimuli or when a suitable substrate is present. The efflux pumps function primarily to rid the bacterial cell of toxic substances, and many of these pumps will

transport a large variety of compounds. The carbon source available influences the resistance capability of these pumps.(Kumar *et al*.,2005).

A bacteria may possess many different types of efflux pumps. There are five main families of efflux pumps in bacteria classified based on structure and energy source: the ATP-binding cassette (ABC) family, the multidrug and toxic compound extrusion (MATE) family, the small multidrug resistance (SMR) family, the major facilitator superfamily (MFS), and the resistance-nodulation-cell division (RND) family. (Kumar *et al*.,2005)

2.5.6 Commonly used antibiotics against *K.pneumoniae* infections

The basics of treatment of infections caused by *K.pneumoniae* depend on the site of infections. Current regimens for community-acquired *K. pneumoniae* pneumonia include a two week treatment with either a third or fourth-generation cephalosporin as monotherapy. A quinolone can be administered as monotherapy or in conjunction with an aminoglycoside. If the patient is penicillin-allergic, then a course of aztreonam or a quinolone should be administered. In the case of nosocomial infections, a carbapenem can be used as monotherapy.(Ashurt *et al.*,2018) When ESBL is diagnosed, carbapenem therapy is given due to its rate of sensitivity across the globe. When carbapenem-resistant Enterobacteriaceae is diagnosed, several antibiotic options to treat CRE include antibiotics from the polymyxin class, tigecycline, fosfomycin, aminoglycosides, or dual therapy carbapenems. Combination therapy of two or more of the agents, may decrease mortality as compared to monotherapy alone.

2.6 Extended spectrum β-lactamases (ESBLs)

ESBLs are enzymes produced by a variety of Gram negative bacteria which confer an increased resistance to commonly used antibiotics. ESBLs are primarily produced by the *Entero bacteriaceae* family of Gram-negative organisms, in particular *K. pneumoniae* and *E. coli* (Shaikh *et al*., 2015). They are also produced by non-fermentative Gram-negative organisms, such as *Acinetobacter baumannii* and *P. aeruginosa* (Leylabadlo *et*

al., 2017). The original method of β-lactamase categorization is the Ambler classification which orders the enzymes into 4 classes (A, B, C, and D) based on molecular structure (Philippon *et al*., 2016).

ESBLs are a worrying global public health issue as infections caused by such enzyme-producing organisms are associated with a higher morbidity and mortality and greater fiscal burden. In Africa, the prevalence of ESBL in *Enterobacteriaceae* has been researched at local levels in various countries, but there is no summarizing research on how prevalent ESBL is on the continent, what type of genes are involved, and where research is missing. In kenya, the scarcity of studies available on children below 5 years warrants caution in drawing conclusions since little information overall is available**,** Wordwide the prevalence of ESBL varies from one region to another. SMART study done between 2009 and 2010 concluded that in Europe, ESBL prevalence among *K. pneumoniae* was 38.9%, in North America, the prevalence was 8.8%. In Asia, the prevalence of ESBL among *K. pneumoniae* varied between 5 and 0%, respectively, in New Zealand 61%. Storberg V. (2014) The increasing prevalence rates worldwide and an ever diminishing supply in the antibiotic armamentarium, these enzymes represent a clear and present danger to public health (Ogbolu *et al.,* 2018). In particular, drug-resistant pathogens are of a major concern, as they carry a higher morbidity and mortality and are more difficult to identify by routine laboratory assays, which can lead to a delay in diagnosis and institution of appropriate antimicrobial therapy. There is also a growing concern regarding the lack of new antibiotics especially for multidrug-resistant Gram-negative bacteria which produce ESBLs (Ogbolu *et al.,* 2018). The β-lactamases are hydrolytic enzymes which cleave the β-lactam ring and are the primary mechanism of conferring bacterial resistance to *β*-lactam antibiotics, such as penicillin and cephalosporin (El-Baky *et al*., 2013). These enzymes can be carried on bacterial chromosomes, that is, inherent to the organism, or may be plasmid-mediated with the potential to move between bacterial populations (Codjoe & Donkor, 2018). This has clear implications regarding spread of infection and infection control and therefore

continuous research is needed so as to understand the ever changing microbial systems and genome.

2.6.1 Types of ESBL

2.6.1.1 TEM beta-lactamase

TEM-1 is the most commonly encountered beta-lactamase in Gram-negative bacteria, most often found in *E. coli* and *K. pneumoniae*. Up to 90% of Ampicillin resistance in *E. coli* is due to the production of *tem-1* (Thenmozhi *et al*., 2014). Also responsible for the Ampicillin and penicillin resistance that is seen in *H. influenzae* and *N. gonorrhoeae* in increasing numbers. The amino acid substitution responsible for the ESBL phenotype clusters around the active site of the enzyme and changes its configuration, allowing access to oxyimino-beta-lactam substrates. Opening the active site to beta-lactam substrates also typically enhances the susceptibility of the enzyme to β-lactamase inhibitors, such as clavulanic acid. Single amino acid substitutions at positions 104, 164, 238, and 240 produce the ESBL phenotype, but ESBLs with the broadest spectrum usually have more than a single amino acid substitution. Based upon different combinations of changes, currently 140 TEM-type enzymes have been described. TEM-10, TEM-12, and TEM-26 are among the most common in the United States (Thenmozhi *et al*., 2014). The most common type of genes in the African strains of ESBL is class A ESBLs. CTX-M-15 is the most prevalent gene in a high proportion of the samples, disregarding country. Storberg V. (2014) The prevelance of these genes among isolates obtained from children under five years in Kenya is yet to be determined.

2.6.1.2 SHV beta-lactamase

SHV-1 shares 68 percent of its amino acids with *tem*-1 and has a similar overall structure. The SHV-1 beta-lactamase is most commonly found in *K. pneumoniae* and is responsible for up to 20% of the plasmid-mediated Ampicillin resistance in this species. ESBLs in this family also have amino acid changes around the active site, most

commonly at positions 238 or 238 and 240. More than 60 SHV varieties are known. SHV-*5* and SHV-12 are among the most common (Thenmozhi *et al*., 2014).

2.6.1.3 CTX-M beta-lactamases

These enzymes were named for their greater activity against Cefotaxime than other oxyimino-beta-lactam substrates. These enzymes are not very closely related to TEM or SHV beta-lactamases, they show only approximately 40% identity with these two commonly isolated beta-lactamases. More than 80 CTX-M enzymes are currently known (Thenmozhi *et al*., 2014).

2.6.1.4 OXA beta-lactamases

OXA beta-lactamases were long recognized as a less common but also plasmid-mediated beta-lactamase variety that could hydrolyze Oxacillin and related penicillins (Lakshmi *et al.,* 2014). These beta-lactamases differ from the TEM and SHV enzymes in that they belong to molecular class D and functional group 2d. The OXA-type beta-lactamases confer resistance to Ampicillins and Cephalothin and are characterized by their high hydrolytic activity against Oxacillin and Cloxacillin and the fact that they are poorly inhibited by clavulanic acid. Amino acid substitutions in OXA enzymes can also give the ESBL phenotype (Lakshmi *et al.,* 2014). Some members of this family confer resistance predominantly to Ceftazidime, but OXA-17 confers greater resistance to Cefotaxime and Cefepime than it does resistance to Ceftazidime (Thenmozhi *et al*., 2014). It is therefore necessary to routinely explore the presence of resistance genes in children so as to apply proper treatment regimens to minimize deaths in such population.

2.6.2 Methods of ESBL detection

Several approaches are used to screen and confirm the presence of Extended Spectrum β–lactamase. These methods can differ between countries and clinical microbiology laboratories. The most common ones are:

2.6.2.1. Double-disk synergy test

On inoculated Mueller-Hinton Agar, disks of third generation cephalosporins and amoxicillin/clavulanic acid (Augmentin) are kept 30 mm apart, centre to centre.
A clear expansion of the cephalosporin inhibition zone towards the augmentin disk is considered positive for ESBL development (Rawat & Nair, 2010)

2.6.2.2 Three-dimensional test

The three-dimensional study provides phenotypic evidence of ESBL-induced inactivation of extended-spectrum cephalosporins or Aztreonam without requiring the use of a -lactamase inhibitor to inactivate the -lactamases. The presence of - lactamase–induced drug inactivation can be seen as a distortion or discontinuity in the usually circular inhibition zone or as the production of discrete colonies in the vicinity of the inoculated slit.

2.6.2.3 E-test

The E-test ESBL strip (AB Biodisk, Solna, Sweden) has two gradients: Ceftazidime on one end and Ceftazidime plus clavulanic acid on the other. The point of intersection of the inhibition ellipse with the E-test strip edge is interpreted as the MIC. The presence of ESBL is indicated by a ratio of Ceftazidime MIC to Ceftazidime-clavulanic acid MIC of 8 or above (Paterson & Bonom, 2005).

2.6.2.4 Molecular detection of ESBLs

Although molecular approaches may detect several ESBL variations, they are labor consuming and expensive to use as standard methods (Bonnet, 2004).
DNA probes, PCR, oligotyping, PCR-RFLPs, and nucleotide sequencing are examples.

2.7 Risk factors Associated with ESBL

2.7.1 Gender

Males have been found to be more likely to develop ESBL Enterobacteriaceae than females. (Shah *et al*., 2002). similarly to Shashwati and Dhanvijay 2014 who found a male preponderance for ESBL production. Different studies have shown conflicting associations between gender and infection due to ESBL-producing Enterobacteriaceae, this may be attributed to the difference in the immune system and other physiological factors between gender. Association between gender with enteric pathogenic bacterial infection among children less than 5 years is an area that requires further research due to various variables attributed such as environmental factors, immunological factors and demographic features. (Mbuthia 2019).

2.7.2 Gastrointestinal colonization with ESBL organism

The colon serves as a reservoir for extra intestinal pathogens and therefore to predict the risk of ESBL infection it is of paramount importance to screen intestinal carriage.(Birgy *et al*., 2012). Intestinal colonization with extended spectrum beta-lactamase producing Enterobacteriaceae makes available a reservoir of bacteria that may cause infections not only to the host but can also be transmitted to others. Such bactria may transmit resistance genes to other bacteria across species. (Weisenberg *et al*.,2012).

2.7.3 Age

Although ESBL significantly varies by age, data suggest that the highest pediatric age risk group is 1 to 5 years (Medernach *et al*,. 2018).Old age is also documented as a risk factor due to the low immunity. Children are protected against bacterial infections by weak maternal antibodies present during early stages of life but their immunity heightens as the age of the child grows. Bacterial infections are minimised as the age of

the child advances. Complementary feeding may also be a risk of exposure to a myriad of ESBL Enterobacteriaceae causing diarrhea. (Mbuthia, 2019).

2.7.4 Treatment with antibiotics

The use of cephalosporins for the treatment of infectious agents has led to the emergence and dissemination of ESBLs producing bacterial pathogens that confer resistance to third generation cephalosporin. (Voets *et al.*, 2012). This could be due to alterations of gastrointestinal flora commensal resulting in selection for antibiotic-resistant organisms. (Chiotos *et al*., 2017). Exposure to antibiotics reduces the drug-susceptible normal flora and enhances the vulnerability of the patient to colonization by resistant organisms. In addition, organisms that are resistant to several drugs are more likely to be selected by the use of one of these antibiotics. (Kuster *et al*,.2010). This phenomena underscores the importance of antibiotic stewardship interventions. (Chiotos *et al*., 2017).Prior antibiotic use, especially of beta-lactams, is a well recognized risk factor for infection with ESBL-producing organisms (Kuster *et al*,.2010).

2.8 Laboratory identification of *K. pneumoniae*

2.8.1 Biotyping

Conventional methods used to detect *K. pneumoniae* are based on the phenotypic characteristics of the microorganism. One of the widely used recgnique is biotyping,the differenciation of strains is based on on differences in biochemicalreaction,morphology and utilization of components in different growth media and carry out certain chemical reactions. Osman *et al*.,(2020). These methods include culture, microscopic examination and biochemical identification. However, these techniques lack specificity, reproducibility and sometimes there is no correlation when comparison is made with commercial methods such as analytical profile index (API) kits (Arenas *et al*., 2009)

2.8.2 Serotyping

Serotyping uses a series of antibodies to detect antigen on the surface of bacteria that show antigen variability. Serotyping is based on the capsule antigens. *Klebsiellae* have well-developed polysaccharide capsules, which give their colonies their characteristic mucoid appearance. Of 82 capsule antigens described, 77 types form the basis for an internationally recognized capsule antigen scheme capsule typing, it shows good reproducibility and is capable of differentiating most clinical isolates. This method has some disadvantages; serological cross-reactions occur among the 77 capsule types, the typing procedure is cumbersome because of the time needed to perform, the test is susceptible to subjective interpretations because of weak reactions that are not always easy to interpret., this technique is practiced mostly in specialized laboratories since anti-capsule antisera are not commercially available. (Osman *et al.,* 2020).

2.8.3 Phage typing

Bacteriophage and bacteriocins is a typing tool where bacteriophage typing classifies bacteria based on the pattern of resistance of susceptibility to a certain set of phages.Bacteriophage are viruses that are able to attach on the cell wall of bacteria,enter,multiply and lyse the cell. Ability of phages to infect cells is based upon the availability of corresponding receptors on the cell surface where the phage can bind. Osman *et al.,* 2020). Although the phage reaction is easily read and the reproducibility of the method is acceptable,this technique shows a poor typing rate of 19 to 67% , it is not commonly used and is useful mainly as a secondary method in combination with serologic testing.

2.8.4 Molecular typing

Molecular techniques are a useful tool for infectious diseases diagnosis; the development of polymerase chain reaction (PCR) based systems have been implemented successfully by their reproducibility, accuracy and specificity for specific recognition of

minimal differences at genotypic level on highly related organisms and to overcome the conventional test limitations, due to their dependence of physiologic and metabolic activity. Arenas *et al*.,(2009)However molecular methods, are not widely used as they are expensive. Plasmid profiles, ribotypes), multilocus enzyme analyses, and applications of pulsed-field gel electrophoresis can be used. The procedures vary from laboratory to laboratory and lack standardization, making it difficult to compare them. (Arenas *et al*., 2009)

2.8.5 Automatic detection methods

Currently, newly developed automatic bacterial identification instruments, such as the VITEK®2 system are also available. The loop-mediated isothermal amplification (LAMP) method is a novel nucleic acid detection method, based on auto cycling strand displacement DNA synthesis using *Bst* DNA polymerase under isothermal conditions within 1hour.This test is rapid, simple, and cost-effective assay that complements current PCR methods. (Dong *et al*., 2015)

2.8.6 Plasmid typing

Plasmid typing is a molecular method used as a bacterial typing tool. Plasmids are self-replicating, often transferable extrachromosomal DNA elements in the prokaryote cytoplasm. Typing if performed through the isolation of plasmid DNA and comparison of the numbers and sizes of the plasmid by gel electrophoresis. (Osman *et al.*, 2020).

2.8.9 Polymerase chain reaction

PCR is a molecular test that permits synthesis of large quantities of targeted nucleic acid sequence. The procedure requires DNA template from the organism being typed, two complimentary oligonucleotide primers designed to flank the sequence on the template DNA to be amplified, and a heat stable DNA polymerase. Primers serve as the starting point for the polymerase to add the bases that make up a strand that is complimentary to the template. Osman *et al.*, (2020) Pulsed-field gel electrophoresis is a tool in which the

approach is to digest chromosomal DNA with restriction enzymes. This results in a series of fragments of different sizes that form different patterns when analyzed by agarose gel electrophoresis. (Osman *et al.*, 2020)

2.9 Prevention and control of K.pneumoniae infections

To prevent the spread of *K.pneumoniae* infections, hand washing should be done with soap and water: before touching eyes, nose, or mouth, before preparing food, before addressing bandaged or wound areas, after using the restroom, and after using the restroom. WHO 2001. Attempts to control the spread of ESBL producing *K. pneumoniae* have concentrated on antibiotic restriction. Restriction of third generation cephalosporins or even cephalosporins as a class has been successfully implemented as a control strategy. Paterson *et al.*, 2014. Replacement of extended-spectrum cephalosporins with piperacillin/tazobactam as empiric treatment has been reccomended. Contact isolation precaution measures should be practiced as a mode of control of spread of ESBL producing *Klebsiella*. Asymptomatic carriers and those colonized of the organism should be isolated. Paterson *et al.*, 2014. Those who enter the room of a patient colonized with an ESBL producing organism should wear gloves and gowns and practice appropriate hand hygiene on leaving the patient's room and removal of the protective apparel. This will help to stop outbreaks of infection and in reducing new infections in ESBL endemic areas. Paterson *et al.*, 2014.

2.10 mechanism of pathogenesis of K. pneumoniae

K. pneumoniae employs several mechanism as Immune evasion strategies.This is shown in table 2.1 below

Table 2.1: mechanism of pathogenesis of *K. pneumoniae*

Immune evasion strategies	Mechanism	Bacterial factor
Preventing the antimicrobial action of soluble innate immune effectors		
Preventing complement bactericidal effect, and opsonisation	Limiting C3b deposition	CPS, LPS O-polysaccharide
Limiting antimicrobial activity of collectins	Blunting interaction with SP-A and SP-D	CPS
Counteracting bactericidal action CAMPs and polymyxins	Limiting the interaction with the bacterial surface. Efflux of CAMPs.	CPS, LPS lipid A decorations,
Attenuating the interaction with immune cells		
Attenuating engulfment by epithelial cells		CPS
Avoiding phagocytosis by neutrophils		CPS
Avoiding phagocytosis by macrophages		CPS, LPS lipid A decorations,
Limiting the activation of PPRs	Limiting the recognition of LPS by TLR4	LPS lipid A 2-hydroxylation
Controlling maturation dendritic cells		CPS, LPS O-polysaccharide
Manipulation phagosome maturation	Activation PI3K-AKT-Rab14 axis	Unknown
Controlling cell death	Cytotoxicity in epithelial cells. Triggering apoptosis in macrophages.	CPS Unknown
Abrogating TLR-controlled inflammatory responses:		
Abolishing TLR signalling		CPS, LPS O-polysaccharide,

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

The study was carried out at Mukuru city council clinic within Mukuru Kwa Njenga slums. Mukuru slum is located on the East of Nairobi in Embakasi constituency. It stretches along the Nairobi Ngong River, situated on waste lands in the industrial area of the city between the Outer Ring Road and the North Airport Road and Mombasa road. It is referred to as the 'industrial slum' because of its proximity to manufacturing facilities.

Figure 3.1: Location of Mukuru slums in Nairobi County

3.2 Study Design

This was a cross-sectional study design that was carried out in Mukuru slums Nairobi, Kenya that analyzed data from the population under study, to assess prevalence of *K. pneumoniae* among children in Mukuru slums and the antimicrobial resistant patterns in *K. pneumonia* isolates and ESBL gene present in children from Mukuru Slums.

3.3 Study Population

The target population included children below 5 years presenting with diarhoea, attending Mukuru clinics and living in Mukuru slums.

3.4 Inclusion and Exclusion criteria

3.4.1 Inclusion criteria

1. Children of 5 years and below who had diarrhea, seeking treatment at outpatient clinics in Mukuru slum.

2. Children whose consent is given by the guardian.

3.Children living in Mukuru slums

3.4.2 Exclusion criteria

1. Children who attended clinic but did not live in Mukuru slums.

2. Children below 5 years, who did not present with diarrhea but seeking treatment at the outpatient clinics in Mukuru slums

3.Children whose consent not given

3.5 Sample Size determination

The sample size was determined using Fishers *et al.* (2002) formulae:

$$n = \frac{Z^2 P (1-P)}{D^2}$$

Where;

n: The desired sample size

Z = the standard normal deviation at the required confidence level. i.e. 1.96 of the new distribution to the significance level [0.05]

D = Normal distribution significance level [0.05]

P = the prevalence of estimated population. [31.4%] (Maina *et al.*, 2013)

N [sample size] =

$$\frac{(1.96)^2(0.314)(1-0.314)}{0.05^2} = 330 \text{ samples}$$

3.6 Sampling procedure

Simple Random sampling was done where a group of children were selected for study from a larger group (a population). Each child was chosen entirely by chance and each child of the population had an equal chance of being included in the sample. The sampling unit was selected using random numbers that were computer generated until the required sample size was attained. In MCC clinic the proportion of male was 24.2% while female 25.7%. Whereas in MMM Clinic, the proportion of females was 27.2%,

males 21.2%.The total number of females was 51.1% while males were 47%. Data regarding socio-demographic factors was collected using a structured questionnaire.

3.7 Ethical consideration and recruitment of participants

This study involving human samples was approved by Kenyatta University Ethical Review Committee. All approved procedures conformed to the required standards (Appendix I). Enrollment to the study was on voluntary basis whereby informed consent was sought from parents/guardians of the participants and information collected kept confidential. There were no monetary gains for those who participated in the study and there were no penalties for those who declined participation**.**

3.8 Specimen collection

3.8.1 Stool collection

One Stool sample was collected per child by the help of parents/guardian in a sterile clean wide mouthed dry leak proof polypot with a tight fitting lid, following instruction from the principal investigator to collect a peas size amount of stool taking care not to contaminate it with urine. The stool sample was transferred using wooden applicator stick. The stool sample was labeled with a unique identifier rather than the name of the participant.

3.9 Microbiological analysis

3.9.1 Plate culture

Stool samples were macroscopically examined for colour and consistency then followed by microbiological analysis. A pea-sized stool sample was transferred into 9 ml Selenite F broth, and incubated overnight at 37°C. A loopful from each of the enrichment medium was then cultured onto MacConkey agar, for the detection of lactose fermentaters. Plating was also done on Xylose Lysine Deoxycholate (XLD) agar and

the plates were incubated for 24 hours at 37°C then a subculture done. Gram staining was carried out for pure isolates and biochemical tests performed for further identification of the isolates. Results obtained from cultural and biochemical reactions were used for identification of strains to species level as previously described (Cheesebrough, 2005) Appendix 111

3.9.2 Antibiotic susceptibility test

Antibiotic susceptibility testing was performed on the isolates using the Kirby – Bauer disc diffusion technique. Zones of inhibitions were measured and the interpretation of results was done according to Clinical Laboratory Standard Institute (CLSI) 2015, guidelines.

Antimicrobial susceptibility testing was done on Mueller-Hinton agar plates (Oxoid). In the first plate, the following antibiotics were used: Ceftazidime (CAZ, 30µg), Cefotaxime (CTX, 30µg), Aztreonam (ATM, 30µg), Cefoxitin (FOX, 30µg) and Ceftriaxone (CRO 30µg) Cefpodoxime (CPD10µg). In order to detect the zone of synergy for ESBL-producers, Amoxicillin-Clavulanic acid disk (AMC, 30µg) was placed at the Centre of this plate at a distance of 15-20 mm from each of the β-lactam antimicrobials. In the second plate, the following antibiotics were used: Tetracycline (TE, 30µg), Sulfamethoxozole/ trimethoprim (SXT, 23.75µg/1.25µg), Gentamicin (CN, 10µg) Ciprofloxacin (CIP, 5µg) Ampicillin (AMP, 10µg), Imipenem (IPM, 10µg) and Nalidixic acid (NA, 10µg). The plates were incubated at 37^0C for 18–24 hours. The innoclum for susceptibility testing were compared against the McFarland 0.5 turbidity standards. *K. pneumoniae* K6 ATCC 700603 (ESBL producer) and *Escherichia coli* ATCC 25922 (non-ESBL producer) served as the positive and negative controls respectively. The interpretation of inhibition zone diameters (potency) results into susceptible, intermediate or resistant was done as per the Clinical and Laboratory Standards Institute guidelines (CLSI, 2015) as seen in (Appendix V).

3.9.3 Detection and confirmation of ESBLs using disc diffusion test

Genotypic characterization was done on *K. pneumoniae* isolates that were resistant to cephalosporins and tested for ESBLs production using the double disk synergy test following the CLSI (2015) guidelines. Only isolates showing synergy zones between amoxicillin/clavulanic and one or more third generation cephalosporins were identified as ESBL-producers (Juma, 2017). Enlargement or distortion of the inhibition zones to form a keyhole appearance/ghost inhibition zone between the cephalosporins discs and the Amoxicillin/Clavulanate disc was interpreted as an ESBL enzyme production phenotype.

3.9.4 Detection of ESBL gene by PCR

ESBL positive phenotypes were subjected for PCR for determination of R- genes. ESBL-producing strains were analyzed by PCR to detect the presence of four beta lactamase ^{bla}SHV, ^{bla}TEM, ^{bla} CTX-M and ^{bla}OXA was performed. The PCR cycling parameters were as follows: an initial denaturation at 94°C for 5 min; followed by 35 cycles at 94°C for 30 s, annealing depended on the primer temperature was done for between 30 seconds and 1 min, before a short extension step at 72°C for 1 min. A final extension temperature was set for 72°C for between 10 min for short fragments and 20min for longer fragments.

3.9.5 DNA extraction and amplification

DNA extraction was done using the boiling method. The heating block was set at a temperature of 95^0C, molecular grade water was added to 2ml eppendorf tube marking each tube with a number corresponding with isolate to be analyzed .A pea size amount of innoclum was scraped using a sterile swab and transferred to corresponding tube and the inoculum heated for a maximum of 12 minutes in a heating block before centrifuged at 26000 RPM for 5 minutes. The tubes were removed without shaking the pellet. The contents were stored without separating pellet from supernatant at -20^0C until use. The

supernatant served as a template for PCR. The amplified PCR products were subjected to electrophoresis.

Table 3.1: Nucleotide sequence for Primers used to analyze ESBL gene

Gene	Primer sequence	Amplicon size	Annealing temperature	Reference
SHV	F:5´-TCAGCGAAAAACACCTTG -3´ R:5´-CCCGCAGATAAATCACCA -3´	498 bp	60	Zhang *et al* 2017
TEM	F:5´GAGTATTCAACATTTCCGTG TC -3´ R:5´TAATCAGTGAGGCACCTATC TC -3´	861bp	52	Zhang *et al* 2017
CTX-M	F:5'TTTGCGATGTGCAGTACCAG T AA-3' R:5'CGATATCGTTGGTGGTGCCA T A-3'	544bp	60	Onyango *et al* 2018
OXA	F5’ATGAAAAACACAATACATAT-3’ R5’GTGTGTTTAGAATGGTGATC GCATT 3’	820	62	Juma *et al* 2017.

F-
5’ATGAAAAACACA
ATACATATCAACT
TCGC3’
R-
5’GTGTGTTTAGAA
TGGTGATCGCATT
3’

Table 3.1 show nucleotide sequences of PCR primers used to amplify ESBL genes with their respective expected band sizes (base pairs) and annealing temperature; bp pairs; F-forward primer; R-reverse primer.

3.9.6 PCR amplification

Amplification reactions was performed in a volume of 25 µL containing Taq DNA polymerase Master Mix with standard buffer 12.5µl, template DNA 2.5 µl, 10 µM forward primer 0.5 µl, 10 µM reverse primer 0.5 µl and nuclease-free water to 25µl. Working solution was prepared from the stock solution with sterile nuclease free water as instructed by manufacturer. Then for 10µM, I dissolved 10µl of each primer in 90µl of nuclease free water. The thermocycling conditions was : *bla shv* at 94^0C for 5 minutes followed by 30 cycles at 94^0C for 30 seconds, 68^0C for 60 seconds, and 72^0C for 60 seconds, with a final extension of 72^0C for 10 minutes; *bla tem* at 94^0C for 2 minutes followed by 30 cycles of 94^0C for 1 minute, 58°C for 1 minute and 72^0C for 1 minute with a final extension at 72^0C to 7 minutes and *bla ctx-m* at 94^0C for 2 minutes followed by 30 cycles of 95^0C for 20 seconds, 51^0C for 30 seconds, 72^0C for 30 seconds with a final extension at 72^0C to 3 minutes .

3.9.7 Electrophoresis analysis

The amplified PCR products were then separated by electrophoresis on a 1.2% agarose gel (AmpliSize; Bio-Rad Laboratories) stained with ethidium bromide in TBE buffer at 100V for ninety minutes. The DNA gel was then visualized by UV transilluminator (Hedge *et al*., 2012) and photographed under ultraviolet light using an instant Polaroid camera. Molecular size marker (DNA ladder; Promega, Madison, Wisconsin, USA) was included in each agarose gel run. A 1kb DNA ladder was used as a standard and visualized under the UV transillumination.

3.9.8 Biosafety issues

During collection, transportation and disposal, the biological specimen was handled using personal protective equipment following Disposal of biological waste was done following KEMRI biosafety guidelines. The bacterial isolates were stored at -40^0C.

3.9.9 Data management and analysis

Data was kept confidential by use of unique identifiers and not participants names in a password protected database and interpreted in form of tables and charts. The data on socio-demographic factors obtained by a questionnaire and test samples were recorded in Microsoft Excel. Data was checked for consistency before analysis. A Chi-square statistical test was used to analyze bivariate data and results presented in tabular and graphical formats. $P \leq 0.05$ were considered to be statistically significant. Odds ratio were generated with 95% confidence interval for the associations between variables and *K. pneumoniae* ESBL by Binary logistic regression analysis. An alpha of less than 0.05 ($P<0.05$) was considered statistically significant. All results were interpreted at 95% confidence interval. The antibacterial susceptibility was reported as sensitive or resistant and was presented in form of tabular and graphical formats. ESBL prevalence was calculated from ESBL positive samples.

3.9.10 Dissemination of findings

Findings from this study was published in a peer-reviewed journal, Advances in Microbiology journal. Findings were also presented at the department of Medical Microbiology and at the School of Biomedical Science, College of Health Science.

CHAPTER FOUR

RESULTS

4.1 Selected demographic characteristics of study participants

Selected socio-demographic profiles of the participants obtained by a questionnair was analyzed to determine the general characteristics of study participants.

4.1.1 Gender

There was disparity in the proportion of males 143(43.33) to females 187(56.67).Out of this the positive cases constituted of 40(12.1) males and 56(16.9) females whereas the negative cases in the proportion of males was 103(31.2) and females was 131(39.6). The total number of positive and negative cases was 96(29.0) and 234(70.9) respectively as shown in table 4.1 below.

Table 4.1: Gender distribution

Gender	Male (%)	Female (%)	Total (n)
Negative cases	103(31.2)	131(39.6)	234(70.9%)
Positive cases	40(12.1)	56(16.9)	96(29.0%)
Total (%)	143(43.3)	187(56.6)	330(100%)

4.1.2 Age

The participant's age was categorized into months.Six months and below, seven to twelve months,thirteen to twenty four months, twenty five to thirty six months,thirty seven to forty eight months, and forty nine to sixty months In terms of age, majority of the participants 85(26.06) were between 49-60 months. Seventy three 73(22.12) of the participants were between the age 13-24 and 25-36months, 63(16.06) were 37-48 months while 42(12.73) were 7-12 months and 14(4.24) 0-6 months. This is shown in the table 4.2 below.

Table 4.2: Age distribution among children in Mukuru slum

Age and number of participants (%)							Total(%)
Sex	0-6 months	7-12 months	13-24 months	25-36 months	37-48 months	49-60 months	
Males	11(78.5)	20(47.62)	39(53.42)	23(36.51)	18(34.62)	32(37.65)	143(43.3)
Females	3(21.43)	22(52.38)	34(46.58)	40(63.49)	34(65.38)	53(62.35)	187(56.7)
Total	14(100)	42(100)	73(100)	63(100)	52(100)	85(100)	330(100)

4.2 Prevalence of *Klebsiella pneumoniae* among children in Mukuru slum

Klebsiella pneumoniae positive cases were 29.0 % (96) while *Klebsiella pneumoniae* negative cases were 70.9% (234) from 330 participants that enrolled into the study. This is shown in the figure 4.1 below.

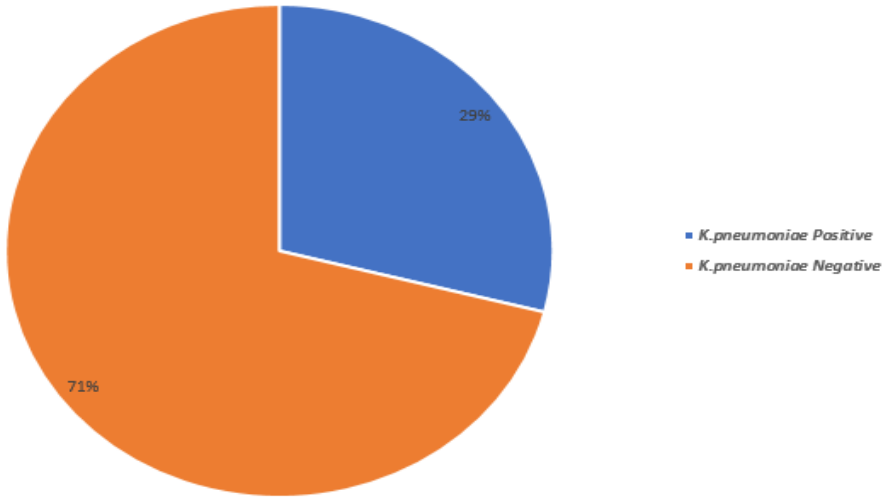

Figure 4.1: prevalence of *Klebsiella pneumoniae* among children in Mukuru slums

4.2.1 Prevalence of *Klebsiella pneumoniae* based on gender

In terms of prevalence of *Klebsiella pneumoniae* among gender, females accounted for 58.3% (56) of study participants while males were 41.6% (40). Using females as the reference group, males have an elevated odds but not significant. P>0.05 as shown in table 4.3 below and appendix vi.

Table 4.3: Prevalence of *Klebsiella pneumoniae* among gender

Gender	N=96	Odds Ratio	95%CI	P Value
Male	40(41.6%)	0.90	0.56 – 1.46	0.696
Female	56(58.3%)	0.42	0.31 – 0.58	Ref

4.2.2 Prevalence of *Klebsiella pneumoniae* based on age

For analysis the study participants were conveniently grouped into age categories: A high prevalence of *Klebsiella pneumoniae* 35.4% (34) was recorded among participants aged 49-60 months, followed by 13-24 months 17.7% (17), 25-36 months16.6% (16), 37-48 months14.5% (14) 7-12months 10.4% (10) and 0-6months recording the least prevalence of 5.2% (5).

With those above 48 months as a reference group, the odds of age group 13-24 months was significantly elevated p< 0.05. On the other hand the Odds are elevated for all other age groups but not significant as shown in the table 4.4 below and also in appendix vi.

Table 4.4: Prevalence of *Klebsiella pneumoniae* based on age

Age(Months)	n=96	Odds ratio	95 %CI	P value
0-6	5(5.2)	0.84	0.26-2.75	0.786
7-12	10(10.4)	0.47	0.20-1.09	0.082
13-24	17(17.7)	0.46	0.23-0.92	0.030
25-36	16(16.6)	0.53	0.26-1.08	0.803
37-48	14(14.5)	0.54	0.25-1.16	0.116
49-60	34(35.4)	0.65	0.42-1.00	Ref

4.3 Proportion of *K.pneumoniae* isolates that were ESBL producers in Mukuru slums

It was notable that among the 96 *Klebsiella pneumoniae* isolates, 42 were ESBL positive recording a prevalence of 44% while the ESBL non producers were 56% as shown in figure 4.2 below.

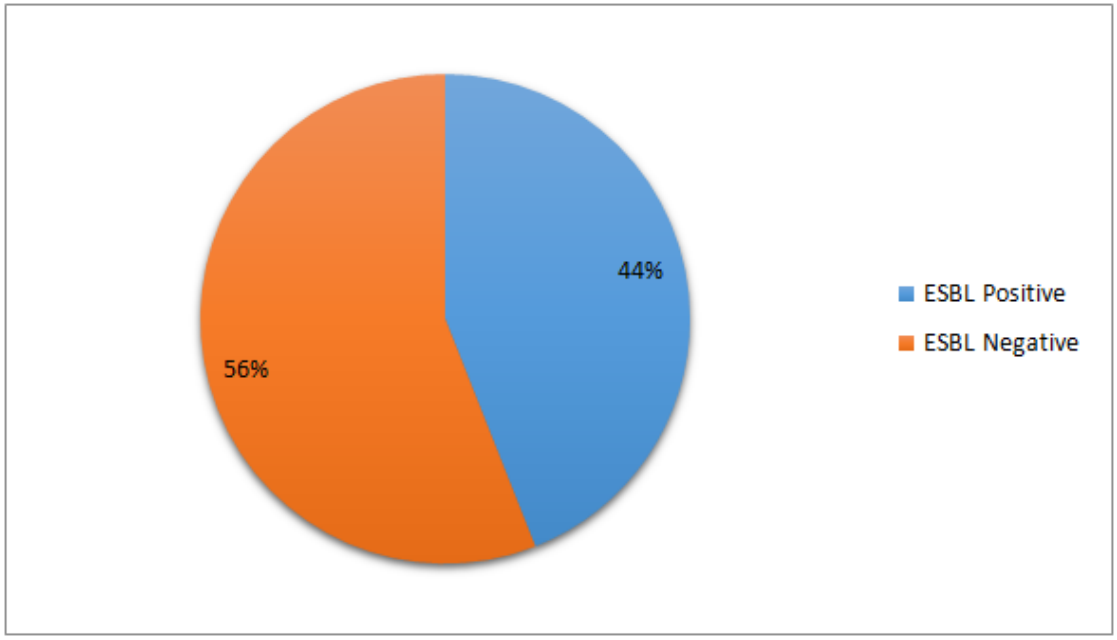

Figure 4.2: Proportion of ESBL producers among children in Mukuru slums

4.4 Overall antimicrobial resistance pattern among children in Mukuru slum

Following susceptibility testing of the *Klebsiella pneumoniae* isolates, recovered from children, high resistances were recorded for Ampicillin (AMP) 97.9% (94), Cefotaxime (CTX), Aztreonam (ATM) and Cefpodoxime (CPD) recorded an equal resistance of 68.8% (66) while Ceftazidime(CAZ) and Ceftriaxone (CRO) recorded 67.7% (65). Ciprofloxacin (CIP) 66.7% (64) ,Sulfamethoxozole (SXT) 65.6% (63),Gentamycin (CN) 60.4% (58), Amoxicillin-Clavulanic (AMC) 43.8% (42),Tetracycline (TE) 55.2% (53), Fox 41.7% (40) Nalidixic acid (NA) 32.3 % (31). It was notable that none of the isolates exhibited resistance to Imipenem (IMP), a Carbapenem. Figure 4.3 below and appendix V11.

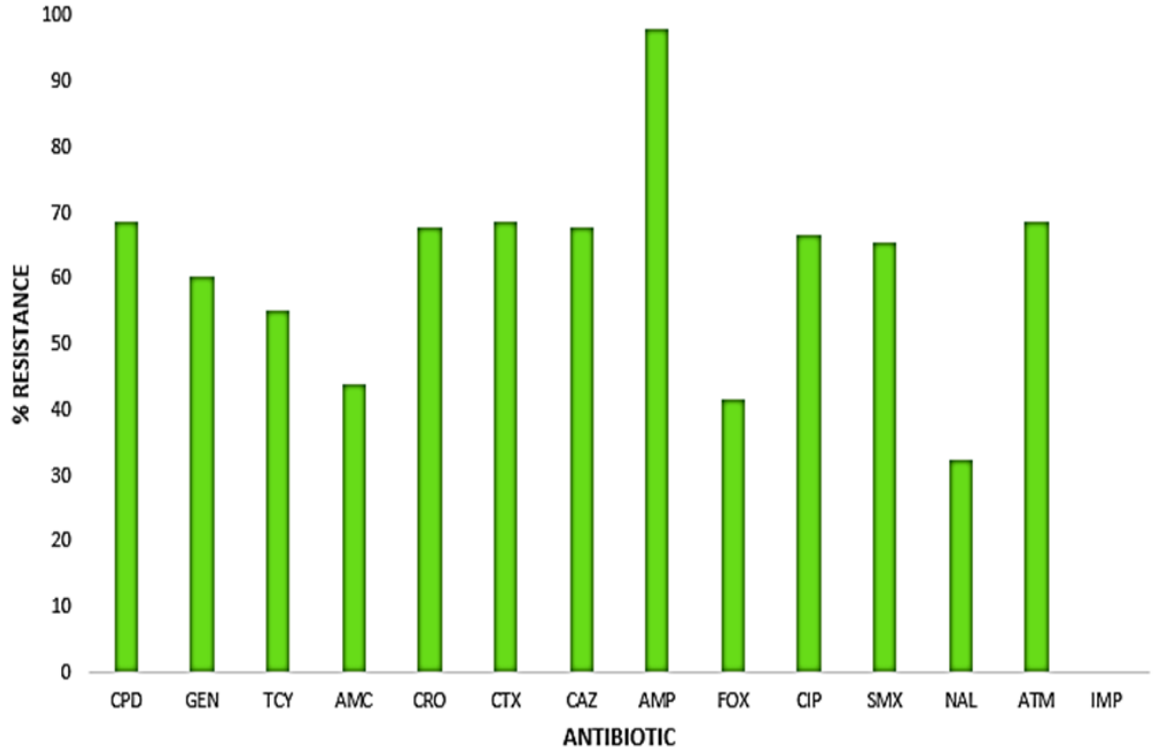

Figure 4.3: overall resistance pattern among children in mukuru slum

AMP- Ampicillin, CTX- Cefotaxime, ATM- Aztreonam CPD- Cefpodoxime, CAZ- Ceftazidime, CRO Ceftriaxone, CIP- Ciprofloxacin SXT- Sulfamethoxozole, CN- Gentamycin, AMC-Amoxicillin-Clavulanic, TE- Tetracycline, Fox-cefoxitin, NA- Nalidixic acid

4.4 Resistance pattern based on gender of children in Mukuru slum

At least 60% of isolates from both sexes were resistant to AMP, CPD, CRO, CTX, CAZ, CIP, SMX and ATM and CPD CRO CTX CAZ AMP CIP SMX ATM GEN TET and AMC. Low resistance < 40%) was recorded for Nalidixic acid and FOX. Resistance of 0.0% was recorded in Imipenem.

The study sought to determine the association between drug resistance and gender (male and female). This was achieved through cross tabulation and chi-square test where a p-value were calculated. A significant value ($p < 0.05$) was taken to indicate statistical

significance between a drug resistances with gender. Generally, there was no significant differences observed between the two genders in terms of resistance. The results are presented in table 4.5 and appendix viii.

Table 4.5: Resistance pattern by gender among children in Mukuru slum

ANTIBIOTIC	Gender	Sensitive (%)	Resistant (%)	p-value
CPD	Male	14 (29.8)	33 (70.2)	0.762
	Female	16 (32.7)	33 (67.3)	
GEN	Male	20 (29.8)	27 (57.4)	0.306
	Female	18 (32.7)	31 (63.3)	
TCY	Male	21 (29.8)	26 (55.3)	0.542
	Female	22 (22.4)	27 (55.1)	
AMC	Male	28 (59.5)	19 (40.4)	0.185
	Female	26 (53)	23(46.9)	
CRO	Male	14 (25.5)	33 (70.2)	0.242
	Female	17 (34.7)	32 (65.3)	
CTX	Male	14 (29.8)	33 (70.2)	0.609
	Female	16 (30.6)	33 (67.3)	
CAZ	Male	14 (29.8)	33 (70.2)	0.710
	Female	16 (33.3)	32 (66.7)	
AMP	Male	1(2.1)	46 (97.9)	0.976
	Female	1 (2.0)	48 (98.0)	
IPM	Male	47 (100.0)	0 (0.0)	0.138
	Female	49 (93.9)	0 (0.0)	
FOX	Male	21 (37.2)	22 (51.2)	0.624
	Female	24 (47.6)	18 (42.9)	
CIP	Male	16 (12.8)	31 (66.0)	0.768
	Female	16 (16.3)	33 (67.3)	
SMX	Male	15 (27.7)	32 (68.1)	0.249
	Female	18 (36.7)	31 (63.3)	
NAL	Male	28 (40.4)	19 (40.4)	0.165
	Female	37 (42.9)	12 (24.5)	
ATM	Male	15 (27.7)	32 (68.1)	0.339
	Female	15 (30.6)	34 (69.4)	

4.3.1 Resistance pattern based on age of children in Mukuru slums

The ages of study participants were grouped into categories of 0-6 months, 7-12 months, 13-24 months, 25-36 months, 37-40months and 41-60 months for easy analysis of resistant pattern. It was notable that none of the isolates recorded resistance to Imipenem. Age 7-12 recorded resistance to most of the antibiotics while age 37-48 recorded lowest resistance to most of the antibiotics. This trend is similar even to the 3rd generation cephalosporins. Generally, there was no significant differences observed between age and resistance. Table 4.6 below and Appendix ix.

Table 4.6: Resistance pattern by age among children in Mukuru slum

ANTIBIOTIC	Sensitivity	Age in Months						*p* value
		Number of isolates (%)						
		0-6	**7-12**	**13-24**	**25-36**	**37-48**	**49-60**	
AMC	Sensitive	8(3.1)	9(2.1)	10(6.3)	7(4.2)	7(6.3)	11(9.4)	0.199
	Resistant	9(9.4)	9(9.4)	6(6.3)	5(5.2)	4(4.2)	11(11.5)	
CRO	Sensitive	3(3.1)	2(1.0)	6(6.3)	5(5.2)	6(6.3)	9(9.4)	0.199
	Resistant	14(14.6)	16(16.7)	10(10.4)	7(7.3)	5(5.2)	13(13.5)	
CTX	Sensitive	2(2.1)	2(2.1)	6(6.3)	5(5.2)	5(5.2)	9(9.4)	0.199
	Resistant	15(15.6)	16(16.7)	10(10.4)	7(7.3)	6(6.3)	13(13.5)	
CAZ	Sensitive	4(3.1)	2(2.1)	6(6.3)	5(5.2)	5(5.2)	9(9.4)	0.199
	Resistant	13(13.5)	16(16.7)	10(10.4)	7(7.3)	6(6.3)	13(13.5)	
SXT	Sensitive	3(3.1)	4(3.1)	6(6.3)	6(6.3)	6(5.2)	8(8.3)	0.199
	Resistant	14(14.6)	14(14.6)	10(10.4)	6(6.3)	5(5.2)	14(14.6)	
ATM	Sensitive	5(3.1)	1(1.0)	5(5.2)	5(5.2)	5(5.2)	9(9.4)	0.199
	Resistant	12(12.5)	17(17.7)	11(11.5)	7(7.3)	6(6.3)	13(13.5)	
AMP	Sensitive	0(0.0)	0(0.0)	1(1.0)	1(1.0)	0(0.0)	0(0.0)	0.223
	Resistant	17(17.7)	18(18.8)	15(15.6)	11(11.5)	11(11.5)	22(22.9)	
IPM	Sensitive	17(17.7)	17(16.7)	16(15.6)	12(12.5)	11(11.5)	22(22.9)	0.223
	Resistant	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	
FOX	Sensitive	16(4.2)	12(2.1)	10(8.3)	9(7.3)	10(6.3)	19(9.4)	0.199
	Resistant	7(7.3)	11(11.5)	7(7.3)	4(4.2)	4(4.2)	8(8.3)	
CIP	Sensitive	13(3.1)	8(3.1)	9(5.2)	10(5.2)	8(6.3)	18(10.4)	0.199
	Resistant	4(4.2)	10(10.4)	7(7.3)	2(2.1)	3(3.1)	4(4.2)	
GEN	Sensitive	4(4.2)	6(3.1)	8(5.2)	5(5.2)	7(5.2)	9(8.3)	0.199
	Resistant	13(13.5)	12(12.5)	8(8.3)	7(7.3)	4(4.2)	13(13.5)	

4.6 Occurrence of selected ESBL genes among children in Mukuru slums

The most prevalent β-lactamase gene in all isolates was *bla*CTX-M42/42 (100%) followed *bla*TEM (97.6%) followed by *bla*SHV, accounting for 90.4%. In all the isolates analysed *bla*OXA was not found to encode any of the isolates. In this study, co-existence of multiple *bla* genes in the same isolate was observed in the following combinations; *bla*CTX-M/TEM, *bla*CTX-M/SHV, and *bla*CTX-M/TEM/SHV. (plates 4.1, 4.2 and 4.3).

The triple combination accounted for 88% while the double combination; *bla*CTX-M/TEM, *bla*CTX-M/SHV, accounted for 9.5% and 2.4% respectively as shown in table 4.7 below.

Table 4.7: Frequency of selected ESBL genes among children in Mukuru slums

Gene n=42	N	%
CTXM	42	100%
TEM	41	97.6%
SHV	38	90.4%
OXA	0	0.00%
CTXM/SHV/TEM	37	88%
CTXM/TEM	4	9.5%
CTXM/SHV	1	2.4%
TEM/SHV	0	0%

Gel electrophoresis was used to identify the bands. The plates below are representative electrophoresis gel images. In plate 4.1,L is the ladder, Lane 1 to 8 are positive isolates, followed by a positive control and a negative control.

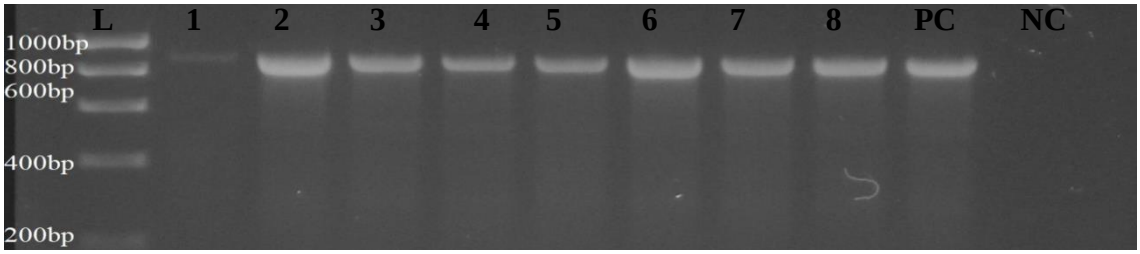

plate 4.1: Electrophoresis gel for blaTEM (865 bp); L-Molecular Ladder; NC Negative Control; PC-Positive Control; bp-base pairs

In plate 4.2, L is the ladder, Lane 1 to 4 are positive isolates, then a positive control and a negative control**.**

Numbers at the top represent random DNA numbers of the isolates

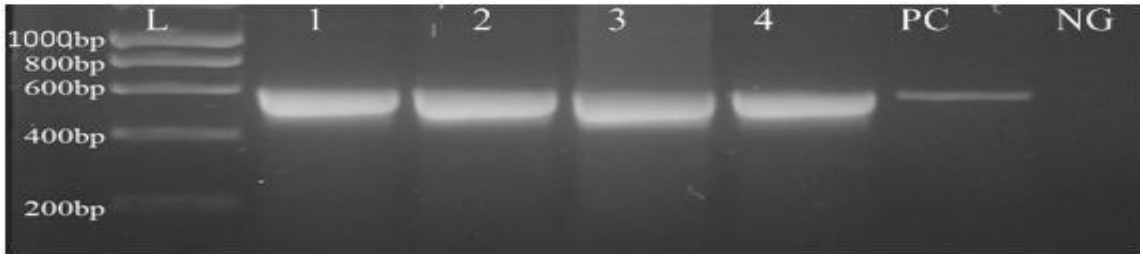

plate 4.2: Electrophoresis gel for *bla*CTX-M (593 bp); L-Molecular Ladder; NC Negative Control; PC-Positive Control; bp-base pairs

Numbers at the top represent random DNA numbers of the isolates

In plate 4.3, L is the ladder, Lane 1 to 24 are positive isolates, followed by a positive control and a negative control**.**

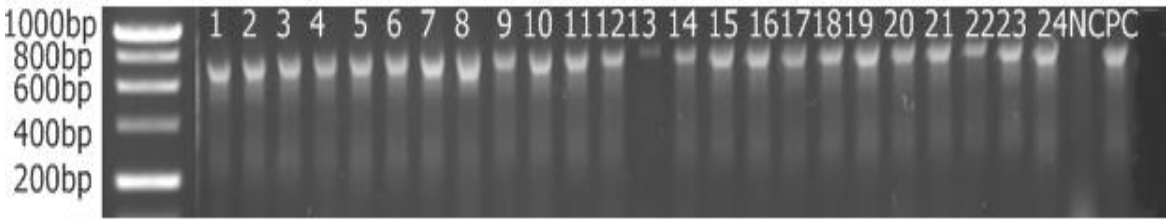

Plate 4.3: Electrophoresis gel for *bla*SHV (747 bp); L-Molecular Ladder; NC Negative Control; PC-Positive Control; bp-base pairs

Numbers at the top represent random DNA numbers of the isolates

4.6.1 Frequency of ESBL genes in relation to gender

Occurrence of genes was compared based on gender. A combination of TEM/CTXM/SHV was more frequent in females 20(47.6%) than males 17(40.5%).TEM/CTXM was also more frequent in females than males. (Table 4.8 below) .Using females as a reference group, males had a significantly elevated odds hence a significance statistical difference between the occurrence of genes and males $P<0.05$ as shown in appendix ix.

Table 4.8: Frequency of genes in relation to gender

Gene occurrence n=42	No of females (%)	No. of males
TEM&CTXM&SHV	20(47.6%)	17(40.5%)
TEM&SHV	0(0%)	1(2.4%)
TEM&CTXM	3(7.1%)	1(2.4%)
CTXM&SHV	0(0%)	1(2.4%)

4.6.2 Occurrence of ESBL genes in relation to age among children in Mukuru Slum

Occurrence of genes was present was irrespective of the age. The most frequent combination of *bla*TEM/CTXM/SHV occurred in all age groups but was high in age 49-60 months and low in age group 25- 36 months. It is notable that *bla*CTXM/SHV combination was found to occur only in age group 0-6 months.Whoever, *bla*TEM/CTXM occurred in age groups 7-12 and 25-36. Table 4.9 below.

With those above 48 months as a reference group, the odds are elevated in all age groups but not significant p>0.05.(Appendix x).

Table 4.9: Occurrence of genes in relation to age in months

Genes present	0-6 months	7-12 months	13-24 months	25-36 months	37-48 months	49-60 months
TEM&CTXM&SHV	6(14.3%)	7(16.7%)	6(14.3%)	3(7.1%)	4(9.5%)	11(26.1%)
TEM&SHV	1(2.4%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
TEM&CTXM	0(0%)	2(4.8%)	0(0%)	1(2.4%)	0(0%)	0(0%)
CTXM&SHV	1(2.4%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Total	8	9	6	4	4	11

CHAPTER FIVE

DISCUSSION CONCLUSION AND RECOMMENDATION

5.1 Social demographics of study participants

Demographics of those surveyed indicated that there was imparity in the numbers of participants based on gender with males and females accounting for 143(43.33) and 187(56.67) respectively. This study is in agreement with a study by Ogallo *et al*.,(2016). at MTRH in western Kenya where majority of participants were females 64.8%. However this study contradicts a study in Dar es Salaam by Tellevik *et al*.,(2016) that shows majority of children colonized were males 365 and females 242 out of 603 children enrolled into the study.

A large proportion (33%)) of the participant recruited in this study was between 49-60 Months of age. However the minority of the study participants were in the age group of 0-6 months (5%) followed by 7-12months (10%). This contradicts a study done in Tanzania Tellevik *et al*.,(2016) where the majority were children were less than 12 months. A similar study by Ogallo *et al.*,(2016) found that the majority of children colonized were newborn, 205 out of 281.This high acquisition rate could be due to hospital exposure and contact with hospital personnel.

5.2 Prevalence of *Klebsiella pneumoniae*

The current study found out that the prevalence of *Klebsiella pneumoniae* among children was 29%. This high prevalence may be due to the emergence of hyper virulent or antibiotic resistant strain. Furthermore this bacteria resides in the environment including surface water and the soil. This finding is in agreement with several previous studies which have demonstrated high *Klebsiella* infections in children. A study conducted by Nielsen *et al.* (2012) in Ghana reported a prevalence of 26% in children. Ogalo *et al*., (2016) has reported a prevalence of 23% in a tertiary teaching hospital in western Kenya. Two more studies reported prevalence of 13% and 26% (Nielsen *et al*.,

2012; Falade *et al*., 2009) respectively in Bangladesh in children aged <5 years. In addition, according to an International Vaccine Access Center (IVAC) report (2015), 25% of deaths in <5 years children were caused by diarrhea and pneumonia in Angola, Chad, Kenya, Somalia, Nigeria, Niger and Demographic Republic of Congo. Findings from this study compares closely with those of Christopher *et al*., (2013) where they reported that *K. pneumoniae* accounted for 33.3% of diarrhea in children < 5 years attending the Bugando Medical Centre, Mwanza in Tanzania However, in US, the prevalence of *K. pneumoniae* diarrhea is reported to be as low as 1.8%.De Baveja *et al*., (2015). However, this global variation of the prevalence of *K. pneumoniae* is due to several factors. Some of these factors include but not limited to patient population, differences in prior antibiotic therapy, heterogeneity in geographical location (Akhter *et al*., 2016). Furthermore, the level of resources available to the country greatly affects the magnitude with higher prevalence reported low-resourced countries and vice versa. This study reveals a significant association between *K.pneumoniae* and ages 13-24months. Possibly due to the immature immune defenses now that they are coming out of a phase of protection of maternal antibodies (Mitchelle *et al*.,2016).In addition children this age start having a significant relationship with the environment which is a reserviour (Moremi *et al*.,2017).

5.3 Proportion of ESBL producers

The proportion of *K. pneumoniae* isolates that are ESBL producers in Mukuru slums was 44% (42/96). Previous studies have shown high frequency of ESBL in hospitals among *Klebsiella* spp. with variation from one continent to another. The proportion of ESBL in the current study is lower compared to a report by (Desta *et al.,* 2016) who reported ESBL in 52% of hospitalized patients in Ethiopia but higher than the study by Isendahl *et al*., (2012) among children <5 years of age attending a pediatric emergency ward in Guinea-Bissau in which 32.6% of the children had ESBL-producing K. *pneumoniae*. These children however had a fever. Also lower than the report in Tanzania, Dar es Salaam which gives the overall prevalence of ESBL carriage to be 34.3% (207/ 603) (Tellevik *et al*., 2016) in children below 2 years of age hospitalized

due to diarrhea and non-hospitalized(community). This variation may be due to the differences in study settings, hospital admitted patients are at a higher risk of acquiring ESBL than community patients.

Studies in South Africa and France has reports a relatively low proportion of ESBL among children 4.7% and 4.6% respectively (Mahomed, 2015; Birgy, *et al*., 2012). The global multi-center surveillance study Tigecycline Evaluation and Surveillance Trial (TEST) reported from 2004 to 2009 an overall prevalence of ESBL producing *Klebsiella pneumoniae* isolated from intensive care units (ICUs), ranging from 12.8% in North America to 26.6% in Europe, 33.8% in the Middle East, 35.6% in the Asia-Pacific region, 45.5% in Latin America an up to 54.9% in Africa. In addition, (Bouchillon *et al*, 2004*)* reported a prevalence of ESBL of 8.8% and 38.9% in North America and Europe respectively with a predominance of CTX-M15 subgroup.

A study in Tanzania (Tellevik *et al*.,2016) reported an overall prevalence of 34.3% (207/603) for *K. pneumoniae* and *E. coli* ESBL-producers. Further analysis reported it at 16.9% and 89.7% in HIV negative and HIV positive children aged below 2 years respectively (Tellevik *et al*., 2016). A study in India reported more than 40% of *K. pneumoniae* ESBL producers which were responsible for clinical sepsis in the infant population. Further analysis using pulse-field gel electrophoresis (PFGE) revealed extensive genetic diversity within the ESBL-producing isolates (Chandel *et al*., 2011). This evidence further increases the need for heightened vigilance and further studies on *K. pneumoniae* producing ESBL in Mukuru slums. Such studies would produce more data on evolution of *K. pneumoniae* in Mukuru slums that may have been missed by the current study. As a matter of fact, investigations related to co-morbidities in the same study site using similar study population are highly recommended. ESBL producing *Klebsiella pneumoniae* is a concern because treatment of children has become difficult. It is therefore necessary for the physicians in Mukuru slums to prescribe with care the antimicrobials for treatment.

5.4 ESBL in relation to age and gender among children in Mukuru Slum

The finding from this study revealed that there was no association between age and ESBL carriage p< 0.05. All age groups have equal chance of getting ESBL, similar to study in Guinea-Bissau and Madagascar that have reported there was no association between age and colonization with ESBL-producing in *K. pneumoniae.* (Isendahl *et al*.,2012) A similar study in Ethiopia by Alemu (2018) found no association between age and ESBL carriage among children. However, this result is inconsistent with the report of Tellevik *et al*., (2016) which reported that age of 12 months or below was significantly associated with ESBL carriage, this differs from a study in French that reported the risk of ESBL carriage to be higher among children above 1 year than in younger children.(Birgy *et al.,* 2012). This could be associated with diet and also intimate hygiene habits.This study also showed that there was significant difference between male and female ESBL carriers p>0.023. This finding is similar to a study by Hijazi *et al*., (2016), which reported that males had a higher colonization frequency than did females. The exact reason for this is not clear and is difficult to explain since there are no studies that have addressed this. This finding is different with a study by (Alemu, 2018; Herindrainy *et al*., 2011; Erdoğan *et al*., 2017) who found no significant difference between male and female ESBL carriers and also states that poor sanitary conditions favors transmission of ESBL by oral route. Many studies in the last decade have reported major genetic diversity in *K.pneumoniae*. This is major source of ESBL-producer in human and the main reservoirs include environment, food, persons, and companion animals (Doi Iovleva *et al*., 2017).

5.5 Antimicrobial resistance profile

Antimicrobial susceptibility of Ampicillin, Ceftazidime, Cefpodoxime, Cefotaxime, Ceftriaxone, Sulfamethoxozole, Aztreonam, Gentamicin, Ciprofloxacin, Amoxicillin-Clavulanic acid, Tetracycline, Cefoxitin, Nalidixic acid and Imipenem varied in resistant pattern. Resistance may be caused by several factors. These factors include over-prescription, patient follow up practices, diagnostic accuracy and poor knowledge

regarding optimal therapies which influences physicians' response to patient demands among others. High resistance of more than 60% was observed in aztreonam, Ampicillin, Sulfamethoxozole, ceftriaxone, ciprofloxacin, Ceftazidime, Cefotaxime, gentamicin and cefpodoxime. Kim *et al*. (2016) reported increased sensitivity of Cefotaxime, nalidixic acid, levofloxacin and kanamycin against *K. pneumoniae*. This findings compare closely to a study conducted in Tanzania by Christopher *et al*., 2013 using similar age group. The current finding contracts the findings of a study conducted by De Baveja *et al.,* (2015). The study reported that *K. pneumoniae* was resistant to Ceftazidime, Cefotaxime, trimethoprim-sulphamethoxazole, nalixidic acid and norfloxacin. However, the children who participated in the study were suffering from acute myeloid leukemia. Therefore, the difference may be attributed to the health status of the children and mostly in immunocompromised patients. In this respect, all pathogens causing diarrhea in patients should be reported to enable the clinicians to prescribe appropriate antibiotics. This would limit the spread and in worst cases mortality among the affected children.

Finding from this study are consistent with the findings of Akhter *et al*. (2016) where they reported a resistance of more than 68%. Ampicillin recorded high resistance of 97% which agrees to the findings of (Christopher *et al.,* 2013) of 95% in Bugando Medical Centre, Mwanza in Tanzania. This indicates that low-resourced countries may share similar risk factors of *K. pneumoniae* epidemiology. It was notable that none of the isolate was resistant to Imipenem, a Carbapenem drug. Carpabenems are considered to be the most reliable last resort treatment for bacterial infections. They are highly effective against many bacterial species and less vulnerable to most beta lactam resistant determinants. For this reason it is important to keep high vigilance in the Mukuru slums since Carbapenem-resistant *K. pneumoniae* strains can easily emerge due to many other risk factors. This finding is consistent to the findings of (Lu *et al.,* 2017). They reported that Imipenem was sensitive to all strains isolated from outpatients' stools including children < 5 years who attended sentinels hospitals during 2010 - 2015 in Beijing. Further, the findings were in agreement to a study by Kumari *et al*., (2013) who

reported 100% effectiveness of Imipenem against *K. pneumoniae.* A recent study in Tanzania, however reported no resistance to carbapenems by *K. pneumoniae.* (Televik *et al*., 2016). According to Wang *et al*.,(2018) admission to intensive care unit, indwelling of urethral catheter, use of β-lactams and β-lactamase inhibitor combination, use of fluoroquinolones and cephalosporins are potential risk factors to such evolution. Further, *K. pneumoniae* can develop resistance by undergoing a complex set of stress-adaptive responses (Adams-Sapper *et al*., 2018). Therefore, continuous vigilance in the community-acquired *K. pneumoniae* strains combined with investigation of pathogens' microbiological characteristics is required to detect any early signals of development of Imipenem resistance. This is because of the fact that the resistance across all the drugs was found to be significant hence resistance control measures are needed in order to avoid the current treatment regiment being rendered ineffective with time. The high resistance observed for most antimicrobials in Mukuru slums may be due to lack of strict policy on sale and use of antibiotics in the country. This therefore leaves few treatment options for infections caused by *K. pneumoniae.*

The age and gender did not show any association with antibiotic resistance level. This finding was consistent with the findings in Guinea Bissau by Isendahl et *al*. (2012) where drug resistance was prevalent in children despite the age group. A similar study in Hacettepe University Children Hospital also reported no difference as far as age and sex of children in relation to antibiotic resistance and ESBL production (Topaloglu *et al*., 2012). This therefore indicates that colonization with ESBL producing bacteria takes place at a young age irrespective of the gender.

5.6 ESBL genes in relation to age and gender among children in Mukuru Slum

In this study ^{bla}CTXM, ^{bla}TEM and ^{bla}SHV genes were detected in ESBL producing *K. pneumoniae*. ^{bla}OXA was not found to encode any of the isolates analysed. ^{bla}CTXM (100%) was the most predominant gene, ^{bla}TEM (97%) and ^{bla}SHV (90.4%) genes responsible for ESBL production. Findings from this study are in accordance with a study done by Maina *et al*., 2013 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 *bla*CTXM (Jihad *et al.,* 2013). In other parts of the world, Philippines reported 35 (95%) *bla*CTX-M. (Cornista *et al*.,2019). In Asia, Taiwan, Japan and China, earlier reports indicated a dominance of *bla*SHV but *bla*CTXM now emerges dominance (Reuland *et al*., 2013. Further reports from South America, Israel, Spain, New York, the United Kingdom, and several parts of Indian subcontinent revealed *bla*CTX-M a the predominant gene.(Villegas *et al*.,2008).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 and *bla*SHV types as the predominant ESBL in many countries (Sharma *et al.*, 2013). *bla*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 (Mathers *et al*., 2015).

However, these findings differ with some studies performed throughout the world. In Machakos hospital, Juma *et al.* (2016) found that *bla*TEM was the most predominant. A report from Canada showed *bla*SHV 22 (43.14%) as the main group of ESBLs, followed by *bla*TEM 18 (35.29%) and *bla*CTXM 16 (31.37%) (Eftekhar *et al.*, 2012). The differences between this 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).

In this 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. (Bali *et al.*, 2010). Contrary to a study done in France that identified the first large outbreak of *bla*OXA among 48-positive Carbapenem-resistant *K. pneumoniae* isolates expressed both *bla*SHV and *bla*CTXM (Cuzon *et al*., 2011). The OXA family of ESBLs has mainly been reported in *Pseudomonas aeruginosa* unlike *bla*TEM and *bla*SHV which are prevalent in Enterobacteriaceae.

Occurrence of more than one beta-lactamase within the same isolate was detected in this study. It is notable that most isolates had a double and a triple combination. Double combination was observed in *bla*CTXM/TEM, *bla*CTXM/SHV. A triple combination was noted in *bla*CTXM/TEM/SHV. Coexistence among the ESBL gene has been reported in various studies. In Africa, Tunisia has reported a Triple combination *bla*TEM/SHV/CTX-M (Alibi *et al*., 2015). In Tanzania, Mshana *et al*., 2013 found a combination of *bla*TEM/CTX-M, 11.96% (11/92) *bla*SHV/CTX-M and 10.87% (10/92) *bla*CTX-M alone. In Togo, combination of *bla*TEM/SHV/CTXM predominating at 61.90%, *bla*TEM/SHV 20.63%, *bla*SHV/CTXM 11.11%, *bla*TEM/SHV 4.76% and *bla*TEM 1.59 has been reported. In Bukinafaso, (Zongo *et al.,* 2015) triple combination of *bla*TEM/SHV/CTX-M was noted.In Tunisia (Alibi *et al*.,2015) reported a triple combination *bla*TEM/SHV/CTX-M represented 44.91% (53/118), and the double combinations were *bla*SHV/CTX-M represented 28.81% (34/118) of cases, *bla*TEM/CTX-M represented 4.23% (5/118) and *bla*TEM/SHV represented 3.39% (4/118) of cases according to (Diagbouga *et al*., 2016). Similarly, in Brazil, Jaskulski *et al*. (2013) 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.

These genes are responsible for drug resistance in children < 5 years within Mukuru slums. These findings are consistent with the findings of Juma *et al*., (2016) who reported presence of TEM and SHV resistant genes from *K. pneumoniae* isolates from diarrhea samples of patients including under 5 years children from Machakos District hospital. A prevalence of 6% in ESBL-producing *K. pneumoniae* and *E. coli* was detected (Juma *et al*., 2016). The findings are also comparable to those of Tellevik *et al*., (2016) which reported presence of *C*TXM gene in 94% of feacal isolates from children aged < 2 years in Tanzania. Another study reported presence of CTXM, SHV, and TEM genes in 19 (14.6%) of *Klebsiella species* ESBL-producers out of 130 isolates from urinary samples collected from different hospitals in Sudan (Doi Iovleva and Bonomo, 2016). In US, the levels reported in different studies are relatively lower at 16.3%

(Castanheira *et al*., 2015). Finding from this study concur with Isendahl *et al*., (2012) who have reported CTXM being prevalent in Guinea Bissau, Madagascar, Niger, Cameroon, Tanzania and even South Africa. The study contributes to the already existing evidence that ESBL-producing *K. pneumoniae* is a major challenge in low-resourced countries and particularly slum settings for treatment of diarrhea due to its ability to resist to treatment of many drugs. As a matter of fact, its multifactorial nature of its expansion presents difficulties in its management. These findings therefore indicate the need for continuous surveillance to identify ESBL-producing *K. pneumoniae* in Mukuru slums for better management of children < 5 years and the general population as whole.

5.7 Conclusion

1. This study reports prevalence of *K. pneumoniae* to be 29 %(96/330) of children under five years in Mukuru slum.
2. This study concluded that the proportion of ESBL among children is 42%(44/96) in Mukuru slum and can vastly contribute to treatment failure leading to serious pathological conditions
3. This study further found high level of resistance against most of the antibiotics including 3rd generation cephalosporins. However all isolates were susceptible to Imipenem. It is possible that use and misuse of antimicrobials is driving resistances to these antimicrobials. Unless an intervention to decelerate this trend of resistance is effected, Carbapenem may be required. It is therefore possible that morbidity and mortality due to such highly resistant strains is likely to rise.
4. ESBL genes, ^{bla}TEM, ^{bla}CTX-M, ^{bla}SHV are associated with varying degrees of resistance to different antibiotics therefore pose the greatest clinical and chemotherapeutic challenges since they limiting therapeutic options. ^{bla}CTX-M (100%) was the predominant gene. Followed by ^{bla}TEM (97%) and ^{bla}SHV (90.4%) genes All the genes exhibited a double and a triple combination of ^{bla}CTXM/TEM, ^{bla}CTXM/SHV and ^{bla}CTXM/TEM/SHV respectively

5. This study found an association between *K. pneumoniae* and children of 13-24months. Howeaver there was no association between age and ESBL carriage.The study further found an association between male children and occurance of ESBL genes.

5.8 Recommendations

1. More research to be done to acertain why ESBL is more prevalent to male children

2. This study has demonstrated the need to combine phenotypic and molecular methods in order to understand important aspects of antimicrobial resistance in developing countries. Continuous surveillance of phenotypic and genotypic drug resistance data, as well as clinical characteristics and treatment outcome for the prevalent strains should be done so as to understand the spread of those successful strains, in order to make better infection control measures.

3. The study emphasizes the need for microbiology laboratories to adequately screen for ESBL-producing strains that cause diarrhea since infections caused by these organisms are not efficiently treated with β-lactam antibiotics. This will assist in revising existing empiric treatment regimens to periodically reflect prevailing resistance strains
4. As antimicrobial resistance among bacterial pathogens is an evolving process, regular monitoring and surveillance is required to establish reliable information about resistance pattern of Enterobacteriaceae for optimal empiric therapy of patients with diarrhea. This approach will help slow the emergence of resistance.
5. Awareness on the risk factors of ESBL production should be emphasized among the population.
6. Measures such as appropriate prescription practices, adequate patient education, adequate diagnostic facilities, limiting unauthorized sale of antimicrobials and appropriate functioning drug regulatory mechanisms should be put in place to

minimize resistance pressure. These measures may help to preserve the potency of these antibiotics and increase/improve successful treatment rates.

7. It is necessary to determine other microorganisms causing diarrhea among children under 5 years in Mukuru slums.
8. In addition, Mukuru slums need to be studied further in order to establish risk factors within Mukuru slum that contribute to drug resistance in order to inform policy regarding treatment and management of infections caused by ESBL producing *K. pneumoniae.*
9. Infection control measures should be implemented to prevent widespread transmission of resistant strains in the community

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APPENDICES

Appendix I: Ethical approval

KENYATTA UNIVERSITY
ETHICS REVIEW COMMITTEE

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Our Ref: **KU/ERC/APPROVAL/VOL.1** (86) Date: 27th September, 2017

Hellen N. Saisi
Jomo Kenyatta University of agriculture & Technology.
P.O. Box 4471,
NAIROBI.

Dear Hellen,

APPLICATION NUMBER PKU/696/E71 "ANTIMICROBIAL RESISTANCE PATTERNS IN EXTENDED-SPECTRUM BETA-LACTAMASE PRODUCING *KLEBSIELLA PNEUMONIAE* ISOLATES FROM CHILDREN IN MUKURU SLUMS, NAIROBI

1. **IDENTIFICATION OF PROTOCOL**

The application before the committee is with a research topic Application Number:

APPLICATION NUMBER PKU/696/E71 "Antimicrobial Resistance Patterns in Extended-Spectrum Beta-Lactamase Producing *Klebsiella pneumoniae* Isolates from Children in Mukuru Slums, Nairobi On 19th July 2017 and approved on 27th September 2017.

2. **APPLICANT**

Hellen N. Saisi

3. **SITE**

Nairobi city county, Mukuru Slums

4. DECISION

The committee has considered the research protocol in accordance with the Kenyatta University Research Policy (Section 7.2.1.3) and the Kenyatta University Review Committee Guidelines **AND APPROVED that the research may proceed for a period of ONE year from 27th September, 2017.**

ADVICE/CONDITIONS

i. Progress reports are submitted to the KU-ERC every six months and a full report is submitted at the end of the study.
ii. Serious and unexpected adverse events related to the conduct of the study are reported to this committee immediately they occur.
iii. Notify the Kenyatta University Ethics Committee of any amendments to the protocol.
iv. Submit an electronic copy of the protocol to KUERC.

When replying, kindly quote the application number above.
If you accept the decision reached and advice and conditions given please sign in the space Provided below and return to KU-ERC a copy of the letter.

DR. TITUS KAHIGA.
CHAIRMAN ETHICS REVIEW COMMITTEE

I ....HELLEN N. SAISI..... accept the advice given and will fulfill the conditions therein.

Signature..... Dated this day of... .....2/10/2017.....2017.

Appendix II: Consent seeking and information form for study participants

Title of project: Antimicrobial Resistance Patterns in Extended-Spectrum Beta-Lactamase Producing Klebsiella Pneumoniae Isolates from Children In Mukuru Slums Nairobi

Principal Investigator: Hellen Nanjala saisi Contact: 0721-446926

Introduction: My name is Hellen Nanjala Saisi. This study intends to evaluate the resistance patterns of *K. pneumonia* in children. The information from the study will provide a better understanding on the multidrug resistance situation and molecular characterization of *K. pneumoniae*. It will also contribute to surveillance and monitor resistance patterns. This will inform proper management and treatment of patients.

Research Procedure: Diarrhea stool sample will be collected in a sterile polypot.

Benefits: This study may directly benefit you, and other patients in this setting. The information from the study will provide a better understanding on the multidrug resistance situation and molecular characterization of *K. pneumoniae*. It will also contribute to surveillance and monitor resistance patterns. This will inform proper management and treatment of patients.

Risk: There will be no pain involved

Your rights: Your participation in this study is voluntary and if you disallow participation, you will not be denied any services that are normally available to you.

Confidentiality: Only the research team and staff will have access to the study results. All collected data will be kept secure and confidentiality ensured by use of passwords. The research team and staff will receive appropriate training on securing and maintaining confidentiality and safeguarding data. Data will be coded in SPSS software for archiving and any other identifiable confidential data will be destroyed.

Consent for parent/guardian: The above details about the study and the basis of participation have been explained to me and **I agree** to take part in the study. I understand that I am free to take part in the study. I also understand that if I do not want to go on with the study, I can withdraw at any time.

Sign/Thumb…… Date……..

Appendix III: Procedures

A. Citrate test

Citrate utilization test is commonly used to distinguish between members of the *Entero bacteriaceae f*amily based on their metabolic by-products. Citrate utilization test is used to determine the ability of bacteria to utilize sodium citrate as its only carbon source. Simmons Citrate Agar was inoculated lightly on the slant by touching the tip of a needle to a colony then incubated at 37 ^{0}C for 18 - 24 hours. Then media was observed for the development of blue color; denoting alkalinization.

B. Triple Sugar Iron Test

The Triple Sugar Iron or TSI test is a microbiological test roughly named for its ability to test microorganism's ability to ferment sugars and to produce hydrogen sulfide. A sterile needle was used to pick a pure isolates from an 18 - 24 hour culture a single colony was used to stab into the medium up to the butt of the TSI tube, and then streaked on the slant and was incubated at 37 ^{0}C for 18 to 24 hours and observed for hydrogen sulfide production, gas production and color change in the media. The *K.pneumoniae* was presumably identified by the following reactions: Acid slant/ Acid butt (A/A); Yellow/Yellow with gas production but no production of hydrogen sulfide.

C. Urease Test

Urea is a diamide of carbonic acid. It is hydrolyzed with the release of ammonia and carbon dioxide. The urease enzyme is able to split urea in the presence of water to release ammonia and carbon dioxide. The ammonia combines with carbon dioxide and water to form ammonium carbonate which turns the medium alkaline, turning the indicator phenol red from its original orange yellow color to bright pink. The broth medium was inoculated with a loopful of a pure culture of the test organism and incubated at 37 ^{0}C for 18 - 24 hours. Then the media was observed for colour change. *E. coli* tested negative since there was no color change in the media.

D. SIM (Sulfur, Indole Motility) Test

The formulation of SIM Medium is designed to allow the detection of sulfide production, indole formation and motility. The SIM Medium was inoculated by stabbing the center of the medium then incubated aerobically at 35 ^{0}C for 18 - 24 hours. H_2S production and motility was observed in the medium before adding three drops of Kovacs Reagent. The formation of a red ring denotes a positive test. The *K.pneumoniae* isolates were positive for sulfur production but negative for both indole and motility.

E. DNA Extraction by Boiling Method

The heating block was set at a temperature of 95 ^{0}C then pure colonies for DNA isolation were Identified and labeled. Approximately 1ml of molecular grade water was added to 2 ml Eppendorf tubes and marked with a number corresponding to the isolate been analyzed. Using a sterile swab, a pea-sized amount of inoculum was scrapped from the culture and transferred to the corresponding tube. The tubes were then placed in the heating block and left to heat for a maximum of 12 minutes. The tubes were then transferred to a table-top centrifuge. The contents were centrifuged at maximum speed for 5 - 6 minutes. The supernatant was transferred to a clean vial and then stored at -80 ^{0}C.

Appendix IV: Clinical and Laboratory Standards Institute (2015) guidelines on Performance Standards for Antimicrobial Susceptibility Testing

Antibiotic	**Sensitive**	**Intermediate**	**Resistant**
Ampicilin (AMP,10µg)	≥17	14-16	≤13
Imipenem (IPM, 10µg)	≥23	20-22	≤19
Cefotaxime (CTX, 30µg)	≥26	23-25	≤22
Ceftazidime (CAZ, 30µg)	≥21	18-20	≤17
Aztreonam (ATM, 30µg)	≥21	18-20	≤17
Cefoxitin (FOX, 30µg)	≥18	15-17	≤14
Ceftriaxone (CRO,30µg)	≥23	20-22	≤19
Cefpodoxime (CPD10µg)	≥21	18-20	≤17
Amoxicillin-Clavulanic (AMC,30µg)	≥18	14-17	≤13
Ciprofloxacin(CIP,5µg)	≥21	16-20	≤15
Nalidixic acid(NA,30µg)	≥19	14-18	≤13
Tetracycline(TE,30µg),	≥15	12-14	≤11
Gentamicin(CN,10g)	≥15	13-14	≤12
Sulfamethoxazole/Trimethoprim (SXT,23.75µg/1.25µg),	≥16	11-15	≤10

NB: Breakpoints (nearest whole mm)

Appendix V: Gender and age distribution among *K. pneumonia* positive cases

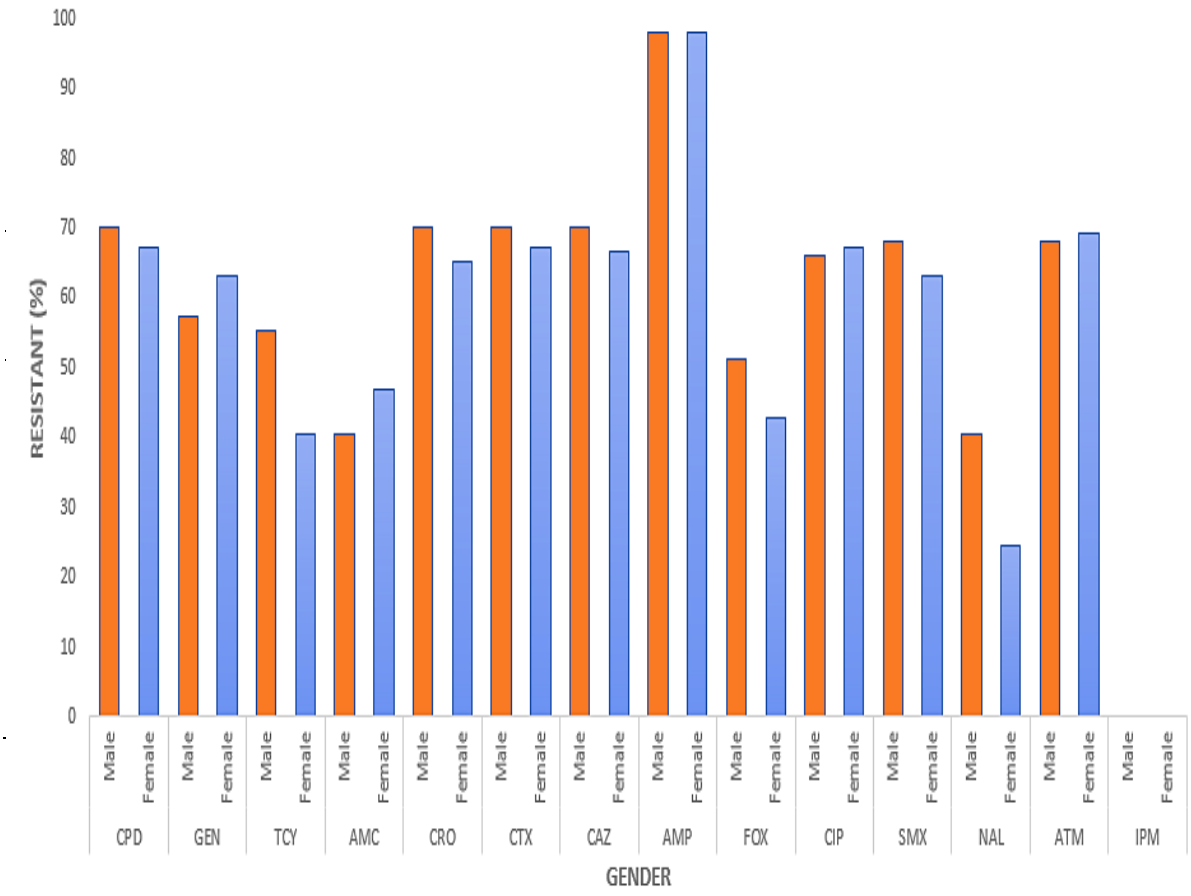
Number of participants (%)							Total (%)
Sex	0-6	7-12	13-24	25-36	37-48	49-60	
Males	3(3.1)	4(4.1)	9(9.3)	6(6.2)	6(6.2)	12(12.5)	40(41.6)
Females	2(2.08)	6(6.2)	8(8.3)	10(10.4)	8(8.3)	21(21.8)	56(58.3)
Total	5(5.2)	10(10.4)	17(17.7)	16(16.6)	14(14.5)	34(35.4)	96(100)

The proportion of ESBL carriage was highest among children of age group 37-48 Months and lowest in age group 51-60 months. About 80.95 % (34) of females and 19.0 %(8) of males were ESBL carriers Table 4.5 below.

Appendix V1: Overall antimicrobial susceptibility pattern among children in Mukuru slum

ANTIBIOTIC	SENSITIVE (%)	RESISTANT (%) N
CPD	30 (31.25)	66 (68.8)
GEN	38 (39.6)	58 (60.4)
TCY	43 (44.8)	53 (55.2)
AMC	54 (56.25)	42 (43.8)
CRO	31 (32.3)	65 (67.7)
CTX	30 (31.3)	66 (68.8)
CAZ	30 (31.3)	65 (67.7)
AMP	2 (20.8)	94 (97.9)
FOX	51 (59.4)	40 (41.7)
CIP	32 (33.3)	64 (66.7)
SMX	33 (34.4)	63 (65.6)
NAL	65 (67.7)	31 (32.3)
ATM	30 (31.2)	66 (68.8)
IMP	96(0.0)	0(0.0)

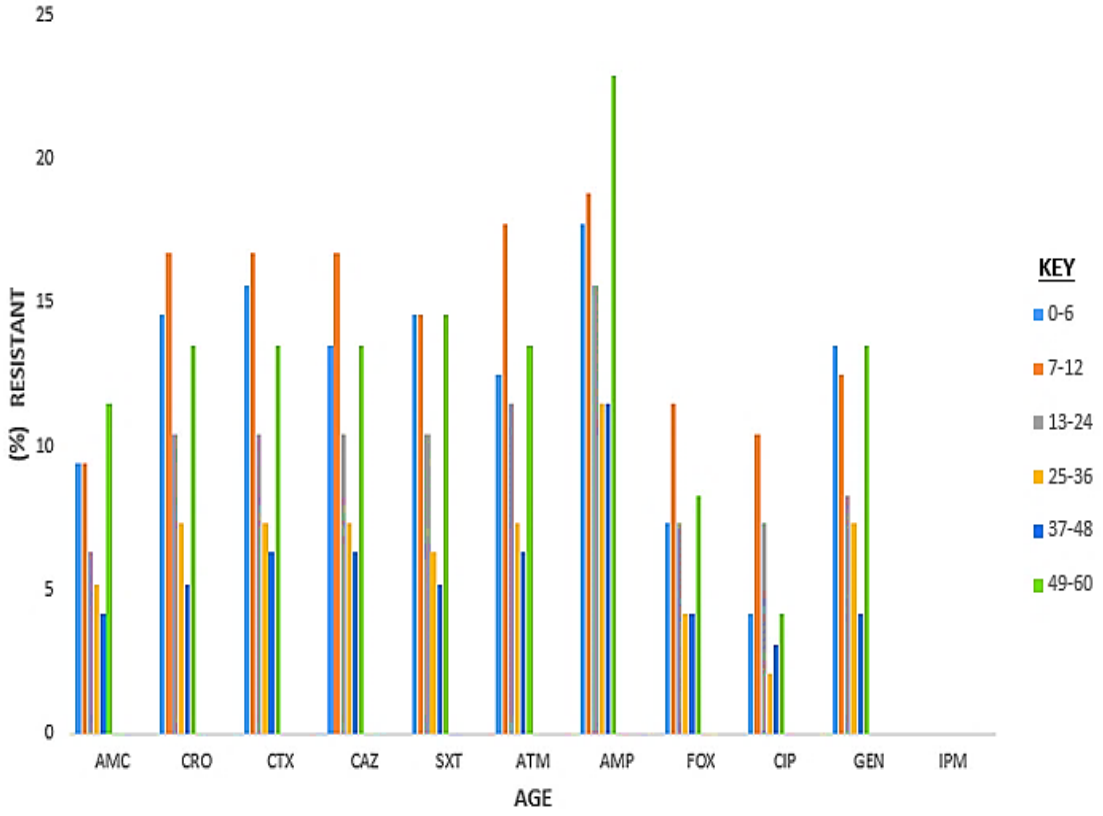
Appendix VII: Resistance pattern by gender among children in Mukuru slum



Resistance pattern by gender among children in Mukuru slum

AMP- Ampicillin, CTX- Cefotaxime, ATM- Aztreonam CPD- Cefpodoxime, CAZ- Ceftazidime, CRO Ceftriaxone, CIP- Ciprofloxacin SXT- Sulfamethoxozole, CN- Gentamycin, AMC-Amoxicillin-Clavulanic, TE- Tetracycline, Fox-cefoxitin, NA- Nalidixic acid

Appendix VIII: Resistance pattern by age among children in Mukuru slum



Resistance pattern by age among children in Mukuru slum

AMP- Ampicillin, CTX- Cefotaxime, ATM- Aztreonam CPD- Cefpodoxime, CAZ- Ceftazidime, CRO Ceftriaxone, CIP- Ciprofloxacin SXT- Sulfamethoxozole, CN- Gentamycin, AMC-Amoxicillin-Clavulanic, TE- Tetracycline, Fox-cefoxitin, NA- Nalidixic acid

Appendix IX: Occurrence of ESBL gene in relation to gender

Gender	n=42	Odds ratio	95 %CI	P value
Male	12(28.5)	0.37	0.15 - 0.87	0.023
Female	30(71.4)	1.15	0.68 - 1.95	Ref

Appendix X: Occurrence of ESBL gene in relation to age

Age Category	n=42	Odds ratio	95 %CI	P value
0-6	2(4.7)	0.59	0.87- 4.00	0.592
7-12	4(9.5)	0.59	0.14- 2.01	0.474
13-24	7(16.6)	0.62	0.19 - 2.01	0.430
25-36	7(16.6)	0.69	0.19 - 2.01	0.545
37-48	4(9.5)	0.35	0.20 - 2.28	0.131
49-60	18(42.8)	1.12	0.09- 1.35	Ref

Appendix X1: Proportion of ESBL producers among children

Number of participants (%)							**Total (%)**
SEX	0-6 months	7-12 month	13-24 months	25-36 months	37-48 months	51-60 months	
MALES	0(0.00)	0(0.00)	1(2.4)	3(7.1)	4(9.5)	0(0.00)	8(19.0)
FEMALES	2(4.7)	2(4.7)	6(14.3)	4(9.5)	19(45.2)	1(2.4)	34(80.95)
Total	2(4.7)	2(4.7)	7(16.6)	7(16.6)	23(54.7)	1(2.4)	42(100)

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

Share and Cite:

Saisi, H. , Makobe, C. , Kangongo, M. and Kariuki, S. (2019) Prevalence of CTXM, SHV, TEM AND OXA Genes among Extended-Spectrum Beta-Lactamase Producing *Klebsiella pneumoniae* from Mukuru Slum, Kenya. *Advances in Microbiology*, **9**, 853-862. doi: 10.4236/aim.2019.910052.

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

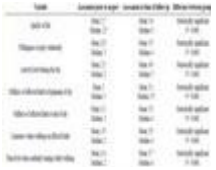

Table 1. Primers used for detection of ^{bla}SHV, ^{bla}CTX-M & ^{bla}TEM genes.

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.

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 $^{bla}_{CTX-M}$ by PCR. Combination of the three resistant genes was also noted. The most common genotype was $^{bla}_{CTX-M}$ 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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