ANTIMICROBIAL SUSCEPTIBILITY AND GENETIC BASIS OF RESISTANCE OF *KLEBSIELLA* SPP ISOLATED FROM DIARRHEIC AND NON-DIARRHEIC PATIENTS AT HEALTH FACILITIES IN MUKURU INFORMAL SETTLEMENT, NAIROBI, KENYA

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Antimicrobial Susceptibility and Genetic Basis of Resistance of *Klebsiella* Spp Isolated from Diarrheic and Non-Diarrheic Patients at Health Facilities in Mukuru Informal Settlement, Nairobi, Kenya

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DECLARATION

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

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DEDICATION

I dedicate this work to God Almighty who has been the source of my strength throughout my MSc study. I also dedicate this work to my all my friends and family especially my mum Mary Wairimu who has always supported me with finances, great encouragement and prayers.

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ABBREVIATIONS AND ACRONYMS

AMR	Antimicrobial resistance
BSI	Bloodstream infections
CA	Community-acquired
CAUTI	Catheter-associated UTIs
CDC	Centers for Disease Control and Prevention
CMR-SASU	Center for Microbiology Research-Salmonella Surveillance Unit
CNS	Central nervous system
CRE	Carbapenem-resistant *Enterobacteriaceae*
CR-Kp	Carbapenem Resistant *Klebsiella pneumoniae*
ESBL	Extended-spectrum beta-lactamase
HAI	Hospital-acquired infections
HIV/AIDS	Human immunodeficiency virus infection and acquired immune deficiency syndrome
ICUs	Intensive care units
IMP	Imipenemase
KPC	*K. pneumoniae* carbapenemase (KPC)
LPS	Lipopolysaccharide
MBL	Metallo-β-lactamase
MDR	Multidrug resistance
NDM	New Delhi metallo-β-lactamase-1

PCR	Polymerase Chain Reaction
PLA	Pyogenic liver abscess
UTI	Urinary Tract Infections
VAP	Ventilator-associated pneumonia
WHO	World Health Organization

ABSTRACT

Antimicrobial resistance (AMR) is a global threat to public health and particularly to children. This study aimed to determine the prevalence of multidrug resistance of fecal *Klebsiella spp* on selected beta-lactam (3rd generation cephalosporins and carbapenems) and fluoroquinolone classes of drugs in four health facilities serving the Mukuru slum community of Nairobi city in Kenya. Additionally, determine the genetic basis for the multidrug resistance observed. A cross-sectional laboratory-based study was undertaken where a total of 1171 children below 16 years were selected, from whom stool samples were collected, tested, and analyzed using various methods namely; culture, biochemical testing, antibiotic sensitivity testing and polymerase chain reaction. A total of 395 (33.73%) *Klebsiella spp* were isolated, consisting of 365 (92.4%) *Klebsiella pneumoniae* and 30 (7.6%) *Klebsiella oxytoca* were isolated. The proportion of multi-drug resistance (MDR) *K. pneumoniae* and MDR *K. oxytoca* was 64.1 % (234/365) and 96.67 % (29/30) respectively. *K. pneumoniae* showed the highest resistance against third-generation cephalosporins namely; cefotaxime (30.7%), ceftriaxone (29.9%), and ceftazidime (27.4%), whereas the least resistance was observed against carbapenems including imipenem (1.6%) and meropenem (1.6%). A significant association was observed in diarrheic children (OR =1.88; p=0.01) and those below 50 months (OR = 0.43; p=0.002) and carrying *K. pneumoniae* resistance to one or more third-generation cephalosporins. Genes associated with resistance included *bla* TEM 100%, *bla* CTX-M 95.2%, *bla* SHV 57.1%, *bla* OXA-1 66.7%, qnrS 54.1%, qnrB 47.6% and *bla* NDM 7.1%. In conclusion, there is a high prevalence of MDR *K. pneumoniae* carrying genes associated with antibiotic resistance, and this poses a threat to the Mukuru community, especially the vulnerable.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Antimicrobial resistance is a phenomenon where microbial organisms develop the ability to survive exposure to antimicrobials designed to kill them or stop their growth. The global burden of AMR is increasing alarmingly and the United Nations (UN) General Assembly AMR report estimates that resistance will be responsible for approximately 10 million deaths by 2050, most of which will occur in a poor resource setting, mainly, Sub-Saharan Africa (O'Neill, 2014). In the United States of America, for example, it is estimated that more than 2 million people are infected with AMR organisms, annually, with approximately 23,000 deaths (CDC, 2016). Major causes in the United States of America are misuse and/or abuse of antibiotics and use of antibiotics in agriculture (Dadgostar, 2019) whereas, in developing countries, the situation is aggravated due to poor implementation of infection control measures and the availability of counterfeit or low-quality drugs (GARP, 2011). In developing countries, the data is limited due to inadequate surveillance and hence likely to be significantly higher than in developed countries.

Klebsiella spp are common intestinal commensals that obtain, accumulate, and disseminate a variety of antibiotic resistance genes such as *bla* KPC. (Salyers *et al.,* 2004). Therefore, they serve as a significant reservoir for resistance in the intestinal tract (Huddleston, 2014; Salyers *et al.,* 2004) and subsequently increase the risk of nosocomial and community-acquired resistant infections (Schjørring *et al.,* 2011).

In vivo dissemination of AMR genes from intestinal *Klebsiella spp* to other bacterial species has been documented (Goren *et al.,* 2010; Haverkate *et al.,* 2015; Schjørring *et al.,* 2008; Sidjabat *et al.,* 2009). In addition, *Klebsiella spp* causes diarrheal disease and a myriad of extraintestinal infections, especially in severely ill patients (Martin *et al.,* 2016; Thi *et al.,* 2003). Apart from diarrheal patients (Huynh *et al.,* 2020; Lee *et al.,* 2021; Zhang *et al.,* 2018), multidrug-resistant *Klebsiella spp* has also been documented in apparently healthy patients including children (Karanika *et al.,* 2016; van Hoek *et al.,* 2015). Multi-drug resistance in slum areas ensures faster spread due

to the high density of humans and livestock living in close proximity, frequent antibiotic misuse, and insufficient drinking water, drainage, and sanitation infrastructure. These settlements, therefore, serve as hotspots for AMR transmission (Nadimpalli *et al.,* 2020; Omulo *et al.,* 2021).

Reports on the emergence and global spread of multidrug-resistant (MDR) and hypervirulent clones of *Klebsiella spp* especially *K. pneumoniae* have been increasing in both nosocomial and community-acquired infections (Martin *et al.,* 2016; Pomakova *et al.,* 2012). As a result, the treatment of *Klebsiella spp* infections has become more difficult with the available options being restricted. Various mechanisms have been implicated in antibiotic resistance including mutation of chromosomal genes and the production of β-lactamases enzymes such as extended-spectrum β-lactamases (ESBLs), cephalosporinases, and carbapenemases (Nathisuwan *et al.,* 2001). Genes encoding for these enzymes are mostly carried on mobile genetic elements such as conjugative plasmids, integrons, transposons, and insertion sequences.

They not only bear resistance genes but also virulence genes, which intensify the ability of an organism to colonize and create infection within the host. (Nathisuwan *et al.,* 2001). Colonization precedes infection in the pathogenicity of disease (Martin *et al.,* 2016), therefore understanding colonization dynamics provides a basis for the identification of colonized patients and the potential establishment of intervention protocols to prevent subsequent infection.

1.2 Problem statement

According to the Global Burden of Disease, AMR accounts for an estimated 4,950 000 (3,620,000–6,570,000) deaths annually and the highest burden is observed in Sub-Saharan Africa at 1,070,000 (847,000–1,340,000) deaths (Murray et al., 2022). Additionally, it is estimated that by 2050, AMR will account for 10 million deaths annually, if no interventions are initiated (O'Neill, 2014). In Kenya, the precise burden is lacking, however, numerous studies have reported varying antimicrobial resistance rates. For instance, the prevalence of extended-spectrum beta-lactamase producing *K.pneomoniae* ranges from 30 -79%, in both urban and rural setting, and community

and hospital settings (Henson *et al.,* 2017; Kagia *et al.,* 2019; Ogalo *et al.,* 2016; Taitt *et al.,* 2017) . *Klebsiella species* is the third leading bacterial pathogen associated with antibiotic-resistant infections including diarrhea, urinary tract infections, bloodstream infections, and respiratory tract infections, particularly in patients with a compromised immune system and children. Of additional concern, *Klebsiella* spp obtain, accumulate, and disseminates a variety of AMR-associated determinants. Therefore, they serve as a significant reservoir for resistance within the gut (Huddleston, 2014; Salyers *et al.,* 2004).

In addition, they have been shown to disseminate resistance genes to other organisms including pathogens in the same environment which aggravates the pathogenicity of these organisms (Sidjabat *et al.,* 2009). This leads to the subsequent increase of resistant infections in both healthcare and community settings. World Health Organization (WHO) recently identified carbapenems and 3rd generation cephalosporins-resistant *Klebsiella pneumoniae* as a tier 1 antibiotic-resistant priority pathogen among other Enterobacteriaceae (Shrivastava *et al.,* 2018). Treatment of *Klebsiella* spp infections has become progressively challenging as a result of the emergence and spread of multidrug-resistant and hypervirulent strains.

Mukuru slum is densely populated and made of temporary structures mostly corrugated metal sheets. Basic services and infrastructure for providing adequate sanitation and clean water are insufficient. In addition to poverty, several factors associated with informal settlements such as overcrowding, substandard housing, unclean and insufficient quantities of water, and inadequate sanitation contribute to a high incidence of infectious diseases and increased mortality among children. Additionally, it is a hotspot for resistance genes. There is limited data on surveillance for MDR *Klebsiella* spp as this is not routinely carried out in Kenya, yet such data is necessary to inform policy on antibiotic-resistant infections and their management in the country.

1.3 Justification

There is a need to reduce and/or eliminate mortality and disabilities associated with antimicrobial resistant-*Klebsiella* spp infections, particularly those caused by multidrug-resistant and hypervirulent strains.

Additionally, there is a need to curb the spread of these strains. Several studies have suggested that the intestinal tract is a reservoir for both pathogens and antibiotic-resistant organisms. They have further suggested mechanisms for the transfer of both virulence genes and resistance-associated determinants (Blair *et al.,* 2014; Donskey, 2004; Salyers *et al.,* 2004). However, few studies in our settings have looked at AMR in the context of diarrhea and asymptomatic Klebsiella infections (Henson *et al.,* 2017; Kagia *et al.,* 2019; Ogalo *et al.,* 2016; Taitt *et al.,* 2017). Data generated from this study will provide knowledge to researchers on the transmission dynamics of Klebsiella spp in the slum setting. Colonization is a subsequent step before infection thus generating data on the former provides a rationale for establishing intervention protocols and driving decisions in policy making. Data obtained from the current study will also be necessary to inform policy on the effectiveness of the available drugs of choice for *Klebsiella* infections. Data generated on the colonization of *Klebsiella spp* will provide necessary information to the Ministry of Health and health stakeholders to guide on patient management of *Klebsiella spp* associated infections. There is a need to reduce/eliminate the economic implications of Multidrug-resistant *Klebsiella* spp specifically, prolonged hospital stays and increased hospital costs which impose a substantial financial burden on the healthcare system as well as the infected individuals.

The drugs selected for this study are; 3rd generation cephalosporins Carbapenems and fluoroquinolones. They were selected because they are the commonly used drugs for the treatment of *Klebsiella spp.*3rd generation cephalosporins are used to treat susceptible non-ESBL strains, while Carbapenems and fluoroquinolones are used to treat ESBL strains.

Children below 5 years are vulnerable to a myriad of infections due to their underdeveloped immunity while children above 5 years are exposed to lifestyle and behavioral risk factors such as eating habits and WASH challenges and hence included in this study.

1.4 Research questions

1. What is the prevalence of Multidrug-resistant *Klebsiella* spp colonizing the intestinal tracts of diarrheic and non-diarrheic children from four health facilities?
2. What are the resistance profiles of *Klebsiella* spp obtained from diarrheic and non-diarrheic in four health facilities*?*
3. What is the molecular genetic basis for multidrug-resistant phenotypes with reference to 3rd generation cephalosporins, fluoroquinolones, and carbapenems classes of drugs?

1.5 Objectives

1.5.1 Broad objective

To determine the antimicrobial susceptibility and genetic basis of resistance of *Klebsiella* spp isolated from diarrheic and non-diarrheic patients at four health facilities in Mukuru informal settlement, Nairobi, Kenya.

1.5.2 Specific Objectives

1. To characterize intestinal *Klebsiella* spp colonizing diarrheic and non-diarrheic children in four outpatient health facilities (Missionaries of Mary Mukuru kwa Njenga clinic, Municipal county council clinic-Mareba, Mukuru kwa Reuben clinic and Mbagathi hospital).
2. To determine the resistance profiles of *Klebsiella* spp from diarrheic and non-diarrheic children in four outpatient health facilities.
3. To determine the genetic basis for the Multidrug-resistant phenotypes with reference to 3rd generation cephalosporins, fluoroquinolones, and carbapenems classes of drugs

CHAPTER TWO

LITERATURE REVIEW

2.1 The burden of antimicrobial resistance

2.1.1 Global burden of AMR

According to the Global Burden of Disease, in 2019, antimicrobial-resistant bacteria were responsible for 4950000 (3620000–6570000) deaths, 192 000 (146 000–248 000) years of life lost, and 189 000 (145 000–245 000) disability-adjusted life-years (Murray *et al.,* 2022). Additionally, the Centers for Disease Control estimated that, in the United States, 2,000,000 infections occur annually, and out of those 23,000 die (CDC, 2016). Low and middle-income countries especially those in Africa and south Asia bear the highest burden. In Contrast, developed countries such as those in Europe, America, and Australia bear the least burden (Murray *et al.,* 2022).

2.1.2 The burden of AMR in Africa

In 2019, Sub-Saharan Africa had the highest burden at 989000 (78600–124200) deaths, 65 800 (51 400–83 600) years of life lost, and 66 200 (51 800–84 000) disability-adjusted life-years while the least burden was observed in Northern Africa (Murray *et al.,* 2022). Deaths associated with extensively drug-resistant are, however, lowest in Sub-Saharan Africa and highest in Northern Africa. Notably, resistance patterns of gram-negative bacteria against ampicillin and trimethoprim are high and those against carbapenems are lowest throughout Africa (Tadesse *et al.,* 2017).

2.1.3 The burden of AMR in Kenya

Data on the precise number of deaths, years of life lost and disability-adjusted life-years is not available. However, numerous studies have reported varying prevalence rates of resistant bacteria. For instance, a study in conducted in Kilifi reported a prevalence of ESBL *K. pneumoniae* associated with nosocomial infections at 79%, with one isolate carrying a plasmid containing *bla*NDM-1 gene. In addition, the prevalence of ESBL *K. pneumoniae* associated with community-acquired infections

was at 37% (Henson *et al.*, 2017). Another study carried out in western Kenya observed an MDR *K. pneumonia* of 36% (Taitt *et al.*, 2017). The available data shows that the trend of resistance rates is trending upward.

2.2 Epidemiology of *Klebsiella* Species

Klebsiella spp are found in a wide range of diverse environments including mucosal membranes of humans where they colonize the gastrointestinal tract and the skin among other sites. It is a major culprit in nosocomial infections and a common pathogen in community-acquired infections, bacteremia, and pneumonia. They are particularly endemic in neonatal wards during outbreaks. Children have exhibited higher colonization rates where *Klebsiella* spp survives up to several months compared to adults where they survive up to a few weeks (Janda *et al.*, 2006). With regard to gender, adult males have shown to be more susceptible to *Klebsiella* spp infections compared to adult females, this difference was attributed to increased levels of alcohol consumption. Stool samples have shown a detection rate of 6% to 38% (Shahab, 2017).In humans, *K. pneumoniae* is the most pathogenic followed by *K. oxytoca*; these species contribute substantially to mortality and morbidity. Other pathogenic species include; *K. ozaenae, K. rhinoscleromatis, K. granulomatis,* and *K. variicola. K. pneumoniae* commonly occurs in the gastrointestinal tract as normal flora.

However, when it crosses the gastrointestinal mucosal membrane into the respiratory system, particularly the lungs, it causes pneumonia. In fact, it is the major cause of pneumonia in the community setting. Lately, it has been implicated in causing diarrhea in HIV-infected persons (Janda *et al.,* 2006). *Klebsiella* spp accounts for 16-43% of central nervous system infections. With regard to community-acquired meningitis, *K. pneumoniae* and *K. oxytoca* are the main causative agents (Shahab, 2017).

Klebsiella granulomatis causes donovanosis; a rare sexually transmitted genital ulcer disease. In recent years the incidence of this disease has decreased. *K. oxytoca* has been implicated in septicemia among preterm babies especially those in neonatal intensive care units. Among the risk factors for infection with *Klebsiella* spp especially the multi-drug resistant strains are the use of broad-spectrum antibiotics for an extended time in hospitalized patients. The infections are particularly endemic when

the MDR strains are extended-spectrum beta-lactamases (ESBLs) producers. In addition, the carriage of these strains has been associated with the overuse and/or abuse of broad-spectrum antibiotics (Shahab, 2017).

These drug-resistant strains are highly virulent because they exhibit the capsule type K55 enabling them to spread extraordinarily fast. They are also able to transfer plasmids and their resistance genes to other organisms. They mostly colonize the gastrointestinal tract through other sites such as the respiratory and urinary tracts are also infected. In addition, they have also caused bacteremia, septicemia, and ultimately increased mortality rates. Other risks of infection have included poor health status, treatment in nursing homes, intensive care units, and the presence of catheters. Transmission of *Klebsiella* spp is mainly through physical contact with contaminated objects (inclusive of medical equipment) or/and surfaces. Transmission through feces has also been reported in a few cases of bacteremia attributed to *Klebsiella* spp (Janda *et al.,* 2006).

2.3 Colonization of *Klebsiella* spp on Human Mucosal Surfaces

The reservoir of *Klebsiella* spp in humans is the environment, upon acquisition, it occurs as colonization which sometimes leads to an infection. *Klebsiella* spp is ubiquitous, it mostly occurs in soil, sewage, plant surfaces, and water (Podschun *et al.,* 2001).*K. pneumoniae* isolated from the environment is identical to the clinical counterpart with regards to biochemical characteristics, virulence, and pathogenicity patterns but the capsule types vary (Struve *et al.,* 2004).*K. pneumoniae* isolated from the clinical setting is more resistant to antimicrobials compared to environmental *K. pneumoniae* which is more susceptible. This is suggestive of selective pressure in clinical settings (Jean *et al.,* 2002).Sources of transmission in hospitals include; contaminated medical equipment and surfaces, and direct physical contact between hospital staff and patients with the hospital staff is a major source (Martin *et al.,* 2016). Upon acquisition in humans, *Klebsiella* spp colonizes the mucus surfaces of the gastrointestinal tract as well as the nasopharynx tract (Podschun *et al.,* 1998). *Klebsiella* spp on the skin is deemed transient as opposed to colonizing (Thurlow *et al.,* 2013). Colonization rates are different in various sites and depending on whether

the *Klebsiella* spp is acquired from the hospital or the community. Hospital-acquired colonization rates of the nasopharynx tract are up to 19% while community-acquired colonization ranges from 3% to 15%, usually higher in adults due to alcohol consumption (Dao *et al.,* 2014; Wolf *et al.,* 2001). In comparison to the nasopharyngeal tract, colonization rates of the gastrointestinal tract are relatively high.

Hospital-acquired ranges from 20% to 70% while community-acquired goes up to 35% (Gorrie *et al.,* 2017; Martin *et al.,* 2018). The increase in gastrointestinal colonization rates has been greatly attributed to antibiotic treatment.

Gastrointestinal tract colonization is, therefore, a significant reservoir with regard to *Klebsiella* spp transmission and infection resistant strains (Dorman *et al.,* 2017).

2.4 Progression from Colonization to Infection

Gastrointestinal tract colonization has long been a notable reservoir for nosocomial infections (Donskey, 2004). Recently, a significant association was shown between gastrointestinal tract carriage and subsequent infection, particularly in hospitalized patients. Up to 80% of infecting *Klebsiella* spp isolates concurred with the colonizing *Klebsiella* spp isolates. Progression from colonization to infection can be majorly attributed to pathogenicity factors (Martin *et al.,* 2016).

2.4.1 Pathogenicity factors

2.4.1.1 Capsule

Klebsiella spp has eminent capsules which are majorly composed of complex polysaccharides and are classified into 79 serological types with K1, K2, K3, K4, and K5 being the most virulent (Hsu *et al.,* 2016). However, out of the 79 types that exist, only a few have been studied with reference to virulence. The capsules serve as protection from the host's immune response specifically, from phagocytosis, and bactericidal serum factors (Rendueles, 2020).In addition, they are presumed to inhibit the activation of C3b complement and differentiation of macrophages. The capsule polysaccharide has been reported to expedite antimicrobial resistance as it serves as a protective barrier thus antimicrobials are not able to diffuse into the cell. For instance,

a study demonstrated that when a gram-negative bacteria cell was exposed to sublethal amounts of kanamycin and streptomycin, the production of capsular polysaccharides increased significantly as a result of the upregulation of certain genes(Campos *et al.*, 2004; Lu *et al.*, 2008; Sachdeva *et al.*, 2017).

2.4.1.2 Serum Resistance

Phagocytosis and bactericidal action by the serum is the primary defense responses of a host against a pathogen. The bactericidal action of the serum is mediated by complement proteins C5b-C9. These proteins collect as membrane attack complexes to cause the lysis of the pathogen (Janeway *et al.*, 2005). Pathogens particularly Enterobacteriaceae have evolved to resist the bactericidal effect of human serum using various strategies including outer membrane proteins. These proteins include lipoproteins such as TraT and lipopolysaccharides(LPS) (Janeway *et al.*, 2005; Rollauer *et al.*, 2015). The LPS is an endotoxin which is majorly implicated in septic shock. LPS-associated septic shock is caused by the host's inflammatory cascade and not the LPS itself. LPS is typically composed of O-antigen and lipid A.O-antigen variations are the basis for O serotypes. *Klebsiella* spp has 9 O serotypes where O1, O2, and O3 have been attributed to 80% of all *Klebsiella* infections (Follador *et al.*, 2016). *Klebsiella* spp isolates with rough LPS (short-length O antigen) are very sensitive to the serum bactericidal effect while those with smooth LPS (full-length O antigen) are resistant. LPS variations also serve to protect pathogens from antimicrobial peptides such as polymyxin A and B (Cheng *et al.*, 2015; Papo *et al.*, 2005).

2.4.1.3 Siderophores

They are defined as high-affinity iron-chelating molecules with low molecular weight, iron acquisition from the host aggravates the virulence of various pathogenic microbes. Examples of siderophores secreted by *Klebsiella* spp include Salmochelin, yersiniabactin, aerobactin, and enterobactin (Holden *et al.*, 2015).

2.4.1.4 Adhesins

Adherence to host surfaces is a step in the process of infection by various pathogens, *Klebsiella* spp utilize fimbriae or pili. They have two types of pili; fim (type 1) and mrk (type 2) adhere to the mucosal surface of the host and therefore, play an important role in colonization. mrk is particularly important in the formation of biofilms (Schroll *et al.,* 2010). Both types have been implicated in the colonization of catheters resulting in catheter-associated infections (Murphy et al.,, 2013).

2.5 Hospital-Acquired Infections (HAI) associated with opportunistic *Klebsiella* spp.

Klebsiella spp is among the major causes of HAIs in developing countries (9.9% in the United States); it's the third leading cause after *Staphylococcus aureus* and *Clostridium difficile*. In developing countries, the extent and magnitude of *Klebsiella*-associated HAIs are largely underestimated or even unknown due to limited resources for diagnosis and surveillance (Nejad *et al.,* 2011). The most implicated species is *Klebsiella pneumoniae* which causes pneumonia, bloodstream infections, and UTIs (Magill *et al.,* 2014).

2.5.1 Pneumonia

Klebsiella pneumoniae is the third leading causative agent of hospital-acquired pnemoniae in the United States. This refers to pneumoniae which occurs within more than 48 hours of hospital admittance (Magill *et al.,* 2014). Among intensive care unit patients, *Klebsiella* spp is the principal cause of ventilator-associated pneumonia (Kalanuria *et al.,* 2014; Selina *et al.,* 2014) which is accountable for approximately 83% of nosocomial pneumonias (Richards *et al.,* 2000). In Egypt, a neonatal intensive care unit reported an incidence rate of 21.4% in reference to ventilator-associated pneumonia caused by particularly *K. pneumoniae* (Abdel-Wahab *et al.,* 2013).

In Kenya, a study conducted in Kilifi examining isolates from 2001-2011 showed that *K. pneumoniae* was responsible for 94% of hospital-acquired pnemoniae and 63% of community-acquired pnemoniae (Henson *et al.,* 2017). In another study conducted in western Kenya studying isolates from 2003-2013, *K. pneumoniae* accounted for 23%

of hospital-acquired pnemoniae, of those 82.6% were from the newborn unit (Ogalo *et al.,* 2016).

2.5.2 Bloodstream infections (BSIs)

BSIs are frequently secondary infections that occur when pathogens disseminate from various tracts including; gastrointestinal, respiratory, and urinary. Among Gram-negative bacteria, *E.coli* is the leading cause of bloodstream infections. It is followed closely by *Klebsiella pneumoniae* (Magill *et al.,* 2014). The population mortality of *K. pneumoniae*-associated bloodstream infections is approximated at 1.3 per 100,000 people while the case mortality rate is approximately 20-30% (Meatherall *et al.,* 2009).

Risk factors for bacteremia include age particularly very young and very old, intravenous drug abuse, and the presence of comorbidities such as malnutrition, catheterization, diabetes, therapeutic immunosuppression, autoimmune disorders, hypertension, reticuloendothelial blockade, organ malignancy (Deku *et al.,* 2019; Gavazzi *et al.,* 2002; Hsu *et al.,* 2003; Uslan *et al.,* 2007; Wester *et al.,* 2013). In adults, cancer is the major co-morbidity associated with nosocomial BSIs while liver disease and diabetes mellitus, are the main co-morbidities associated with community-acquired *K. pneumoniae* bloodstream infections (Kang *et al.,* 2006).In South Africa, a 6-year study reported that 86.1 % of *K. pneumoniae* associated bloodstream infections were hospital-acquired (Lochan *et al.,* 2017). In Malawi, *Klebsiella* spp was accountable for 4.4% of all BSIs from 1998 to 2016 with 90.5% of the isolates being extended-spectrum beta-lactamases (ESBL) (Musicha *et al.,* 2017).

In Nigeria, *Klebsiella pneumoniae* was the most common causative agent of neonatal bloodstream infections (West *et al.,* 2012). A study conducted in Kilifi, between 2002 and 2009 showed that *Klebsiella pneumoniae* was accountable for 20% of all hospital-acquired bloodstream infections (Aiken *et al.,* 2011).

2.5.3 Urinary Tract Infections

K. pneumoniae commonly invades the urinary tract to cause infections when it finds its way from the gastrointestinal tract. The major predisposing factors include diabetes mellitus, long stays in hospitals, indwelling urinary catheterization, and chronic

institutional residence (Kodner *et al.,* 2010). Indwelling catheter-associated urinary tract infections (CAUTIs) are primarily expedited by the formation of biofilms on the catheters (Schroll *et al.,* 2010). In women, *Klebsiella pneumoniae* associated UTIs are often recurrent due to their anatomy and are responsible for significant morbidity (Kodner *et al.,* 2010). In Morocco, a retrospective study covering 3 years reported that *K. pneumoniae* was responsible for 22% of all the urinary Enterobacteriaceae, out of which 25.5% were ESBL while 7% were carbapenem-resistant (El Bouamri *et al.,* 2015).

2.5.4 Diarrhoea

Hospitalization predisposes patients to various infections, among them is nosocomial diarrhea. *K. oxytoca* and *K. pneumoniae* are the most implicated species. *Klebsiella oxytoca* pathogenic strains act by producing a toxin that impedes DNA synthesis. They are responsible for 50-80% of cases of hemorrhagic colitis not caused by *Clostridium difficile* after antibiotics use (Smith *et al.,* 2009). Diarrheic strains of *K. pneumoniae* produce thermostable or thermolabile toxins, which cause bloody or watery chronic diarrhea, particularly in HIV-infected patients (Thi *et al.,* 2003).

2.6 Emergence of Hypervirulent Strains in the community setting.

In the 1980s and 90s, severe infections caused by *K. pneumoniae* were reported in Asia (Wang *et al.,* 1998). These infections were community-acquired, diverging from the typical presentation of *K. pneumoniae* associated nosocomial infections. These strains are now referred to as hypervirulent *K. pneumoniae* (hvKP). They are implicated in infections such as meningitis, bloodstream infections, pyogenic liver abscess (PLA), and endophthalmitis (Fang *et al.,* 2007). An estimated 3-11% of PLA patients further develop endophthalmitis (Sheu *et al.,* 2011).

The emergence of these hypervirulent *K. pnemoniae* strains has started to occur worldwide (Bialek-Davenet *et al.,* 2014; El-Mahdy *et al.,* 2018; Russo *et al.,* 2019). Previously, these strains were very susceptible to most antibiotics of choice (Fang *et al.,* 2007). However, more resistant strains have emerged in the last decade (Gu *et al.,* 2018; Juan *et al.,* 2020; Yu *et al.,* 2018) For instance carbapenem-resistant ST 11 hvKP

isolates have recently caused an outbreak in China. This signals the potential of double-risk isolates i.e. those that are resistant to most antibiotics and capable of causing very severe infections (Gu *et al.,* 2018).

2.6.1 Hypervirulent *K. pneumoniae* virulence Factors

Hypervirulent *K. pneumoniae* possesses a unique property in that; it can cause serious infection in healthy people**.** This property is primarily associated with the accessory genome which encodes for various virulence factors (Shon *et al.,* 2013). These isolates are hypermucoviscous; trying to pick a colony in a strand using a loop typically results in the bacteria clinging on to the media. This is referred to as the string test commonly used to characterize the hvKP phenotype. RmpA and MagA are the two proteins associated with the hvKP phenotype (Shon *et al.,* 2013) and are involved in the regulation of capsule production (Yu *et al.,* 2006).

K1 and K2 are the most implicated capsule types in hvKP isolates, they play a significant role in virulence (Yu *et al.,* 2006). Aer is the most common siderophore associated with hvKP (Russo *et al.,* 2015). Another siderophore identified to be secreted by the hvKP is Ybt (Holt *et al.,* 2015), which not only occurs in hvKP but also in the classical *K. pneuomoniae.* Allantoin is a metabolism product in various pathogens including *K .pneumoniae* (Navone *et al.,* 2014). The existence of an allantoin utilization operon has been reported in hvKP strains, particularly those implicated in PLA. Further reports show that when the regulator gene of the operon is deleted in a mouse model, the virulence decreases significantly. This stipulates that the ability of *K. pneumoniae* to utilize a nitrogen source aggravates its virulence in particular sites of infection. (Chou *et al.,* 2004).

2.7. Antibiotic Resistance in *Klebsiella* spp

According to the Centers for Disease Control and Prevention (CDC), in the United States, more than two million people are infected by antibiotic-resistant organisms yearly. It further estimates that out of the infected, 23,000 die (CDC, 2016). In the past decades, various microorganisms particularly *Klebsiella* spp have experienced significant changes in antibiotic resistance. Several factors have contributed to the

dissemination of these antibiotic-resistant organisms including misuse and abuse of antibiotics in human health, animal health, and plant sectors (Dadgostar, 2019). Antibiotic resistance in *Klebsiella* spp is facilitated by different mechanisms; those that expedite B-Lactams resistance have the most significant effect on efficient and effective treatment (Salyers *et al.,* 2004). In hospitalized patients, infection with antibiotic-resistant *Klebsiella* spp is often preceded by colonization with antibiotic-resistant *Klebsiella* spp. The accessory genome particularly plasmids play a very significant role in the dissemination of resistance genes which has resulted in limited treatment options (Martin *et al.,* 2016).

2.7.1 β-Lactamase-Producing *Klebsiella* spp

Resistance to penicillin was first noted by Alexander Fleming. He reported that some bacteria including *E.coli* that were previously susceptible to penicillin could no longer be inhibited by the antibiotic. Later, this phenomenon was defined as antibiotic resistance and associated with enzymes secreted by these organisms. The enzymes were defined as Beta-lactamases. Genes encoding for these enzymes are found in both the core and accessory genome. For instance, all *Klebsiella* spp have the SHV gene in the chromosome which encodes for ampicillin resistance (Babini *et al.,* 2000; Bialek-Davenet *et al.,* 2014). Among plasmid-mediated B-Lactamases is the AmpC enzyme which confers resistance to penicillin, 2^{nd,} and 3rd generation cephalosporins, and cephamycins. AmpC enzymes are mainly present in *K. pneumoniae*. Besides B-Lactamases, other mechanisms include; the alteration of penicillin-binding protein thus reducing B-Lactam affinity (Meroueh *et al.,* 2003) and efflux pumps.

2.7.2 Extended-Spectrum β-Lactamases

Extended-spectrum B-lactamases are enzymes with the ability to hydrolyze penicillin, monobactams, and oxymino-cephalosporins which are the 3rd generation cephalosporins; however, they are inhibited by carbapenems and B-Lactamases inhibitors such as amoxicillin-clavulanate,ticarcillin-clavulanate, ampicillin-sulbactam,piperacillin-tazobactam, cefoperazone-sulbactam. B-Lactamses encoding genes are mainly found in plasmids which also harbor resistance genes to heavy metals. This is a characteristic that enhances the fitness of strains possessing these

plasmids (Bush *et al.*, 2010). Examples of Extended-Spectrum β-Lactamases include; TEM, SHV, OXA, CTX-M which are encoded by the following genes *bla* TEM-1, *bla* SHV-1, *bla* OXA-1, *bla* CTX-M respectively. (Bush *et al.*, 2010) In Kenya, infections associated with ESBL-producing organisms are typically treated with Carbapenems (MOH-Kenya, 2009).

2.7.3 Carbapenem-Resistant *Klebsiella pneumoniae* (CR-Kp)

The leading carbapenem-resistant Enterobacteriaceae (CRE) is *Klebsiella pneumoniae.* The selective pressure associated with the treatment of ESBL infections with carbapenems antibiotics may have resulted in their resistance. Centers for Disease Control declared carbapenem-resistant Enterobacteriaceae an urgent threat to public health in 2013 (CDC, 2016; Health & Services, 2013). CRE is estimated to cause 9,000 infections, 80% of these infections are caused by *Klebsiella* spp (CDC, 2016).

K. pneumoniae resistant to carbapenems utilizes various mechanisms which include; efflux pumps up-regulation (Filgona *et al.,* 2015), outer membrane porins alteration (Kaczmarek *et al.,* 2006) and carbapenemase enzymes hyperproduction. *K. pneumoniae* Carbapenemase (KPC) is majorly plasmid-mediated (Bush *et al.,* 2010).

In addition to KPC enzymes, other plasmid-mediated carbapenemases have emerged globally. The New Delhi-B-lactamase 1 (NDM-1) emerged in India, VIM carbapenemases in Greece and Italy, IMP (Imipinases) in Japan (Limbago *et al.,* 2011) these strains of infection are often associated with hospitalization and travel to endemic areas (Van der Bij *et al.,* 2012).VIM and IMP types are encoded on an integrin which can either be integrated into a chromosome or a plasmid (Pournaras *et al.,* 2005). OXA types particularly OXA-48 are encoded on a plasmid, this makes its dissemination to other species relatively easy (Poirel *et al.,* 2004). In Kenya, the first NDM-producing Klebsiella pneumoniae was detected in 2011(Poirel *et al.,* 2011). In recent years, a study conducted in Kilifi examined isolates from 2001-2011also observed NDM-producing isolates (Henson *et al.,* 2017).

2.7.4 Colistin Resistance

Colistin belongs to the polypeptides class of antibiotics known as polymyxins. In the 1970s it was effective in treating Gram-negative bacteria. However, it was phased out due to its damaging effect on the nervous system (Jerke *et al.,* 2016). CRE's emergence has led to the comeback of colistin for use as a last resort. This use has subsequently led to resistance, which is mediated by genes such as *mgr*B and *mcr* (Poirel *et al.,* 2014; Wright *et al.,* 2014).In china an *E.coli* spp isolate resistant to colistin was discovered in 2015, this resistance was mediated by mcr-1 gene which is plasmid-mediated (Liu *et al.,* 2016). In 2016, *K. pneumoniae* resistant to colistin was discovered but this resistance was not conferred by *mcr-1* gene (Chen *et al.,* 2017).

2.8 Risk Factors for infection with Antibiotic-Resistant *Klebsiella pneumoniae*

The risk factors for infection with endemic antibiotic-resistant *Klebsiella* spp in hospital and community settings are similar. They include extended hospitalization and/or ICU admission (Hu Y *et al.,* 2016; Jahani-Sherafat *et al.,* 2015), mechanical ventilation (Michalopoulos *et al.,* 2011), renal dysfunction (Lautenbach *et al.,* 2001; Nathisuwan *et al.,* 2001), old age usually above 65 years and /or residing in a nursing facility, surgical procedures such as organ transplant, dialysis, malignancy and dermatitis, diabetes mellitus(Wolfe *et al.,* 2014) and prior antibiotic use(Jiao *et al.,* 2015; Kofteridis *et al.,* 2014). These risk factors apply for both ESBL and carbapenem-resistant *Klebsiella* spp

2.9 Laboratory diagnosis of *Klebsiella spp*

According to the Infectious Diseases Society of America and the American Society for Microbiology guide for Diagnosis of Infectious Diseases the specimen of choice based on the site of infection is as follows: Blood is the specimen of choice for bloodstream infections, midstream urine is preferred for urinary tract infections and diarrheal stool is preferred specimen for diarrhea illness. Rectal swabs are not recommended for adults as they are less sensitive to culture methods, however, in symptomatic children, rectal swabs and stool samples are equally sensitive (Kotton *et al.,* 2006). Sputum and blood are the specimen of choice for community-acquired

pnemoniae however, in ventilated patients endo-nasal tracheal aspirates are preferred (Langelier *et al.,* 2018; Metlay *et al.,* 2019).

The most common phenotypic methods for identifying *Klebsiella* spp are culture and biochemical testing. For culture, macConkey media is the preferred media, where *Klebsiella spp* appear mucoid and ferments lactose. Biochemical testing is performed using a panel of a wide range of tests including Triple sugar iron (TSI), Urea test, Sulphur indole motility (SIM), Methyl red, Voges-Proskauer, and Citrate utilization test. It is either conducted using test tubes or the commercially available API 20E identification system (Shahab, 2017).

Up until recently, serology testing was performed based on the various capsule antigens internationally recognized. However, due to various challenges such as cross-reactions among the antigens, it was replaced with molecular methods such as Polymerase chain reaction (PCR). These molecular methods either use singleplex or multiplex primers to target various virulence genes such as *irp2, wcaG, rmpA, rmpA، allS* , and *fimH* (Hormozi *et al.,* 2018). In addition, they also target various capsular types ranging from K1-K20 as well as 16S–23S conserved regions. Fingerprinting by GTG 5 targets variable number tandem repeat (VNTR) regions (Turton *et al.,* 2010).

With regard to resistance genes, these methods can detect genes on chromosomes as well as in mobile genetic elements such as plasmids, integrons, transposons, and insertion sequences. Such resistance genes include *bla* TEM, *bla* SHV, *bla* OXA, *bla* KPC, *bla* NDM, (Smalla *et al.,* 2000). The challenge with utilizing these assays is that they detect both viable and non-viable organisms' despite being more sensitive than culture. Whole genome sequencing allows for the determination of the complete DNA sequence of an organism and thus facilitating its detection and characterization This is inclusive of not only virulence genes but also AMR genes (Shahab, 2017).

2.10 Treatment of Klebsiella Infections

The choice of treatment is based on various factors including local antimicrobial sensitivity, site of infection, and comorbid conditions. Generally, susceptible strains are treated with drugs such as aminoglycosides (e.g., gentamicin), fluoroquinolones

(e.g. ciprofloxacin), third-generation cephalosporins (e.g., cefotaxime, ceftazidime, ceftriaxone), monobactams (aztreonam) macrolides (erythromycin), carbapenems (e.g., imipenem/meropenem). Monobactams and fluoroquinolones are particularly used in patients with beta-lactam drug allergies. These drugs are either be used individually (monotherapy) or together with others(combination therapy). For Extended-spectrum beta-lactamase (ESBL) producing strains, carbapenems are used particularly meropenem and imipenem. For carbapenemase-producing strains, colistin may be used as monotherapy or in combination with tigecycline. In Kenya, the choice of treatment for *Klebsiella* spp infections is 3rd generation cephalosporins and fluoroquinolones especially when the infections are bacteremic (MOH-Kenya, 2009).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

Mukuru slum is one of the largest urban settlements in Nairobi with a population of approximately 700,000 (KNBS, 2019). It stretches along the Nairobi River and it is located on wastelands in the industrial area of the city between the Outer Ring Road, North Airport Road, and Mombasa road. It is divided into 2 major villages namely; Mukuru kwa Njenga and Mukuru kwa Reuben villages. It is densely populated and made of temporary structures mostly corrugated metal sheets. Based on unpublished data Mukuru has approximately five public schools and five health facilities. The collection sites were outpatient health facilities namely:

1. Municipal city council (MCC).
2. Missionaries of Mary Mukuru kwa Njenga clinic (MMM),
3. Mukuru kwa Reuben clinic (MR)
4. Mbagathi hospital (MB)

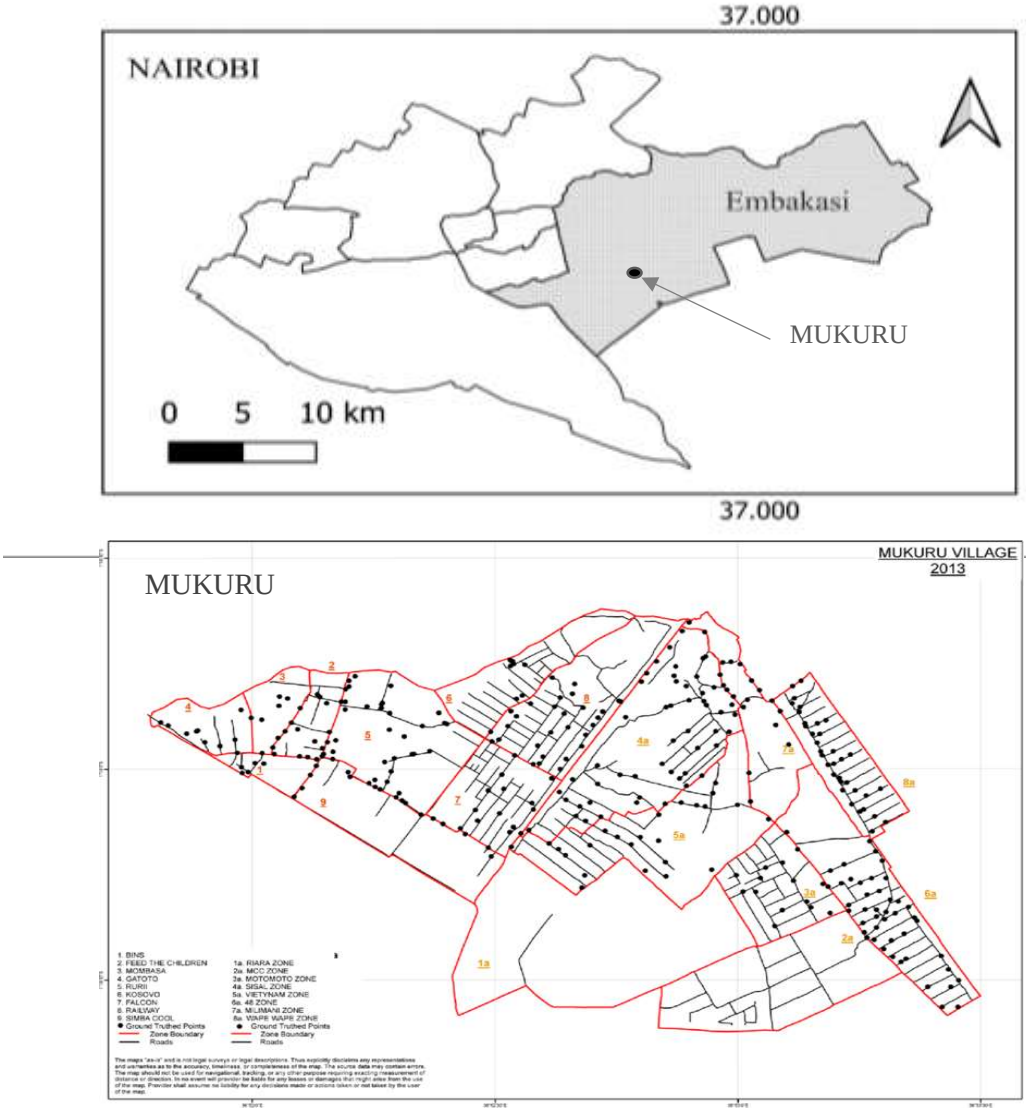


Figure 3:1: A map showing location of Mukuru Slums in Nairobi.

3.2 Study design

A cross-sectional laboratory-based study analyzed stool samples collected from the field during the study period.

3.3 Study population

The study participants were children and minors under the age of 16 years. Children below 5 years are vulnerable to a myriad of infections due to their underdeveloped immunity while children above 5 years are exposed to lifestyle and behavioral risk factors such as eating habits and WASH challenges and hence included in this study. Included in the study were children and minors below the age of 16 years and who must have been residing in Mukuru slums for at least 3 months prior to the study. For diarrheic cases, participants must have presented with episodes of loose or watery diarrhea within the last three days. The mode of entry into the health facilities was their management. Eligible participants were identified after assessment by the clinical officers on duty. The purpose of the study was explained and subsequently, consent was sought.

3.3.1 Inclusion criteria.

- Children and minors from 1- 16 years of age, those that reside in Mukuru slums.
- Diarrheic children are those that presented with episodes of loose or watery diarrhea within the last three days.
- Consent from parent or guardian.
- Non-diarrheic children especially those that attended the mother and child health clinic.
- The study participants who had not taken antibiotics for the current episode of diarrhea.

3.3.2 Exclusion criteria

- Children who were too weak to give a sample.
- Children who did not reside in Mukuru Slums.

- The children who had taken antibiotics for the current episode of diarrhea.

3.4 Sample size determination

The sample size was determined using the Fisher exact test formulae (Sprent, 2011)

$$n = \frac{Z^2PQ}{d^2}$$

Where;

n = Estimated sample size

Z = 1.96 at 95% confidence level

P = Estimated prevalence of MDR in *Klebsiella* spp is 36.7% (Taitt *et al.,* 2017).

Q = 1-P

d = degree of accuracy which is 0.05 at 95% confidence level.

$$= \frac{1.96^{2*}0.367*0.633}{0.05^2}$$

$$= \frac{3.8416*0.367*0.633}{0.05}$$

=356.9

=357

3.5 Sampling method

This study utilized purposive sampling. Participants who met the inclusion criteria were purposively sought during hospital visits and stool samples were collected before the initiation of treatment. The mode of entry into the health facilities was their management. Illegible participants were identified after assessment by the clinical officers on duty. The purpose of the study was explained in detail and subsequently, consent/assent was sought. Thereafter, participants' guardians were given poly pots and instructed on how to collect a stool sample.

3.6 Collection and transport of stool specimen.

Five grams of stool samples were collected from the participants and transported to the Salmonella surveillance unit I (SASU I) laboratory in the Center for Microbiology Research (CMR) of the Kenya Medical Research Institute (KEMRI) at 4^{o}C in Carry Blair transport media.

3.7 Laboratory Procedures

All bacteriological media was prepared at least two days before sampling. After preparation, it was quality controlled using positive and negative test organisms to ensure sterility.

3.7.1 Specimen Enrichment

Upon reception, the stool specimen was enriched in selenite fecal broth (Oxoid, Basingstoke, UK) and then incubated for 18-24 hours at 37^{0}C.

3.7.2 Culture and subculture

After enrichment, the samples were inoculated onto MacConkey media (Oxoid) and then incubated overnight at 37^{0}C.

Discrete suspected colonies of *Klebsiella* spp were subcultured onto Meuller Hinton agar (Oxoid) to get pure colonies. *Klebsiella* spp colonies were lactose fermenters, large, mucoid, and doom shaped.

3.7.3 Gram Stain

Pure colonies of *Klebsiella spp* suspects were subjected to various steps of gram stain i.e. application of primary stain (Crystal violet), mordant (Iodine), decolorizer (Acetone), and secondary stain (Safranin). Rod bacillus in singles, pairs or short chains proceeded to biochemical testing.

3.7.4 Biochemical testing

Pure colonies of Klebsiella spp suspects were subjected to the following biochemical tests; Triple sugar iron (TSI) (Oxoid), Urea test (Oxoid), Sulphur indole motility (SIM) (Oxoid), Methyl red (Sigma Aldrich, USA), Voges-Proskauer (Sigma Aldrich) and

Citrate utilization test(Oxoid). Analytical Profile Index (API 20 E) was used to confirm the *Klebsiella* isolates.

3.7.4.1 Triple sugar iron (TSI) test

TSI detected the ability of an organism to produce hydrogen sulphide and/ or ferment sugars such as glucose, sucrose, and fructose. The surface of the medium was inoculated using a straight loop on the slant and a needle stab at the butt. Fermentation of various sugars resulted in a change of colour, from red to yellow in both the slant and butt and the production of gas was demonstrated by the appearance of cracked media. Both *K. pneumoniae* and *K. oxytoca* appeared yellow in both slant and butt and showed gas production.

3.7.4.2 Citrate utilization test

Citrate utilization was used to detect the ability of an organism to utilize citrate as a carbon source. Simmons citrate agar was used. The medium was inoculated by a loop on the slant on the surface of the medium. Utilization of citrate resulted in color change from blue to green. *K. pneumoniae* and *K. oxytoca* were positive for this test

3.7.4.3 Sulphur indole motility (SIM)

Sulphur indole motility (SIM) is a combination of various tests i.e. motility, indole, and hydrogen sulphide. The medium was inoculated using a needle stab at the butt. Colour changes to black indicated the presence of hydrogen sulphide gas. Upon addition of Kovacs reagent, the formation of a pink ring was indicative of a positive indole test. Turbidity was indicative of a positive motility test. Both *K. pneumoniae* and *K. oxytoca* were negative for the hydrogen sulphide and motility test. For the indole test, *K. pneumoniae* was negative while *K. oxytoca* was positive.

3.7.4.4 Methyl red test

Methyl red test was used to detect the ability of an organism to utilize glucose and convert it to a stable acid such as acetic and lactic acid as an end product. The medium was inoculated using a loop. Upon addition of methyl red indicator, color change to red was indicative of a positive methyl red test. Both *K. pneumoniae* and *K. oxytoca*

were negative. This test was particularly useful in the differentiation between *E. coli* and *Klebsiella ssp*.

3.7.4.5 Voges-Proskauer test

Voges-Proskauer was used to detect the ability of an organism to produce acetoin. The broth was inoculated using a loop.

Upon addition of 2 drops of α-Naphthol Reagent and 3 drops of 40%, Potassium Hydroxide to the incubated broth, colour change to red on the surface of the broth was indicative of a positive Voges-Proskauer test. Both *K. pneumoniae* and *K. oxytoca* were positive.

3.7.4.6 Urea test

This test was used to detect the ability of an organism to split urea by producing urease enzyme. The medium was inoculated using a stab needle. Production of urease resulted in a colour change from yellow to pink. Both *K. pneumoniae* and *K. oxytoca* were positive.

3.7.4.7 Analytical Profile Index (API 20 E)

API-20E test (bioMerieux, France) was used to confirm *Klebsiella pneumoniae* and *Klebsiella oxytoca*. The incubation chamber was prepared by distributing about 5mls of distilled water into the wells of the tray to create a humid chamber. The strip was removed from its packaging and placed in the tray. Two discrete colonies of Klebsiella suspects were emulsified in 5ml of normal saline to achieve a homogenous suspension. With a sterile pipette, filled both the tubes and the cupules for CIT, VP, GEL tests with the suspension. Filled only the tubes (and not the cupules) of the other tests with the suspension. The following tests: ADH, LCD, ODC, UREA, and H2S, were covered with the mineral oil to create an anaerobic environment. Closed the lid and incubated the strip at 37^{o}C overnight. After incubation, TDA, VP1&2, JAMES reagents were added to the appropriate wells. Depending on colour changes, the scores were obtained

and recorded. Thereafter, the scores were entered into API 20E web for identification and a threshold of 90% was utilized.

3.7.5 Antibiotic Sensitivity Testing

Kirby – Bauer disc diffusion technique was used on the *Klebsiella spp* isolates (Schwalbe, Steele-Moore, & Goodwin, 2007). Using a sterile swab, a pure colony of *Klebsiella spp* was emulsified in a normal saline solution. The suspension was adjusted to a 0.5 McFarland standard. It was inoculated onto Mueller-Hinton agar plates (4mm depth) by swabbing and rotating several times to ensure the entire surface of the agar was covered including the rim. Antibiotic disks were dispensed appropriately using a disk dispenser and sterile forceps. A panel of antibiotic disks for Ampicillin (AMP, 10 µg), Cefotaxime (CTX 30 µg), Ceftriaxone (CRO 30 µg), Ceftazidime (CAZ 30 µg), Cefoxitin (FOX 30 µg), Imipenem (IPM 10 µg, Meropenem (MEM 10 µg), and Amoxicillin-Clavulanate acid (AMC 30 µg) were used on the first plate. This facilitates the observation of a synergistic zone that typically forms when a cephalosporin antimicrobial combines with a Beta-Lactamase inhibitor. The second plate had: Gentamicin (CN 10 µg), Ciprofloxacin (CIP 5 µg), Nalidixic acid (NA 30 µg), Chloramphenicol (C 30 µg), Streptomycin (STR 30 µg) Co-trimoxazole: (STX 25 µg), Tetracycline (TE 30 µg) and Aztreonam (ATM 30 µg). All discs were obtained from Oxoid, UK

All the plates were incubated at 37°C for 18 hours, and inhibition zones were measured and interpreted according to Clinical Laboratory Standard Institute (CLSI) 2019, guidelines. The standard control strain *E. coli* (ATCC-25922) was used to assure the testing performance of the potency of antibiotics discs and the quality of the media.

Table 3.1: Panel of antibiotics used for sensitivity testing

PLATE A		PLATE B	
Antibiotic	Class of antibiotic	Antibiotic	Class of antibiotic
Ampicillin (AMP)	Penicillin	Gentamicin(CN)	Aminoglycosides
Cefotaxime (CTX)	3rd Generation	Ciprofloxacin (CIP)	Fluoroquinolone
Ceftriaxone(CRO)	3rd Generation	Nalidixic Acid (NAL)	quinolone
Ceftazidime (CAZ)	3rd Generation	Chloramphenicol (CHL)	Chloramphenicol
Amoxicillin/Clavulanic acid (AMC)	β-lactam/β-lactamase inhibitor combination	Streptomycin (STR)	Aminoglycosides
Cefoxitin (FOX)	Cephamycin	Sulfamethoxazole/Trimethoprim (SXT)	Folate and dihydofolatebiosynthesis
Imipenem	carbapenem	Tetracycline (TE)	Macrolide
Meropenem	Carbapenem	Aztreonam	Monobactam

3.7.6 Phenotypic screening for ESBL-producing *Klebsiella spp*

The double disk synergy method was used to detect ESBL-producing *Klebsiella spp* where 4 antibiotics discs were used including Cefotaxime (CTX) (BD), Cefotaxime/Clavulanic acid (CTX/CLA) (BD), Ceftazidime (CAZ) (BD) and Ceftazidime/Clavulanic acid (CAZ/CLA) (BD). These antibiotic discs were placed 30mm from each other on Mueller Hinton agar media plates on which a confluent layer of the test isolates had been swabbed. The test was considered positive when the difference of inhibition zones between CAZ/CLA and CAZ or CTX/CLA and CTX was greater or equal to 5mm.

The 42 isolates that were ESBL positive and were resistant to at least one fluoroquinolone and or carbapenems were then subjected to Minimum Inhibitory Concentration test (MIC) using the Vitek 2 machine (bioMerieux) using the GN83 card for antibiotic susceptibility testing (AST).

3.7.7 DNA Extraction

Extraction was performed for 42 isolates ESBL positive and which were resistant to at least one fluoroquinolone and or carbapenems. The boiling method at 95^0C for 15 minutes was utilized to extract DNA from pure isolates. Bacteria were grown on Muller Hinton and then incubated at 37 ^{o} C. Using a sterile loop an inoculum of pea size was scrapped from the culture and transferred into a 2ml Eppendorf tube. The tubes were then placed on a heating block and left to heat for a maximum of 15 minutes. After cooling, they were placed in a centrifuge, and the contents were centrifuged at 14000rpm for 5 minutes. The supernatant included the extracted DNA which was transferred to a sterile tube and stored at 4^0C awaiting PCR.

3.7.8 Detection of resistance-associated genes

After extraction, DNA amplifications were done using sets of different primers targeting resistance genes against 3rd generation cephalosporins (Table 3.2), fluoroquinolones (Table 3.3), and carbapenems (Table 3.4). A reaction mixture of 25 µL was used in a master mix containing 1 µl forward primer(0.2µM), 1 µl reverse primer (0.2µM),11 µl pcr water,11 µl pcr mix (QIAGEN, Germany) which includes *Taq* DNA Polymerase(2.5units), PCR Buffer(1x), $MgCl_2$ (0.2µM), and ultrapure dNTPs(200µM),) followed by addition of 1 µl template DNA.

Table 3.2: Primers used for detection of 3rd Generation cephalosporins resistance genes.

Gene	Primer sequence	Expected size (bp)	Annealing Temp(°C)	References
blaTEM	F-5'GCGGAACCCCTATTTG3'	793	50	(Moubareck
	R-5'TCTAAAGTATATATGAGTAAACTTGGTCTGAC 3'			*et al.*, 2009)
bla	F-5'TTCGCCTGTGTATTATCTCCCTG 3'	854	50	(Celenza *et*
SHV	R-5'TTAGCGTTGCCAGTGYTCG 3'			*al.*, 2006)
bla	F-5'ATGAAAAACACAATACATATCAACTTCGC 3'	820	50	(Yu a *et al.*,
OXA-1	R-5'GTGTGTTTAGAATGGTGATCGCATT 3'			2006)
bla	F-5'ATGTGCAGYACCAGTAARGTKATGGC 3'	593	60	(Iraz *et al.*,
CTX-M	R-5'TGGGTRAARTARGTSACCAGAAYCAGCGG 3'			2015)

Table 3.3: PCR Primers used for detection of fluoroquinolones resistance genes.

Gene	Primer sequence	Expected size	Annealing temperature	Reference
aac(6')-1b-cr1	F-5'ATATGCGGATCCAATGAGCAACGCAAAAACAAAGTTAG3' R-5'ATATGCGAATTCTTAGGCATCACTGCGTGTTCGCTC-3'	482	55	(Robicsek *et al.*, 2006)
aac(6')-1b-cr2	F-5′-TTGCAATGCTGAATGGAGAG-3′ R-5′CGTTTGGATCTTGGTGACCT-3′	482	55	(Robicsek *et al.*, 2006)
qnrA	F-5′-ATAAAGTTTTTCAGCAAGAGG-3′ R-5′-ATCCAGATCGGCAAAGGTTA-3′	624	55	(Cavaco *et al.*, 2008)
qnrB	F-5′-GGMATHGAAATTCGCCACTGC-3′ R-5′-TTTGCYGYYCGCCAGTCGAAC-3′	469	55	(Cavaco *et al.*, 2008)
qnrS	F-5′-GCAAGTTCATTGAACAGGGT-3′ R-5′-TCTAAACCGTCGAGTTCGGCG-3′	417	55	(Cavaco *et al.*, 2008)
parC1 parC2	5'-ATGAGCGATATGGCAGAGCG-3 5'-TGACCGAGTTCGCTTAACAG-3	412	57	(Cavaco *et al.*, 2008)
parE1 parE2	5'-GACCGAGCTGTTCCTTGTGG-3 5'-GCGTAACTGCATCGGGTTCA-3	272	55	(Cavaco *et al.*, 2008)

Table 3.4: Primers used for detection of carbapenems resistance genes.

Gene	Primer sequence	Expected size (bp)	Annealing Temp(°C)	References
bla KPC	F-5 TGTTGCTGAAGGAGTTGGGC'3'	863	61	(Moubareck *et al.*, 2009)
	R-5' TGTTGCTGAAGGAGTTGGGC3'			
bla NDM-1	F-5'GAGATTGCCGAGCGACTTG 3'	591	61	(Iraz *et al.*, 2015)
	R-5'CGAATGTCTGGCAGCACACTT 3'			

Amplification conditions consisted of 30 cycles of 94 ℃ for 30 seconds, 55 °C for 30 seconds, and 72 ℃ for 30 seconds, with a final extension step of 72 °C for 10 min (Robicsek *et al.*, 2006).

Gel electrophoresis of PCR products was carried out to view the detected resistance genes. 1-2% agarose gel was prepared depending on the expected size of the target DNA and stained with SYBR Green solution (7.5 µl for 75ml of gel). 5 µl of loading dye was mixed with 10 µl of amplified PCR products on a sterile aluminum foil and then loaded into the wells of the gel. The gel was electrophoresed at a voltage of 100V for 30 minutes and UV Tran illuminator was used to visualize the bands of the amplified DNA. A digital camera was used to take photographs of the gels for documentation.

3.7.9 Genetic relatedness of bacteria isolates

This was performed using GTG 5 5'-GTGGTGGTGGTGGTG-3'primers. A total volume of 25 µl reaction mixture was used, composed of 1 µl primer(0.2µM),11.5 µl PCR water, 11.5 µl PCR mix (QIAGEN) which includes *Taq* DNA Polymerase (2.5 Units), PCR Buffer, $MgCl_2$ (0.2µM) and ultrapure dNTPs(200µM) with 1 µl template DNA. Amplification conditions constituted; initial denaturation at 95^0C for 2 minutes, final denaturation for 30 seconds, annealing of primers at 40^0C for 30 seconds, initial extension at 65^0C for 5 minutes, and final extension at 65^0C for 15 minutes. The amplified products were electrophoresed in 2% agarose gel and stained using SYBR Green solution. 5 µl of loading dye was mixed with 10 µl of amplified PCR products. Gel electrophoresis was done on 100V for 30 minutes and UV Tran illuminator was used to visualize the bands.

3.8 Biosafety issues

Stool samples and isolates were considered infectious and handled using appropriate personal protective equipment at the time of collection, transportation, and processing. Disposal of biological waste was done following KEMRI biosafety guidelines.

3.9 Ethical Approval

Ethical approval was obtained from the Kenya Medical Research Institute-Scientific and Ethics Review Unit (Appendix 3). This study did not involve invasive procedures and thus no harm was done to the participants. Confidentiality was maintained throughout and after the study period. Samples were assigned unique codes only identified by the principal investigator.

Although no direct benefit was availed to the participants, the results of this study were of benefit to the general population. Finally, only those participants whose consent was sought and ensured through assent by their parents were recruited into the study.

3.10 Intellectual Property Rights (IPR)

All IPR issues were handled in accordance with the KEMRI guidelines.

3.11 Data management and analysis

Participants' data were recorded in Microsoft Excel and WHO-NET softwares with password protection. Descriptive analysis of the data was performed where measures of central tendency and variability were determined. This data was presented in bar graphs. Logistic regression was performed to test for significant associations for AMR for multiple variables including diarrheic versus non-diarrheic patients ($p<0.05$) was considered significant. This was done using STATA software. Antibiotics susceptibility patterns data was analyzed using the WHO-NET software to determine resistance, intermediate and susceptible frequencies, and proportions. Phylogenetic relatedness/similarities was determined using BioNumerics tool.

3.12 Dissemination of findings

Results obtained from this study were shared with the health facilities for clinical care purposes. A manuscript was prepared for publication in a relevant journal to create wider awareness of the findings.

CHAPTER FOUR

RESULTS

4.1 Demographic characteristics of children from Mukuru slums

A total of 1171 children were recruited into this study comprising 592(50.56%) males and 579(49.44%) females. Distribution of participants among 1-50, 51-100, 101-150 and 151-200 age categories (in months) was as follows; 576(49.19%), 364(31.08%), 138(11.79%) and 93(7.94%) respectively. Diarrheic children were 514(43.89%) while non-diarrheic children were 656 (56.02%). Distribution between resident villages namely; Mukuru kwa Njenga village (MN) and Mukuru kwa Reuben village (MR) was 413(35.27%) and 196(16.74%) respectively. 562 (47.99%) children's guardians did not provide their exact residence in Mukuru (Table 4.1).

Table 4.1: Demographic characteristics of study participants'

Variable		**Frequency(n)**	**Percentage (%)**
Gender	Male	592	50.56%
	Female	579	49.44%
*Age category	1-50	576	49.19%
	51-100	364	31.08%
	101-150	138	11.79%
	151-200	93	7.94%
Residence	*MN	413	35.27%
	*MR	196	16.74%
	*Village unknown	562	47.99%
Symptoms	Diarrheic	515	43.89%
	Non diarrheic	656	56.02%

*Age category is in months *MN= Mukuru kwa Njenga village *MR= Mukuru kwa Reuben village * Village unknown= Village information not provided in the questionnaire.

4.2 Prevalence of *Klebsiella spp* isolated in Children from Mukuru slums

Of the 1171 participants recruited in the study, the prevalence of *Klebsiella* spp carriage was 33.7% (395/1171). The prevalence of *K. pneumoniae* was established at 31.2% (365/1171) while that of *K. oxytoca* was at 2.6% (30/1171). Within *Klebsiella* spp therefore children were significantly 12 times more likely to be colonized with *K. pneumoniae* (OR 12.2; p=0.0001).

Although a significant association was statistically derived between *Klebsiella* intestinal carriage and the residential area, this association could not be concluded due to the number of participants whose villages were not captured (Table 4.2). Further, no significant association was observed between carriage and presentation type (OR 1.2; p=1.3). All other correlates of carriage included age and gender (Table 4.2).

Table 4.2: Prevalence of Klebsiella spp in Children from Mukuru slum (n = 1171)

Variable		Frequency(n)	Percentage (%)	*O.R	P value
Serotype	*K. pneumoniae*	365	31.16%	12.17	0.0001
	K. oxytoca	30	2.56%	R	
Gender	Male	202	17.25%	1.05	0.07116
	Female	193	16.48%	R	
*Age category	1-50	238	60.25%	2.7	0.001
	51-100	88	22.28%	R	
	101-150	59	14.94%	0.67	0.0001
	151-200	10	2.53%	0.11	
Residence	*MN	135	11.52%	0.78	*0.0001
	*MR	88	7.51%	0.51	0.0732
	*Village unknown	172	14.68%	R	
Symptoms	Diarrheic	216	18.45%	1.21	0.1285
	Non diarrheic	179	15.29%	R	

*Age category is in months *MN= Mukuru kwa Njenga village *MR= Mukuru kwa Reuben village * Village unknown= Village information not provided in questionnaire *O.R = Odds Ratio

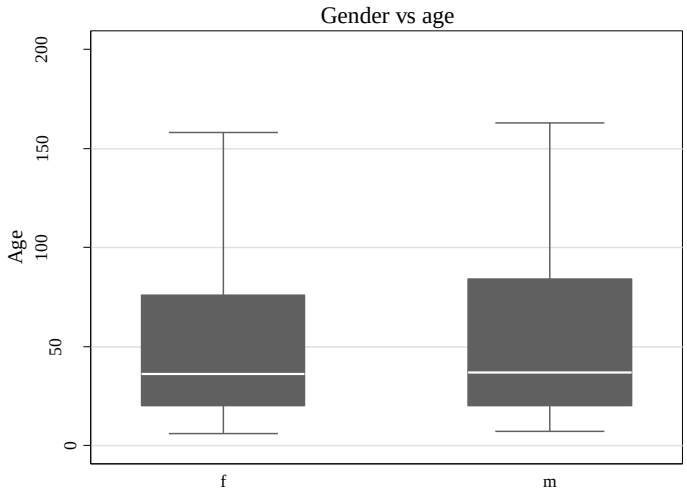


Figure 4:1: Distribution of children's age between genders; those colonized with *Klebsiella* spp.

4.3 Antibiotic resistance patterns of *K. pneumoniae* and *K. oxytoca*

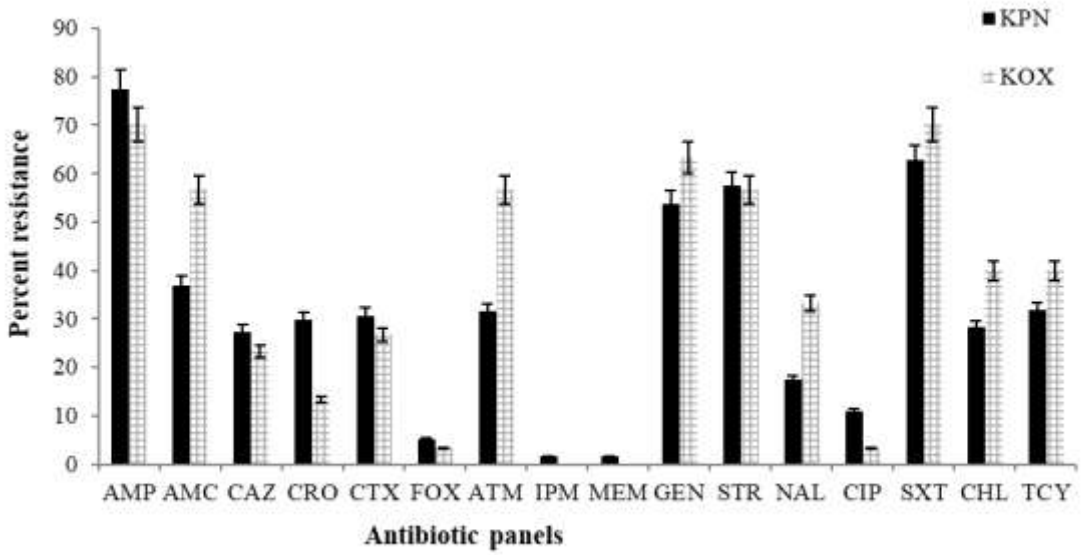


Figure 4:2: Resistance patterns of Klebsiella pneumoniae and Klebsiella oxytoca isolated from children and minors from Mukuru slums, Nairobi Kenya.

Highest percentage resistance (with a 5% margin of error) is observed for AMP with lowest resistance shown for IPM and MEM. Key: Ampicillin (AMP), Amoxicillin-Clavulanate acid (AMC), Ceftazidime (CAZ), Ceftriaxone (CRO), Cefotaxime (CTX), Cefoxitin (FOX), Aztreonam (ATM), Imipenen (IPM), Meropenem (MEM), Gentamicin (GEN), Streptomycin (STR), Nalidixic acid (NA), Ciprofloxacin (CIP), Trimethoprim Sulphamethoxazole: (SXT), Chloramphenicol (CHL) and Tetracycline (TCY). KOX= *K. oxytoca* KPN= *K. pneumonia*

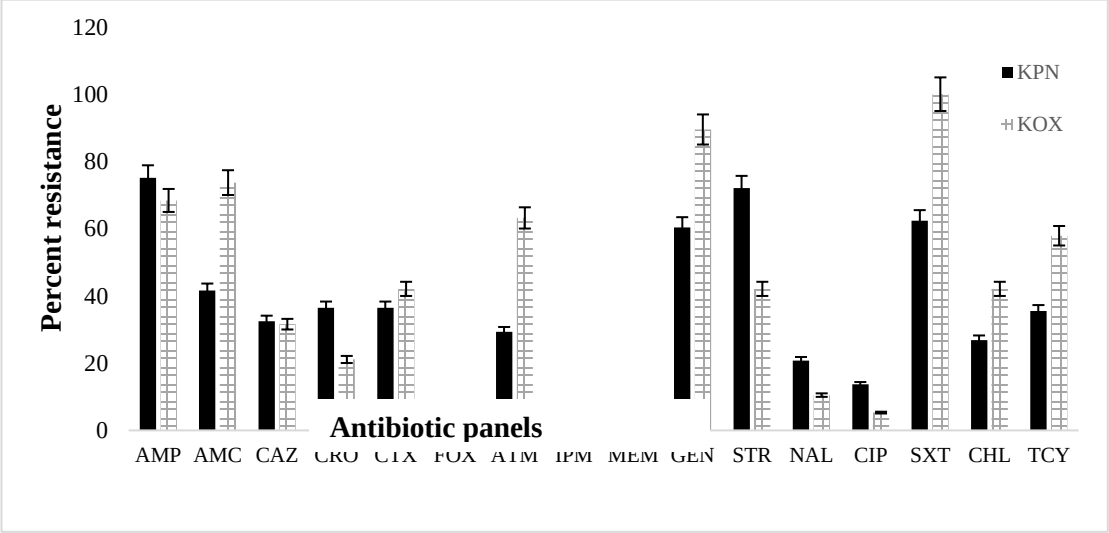


Figure 4:3: Resistance patterns of Klebsiella pneumoniae and Klebsiella oxytoca isolated from diarrheic children and minors from Mukuru slums, Nairobi Kenya.

Key: Ampicillin (AMP), Amoxicillin-Clavulanate acid (AMC), Ceftazidime (CAZ), Ceftriaxone (CRO), Cefotaxime (CTX), Cefoxitin (FOX), Aztreonam (ATM), Imipenen (IPM), Meropenem (MEM), Gentamicin (GEN), Streptomycin (STR), Nalidixic acid (NA), Ciprofloxacin (CIP), Trimethoprim Sulphamethoxazole: (SXT), Chloramphenicol (CHL) and Tetracycline (TCY). KOX= *K. oxytoca* KPN= *K. pneumonia*

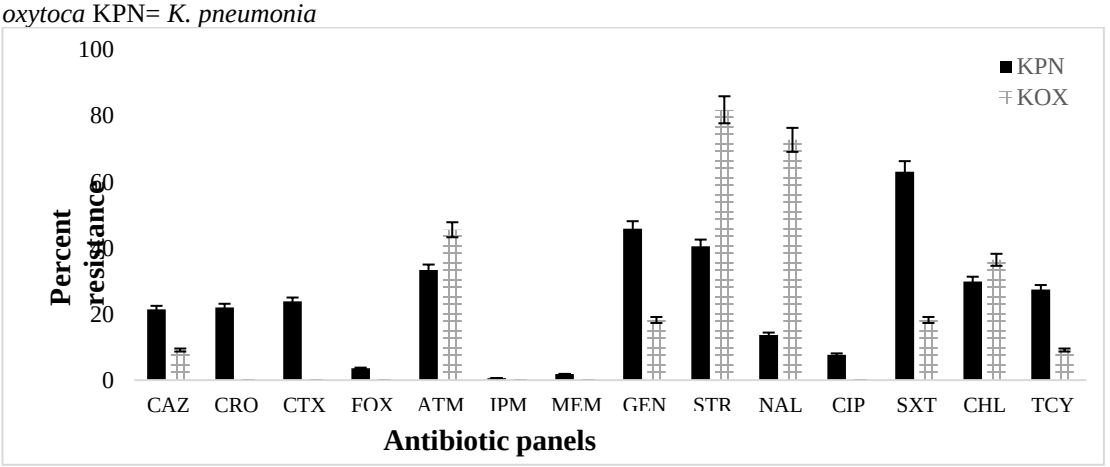


Figure 4.4: Resistance patterns of Klebsiella pneumoniae and Klebsiella oxytoca isolated from non-diarrheic children and minors from Mukuru slums, Nairobi Kenya.

Key: Ampicillin (AMP), Amoxicillin-Clavulanate acid (AMC), Ceftazidime (CAZ), Ceftriaxone (CRO), Cefotaxime (CTX), Cefoxitin (FOX), Aztreonam (ATM), Imipenem (IPM), Meropenem (MEM), Gentamicin (GEN), Streptomycin (STR), Nalidixic acid (NA), Ciprofloxacin (CIP), Trimethoprim Sulphamethoxazole: (SXT), Chloramphenicol (CHL) and Tetracycline (TCY). KOX= *K. oxytoca* KPN= *K. pneumonia*

K. pneumoniae showed highest resistance to ampicillin at 77.5% and moderate resistance to one of the most commonly prescribed amoxicillin/clavulanic acid at 37% with low or close to no resistance for imipenem and meropenem each recording percentage resistance of 1.6% (Figure 4.2). Generally, *K. pneumoniae* showed high resistance to 3rd generation cephalosporins (cefotaxime, ceftriaxone, and ceftazidime) compared to fluoroquinolones (nalidixic acid and ciprofloxacin).

The least possible resistance from *K. pneumoniae* was shown for cephamycin (cefoxitin) and carbapenems (imipenem and meropenem) (Figure 4.2). A similar trend was shown for *K. oxytoca* which again showed high resistance to ampicillin at 70% with resistance to the most commonly empirically prescribed amoxicillin/clavulanic acid also being relatively high at 56.7%. Moderate resistance was observed for Nalidixic acid and cefotaxime at 33.3% and 26.7% respectively, with low resistance observed for Ciprofloxacin and cefoxitin both at 3.3%. No resistance was observed to the carbapenems (imipenem and meropenem) by *K. oxytoca* (Figure 4.2).

4.4 Prevalence of Multidrug-resistant (MDR) *K. pneumoniae and K. oxytoca* and their resistance patterns across different antibiotic panels

Multidrug resistance (MDR) was defined as an isolate non-susceptible to at least one agent in three or more antibiotic categories/classes (Magiorakos *et al.,* 2012). The prevalence of MDR *K. pneumoniae* in the population was 20.75%. (243/1171) while that of *K. oxytoca* was 2.47% (29/1171). Among the isolates, MDR *Klebsiella pneumoniae* was 64 % while MDR *K. oxytoca* was 96.7% (29/30). *K. pneumoniae* multidrug resistance was high accounting for 77.5% (283/365) of samples exposed to Penicillin, 73.7% (269/365 among Aminoglycosides, and 62.7% (229/365 among Folate biosynthesis inhibitor. Beta-lactam inhibitor combination, Tetracycline and Monobactam showed a rate of resistance of 37% (135/365, 31.8% (116/365), and 31.5% (115/365) respectively. Third-generation cephalosporins recorded a rate of resistance of 30.9% (113/365) while Quinolone and Fluoroquinolone were 18.4% (67/365). Less resistance rate was demonstrated against Cephamycin at 5.2% (19/365 and Carbapenem at 3.3% (12/365 (Table 4.3).

Multidrug resistance for *K. oxytoca* was highest against Aminoglycosides at 96% (29/30), Penicillin, and Folate Biosynthesis Inhibitor each at 70% (21/30).

The rate of resistance to monobactam and Beta-Lactam Inhibitor were each 57% (17/30). *K. oxytoca* showed minimal resistance to the 3rd generation cephalosporins, Quinolones and Fluoroquinolones, each group standing at 33.3% (10/30), with no resistance recorded against Carbapenems (Table 4.3).

Table 4.3: Multidrug Resistance frequency of *K. pneumoniae and K. oxytoca* to various classes of antibiotics

Class of antibiotics	*K. pneumoniae* n=365 (%)	*K. oxytoca* n=30 (%)
Penicillin	283(77.5)	21(70)
Beta-Lactam Inhibitor	135(37)	17(56.7)
Monobactam	115(31.5)	17(56.5)
Cephamycin	19(5.2)	1(3.3)
Third generation cephalosporins	113(30.9)	10(33.3)
Quinolone and Fluoroquinolone	67(18.36)	10(33.3)
Folate biosynthesis Inhibitor	229(62.7)	21(70)
Phenicol	103(28.2)	12(40)
Tetracycline	116(31.8)	12(40)
Aminoglycosides	269(73.7)	29(96.7)
Carbapenems	12(3.3)	0

There was a significant difference in resistance to monobactam (OR=0.56; p=0.02), third generation cephalosporins (OR=1.88; p=0.01), aminoglycosides (OR= 3.6; p=0.00) and beta-lactam inhibitor (OR=1.54p=0.05) observed in *K. pneumoniae* isolated from diarrheic children. This means that diarrheic children have a higher chance of colonization with *K. pneumoniae* resistant to the antibiotics stated above.

There was a significant difference noted in resistance to third-generation cephalosporins, among *K. pneumoniae* isolates obtained from children between 1 and 50 months (OR=0.43; p=0.002). Children in in this age group have higher odds of carrying *K. pneumoniae* resistant to third-generation cephalosporins.

Additionally, a significant difference was observed in resistance to phenicol (OR=1.81; p=0.02), tetracycline (OR=3.14; p=0.00), aminoglycosides (O. R=4.35;

p=0.000) and folate biosynthesis inhibitor (O. R=3.6; p=0.000) among *K. pneumoniae* isolates obtained from children residing in Mukuru kwa Njenga village. Male children (OR=4.69; p=0.05) showed a higher chance of colonization with *K. pneumonia* resistant to carbapenems (Table 4.4, 4.5, 4.6, and 4.7).

There was no significant difference in resistance to cephamycin from isolates obtained from participants among the various age categories, gender, resident villages, and symptoms. There was no significant difference in resistance to third-generation cephalosporins among isolates obtained from various resident villages and gender. In addition, no significant difference in resistance to quinolone and fluoroquinolone among isolates obtained from children among various age categories, resident villages, and gender. With regard to carbapenems resistance, no significant difference was observed among isolates obtained from children among various age categories, resident villages, and symptoms (Table 4.4, 4.5, 4.6, and 4.7). Although association was observed with regards to residence, it could not effectively be interpreted since a majority of the participants did not indicate their areas of residence.

Table 4.4: Frequency of resistance to Beta-Lactam class of drugs in *Klebsiella pneumoniae* isolated from Children in Mukuru slums

		Penicillin			**Monobactams**			**Cephamycin**			**3rd Gen cephalosporins**			**Carbapenems**		
		N (%)	O.R	P.V	N (%)	O.R	P.V	N (%)	O.R	P.V	N (%)	O.R	P.V	N (%)	O.R	P.V
Gender	Male	151(53.36)	1.14	0.592	70(61.90)	0.80	0.64	9(47.37)	0.80	0.64	60(53.10)	1.03	0.89	10(10.33)	4.69	0.05
	Female	132(46.64)	R	-	45(39.2)	R	-	10(52.63)	R	-	53(46.90)	R	-	2(16.67)	R	-
Residence	MN	102(36.04)	-	-	25(21.43)	0.56	0.09	11(57.89)	-	-	60(53.10)	0.48	0.05	4(33.33)	1.19	0.77
	MR	65(22.97)	-	-	31(27.38)	-	-	0	-	-	38(33.63)	0.93	0.86	0	-	-
	VU	116(40.99)	-	-	59(51.19)	-	-	8(42.11)	-	-	15(13.27)	R	-	8(66.67)	-	-
Age category	0-50	164(57.95)	0.59	0.12	43(37.39)	0.80	0.62	11(57.89)	2.21	0.31	53(46.90)	0.43	0.002	7(58.33)	1.37	0.28
	51-100	71(25.09)	R	-	54(46.96)	R	-	2(10.53)	R	-	36(31.36)	R	-	2(16.67)	R	-
	101-150	43(15.19)	0.56	0.18	13(11.30)	-	-	4(21.05)	3.09	0.20	20(17.70)	0.72	0.35	3(25)	2.2	0.37
	151-200	5(1.77)	0.23	0.04	5(4.35)	-	-	2(10.53)	11.71	0.02	4(3.54)	1.06	0.93	0	-	-
Symptoms	D	148(52.30)	0.74	0.23	49(42.86)	0.56	0.02	13(68.42)	1.91	0.20	73(64.30)	1.88	0.01	12(100)	-	-
	ND	135(47.70)	R	-	66(57.14)	R	-	6(31.56)	R	-	40(35.40)	R	-	-	-	-

*Age category is in months *MN= Mukuru kwa Njenga village *MR= Mukuru kwa Reuben village * VU-Village unknown; These were persons who declined to provide their exact residence in Mukuru. kox= *K. oxytoca* kpn= *K. pneumoniae* OR-Odds Ratio P v- P value

Table 4.5 Frequency of resistance to Quinolone &fluoroquinolone class of drugs and commonly antibiotics in *Klebsiella pneumoniae* isolated from Children in Mukuru slums

		Quinolone &fluoroquinolone			**Folate biosythesis inhibitor**			**Phenicol**			**Tetracyline**			**Aminoglycosides**			**Beta-Lactamase Inhibitor**		
		N (%)	O.R	P.V	N (%)	O.R	P.V	N (%)	O.R	P.V	N (%)	O.R	P.V	N (%)	O.R	P.V	N (%)	O.R	P.V
Gender	Male	33(49.25)	0.84	0.54	122(53.28)	1.07	0.73	59(57.28)	1.3	0.26	56(48.28)	0.77	0.26	134(19.81)	0.65	0.07	69(51.11)	0.91	0.66
	Female	34(50.75)	R	-	107(46.72)	R	-	44(42.72)	R	-	60(51.70)	R	-	135(50.19)	R	-	66(48.89)	R	-
Residence	MN	26(38.81)	1.67	0.07	86(37.50)	-	-	40(38.83)	1.81	0.02	54(46.55)	3.14	0.00	97(36.06)	4.35	0.00	61(45.19)	3.2	0.00
	MR	9(13.43)	R	-	44(19.21)	-	-	19(18.45)	R	-	13(11.21)	R	-	52(19.33)	R	-	21(15.56)	R	-
	VU	32(47.76)	-	-	99(43.23)	-	-	44(42.72)	-	-	49(42.24)	-	-	120(44.61)	-	-	53(39.20)	-	-
Age category	0-50	38(56.72)	0.78	0.46	131(57.21)	0.62	0.09	64(62.14)	0.95	0.84	66(56.90)	0.98	0.97	156(57.99)	1.19	0.55	73(54.07)	0.925	0.773
	51-100	18(26.87)	R	-	60(26.20)	R	-	26(25.24)	R	-	26(22.41)	R	-	58(21.56)	R	-	30(22.22)	R	-
	101-150	7(10.45)	0.51	0.20	34(14.85)	0.59	0.15	10(9.71)	0.47	0.08	19(16.38)	1.11	0.77	46(17.10)	1.87	0.13	27(20.00)	1.62	0.17
	151-200	4(5.95)	2.9	0.14	4(1.75)	0.32	0.11	3(2.91)	1.12	0.88	5(4.31)	2.79	0.15	9(3.35)	-	-	5(3.70)	2.25	0.25
Symptoms	D	33(49.25)	1.67	0.07	123(53.71)	0.97	0.89	53(51.5)	0.86	0.54	70(60.34)	1.46	0.09	167(62.08)	3.6	0.00	82(60.74)	1.54	0.05
	ND	34(50.75)	R	-	106(46.29)	R	-	50(48.5)	R	-	46(39.66)	R	-	102(37.92)	R	-	53(39.26)	R	-

*Age category is in months *MN= Mukuru kwa Njenga village *MR= Mukuru kwa Reuben village * VU-Village unknown; These were persons who declined to provide their exact residence in Mukuru. kox= *K. oxytoca* kpn= *K. pneumoniae* OR-Odds Ratio P v- P value

Table 4.6 Frequency of resistance to Beta-Lactam class of drugs in *Klebsiella oxytoca* isolated from Children in Mukuru slums

		Penicillin			**Monobactams**			**Cephamycin**			**3rd Gen cephalosporins**			**Carbapenems**		
		N (%)	O.R	P.V	N (%)	O.R	P.V	N (%)	O.R	P.V	N (%)	O.R	P.V	N (%)	O.R	P.V
Gender	Male	13(61.90)	2.15	0.40	6(35.29)	1.22	0.79	1(100)	-	-	4(40.00)	1.55	0.58	0	-	-
	Female	8(38.10)	R	-	11(64.71)	R	-	0	-	-	6(60.00)	R	-	0	-	-
Residence	MN	18(85.71)	-	-	14(82.35)	-	-	1(100)	-	-	8(80)	-	-	0	-	-
	MR	1(4.70)	-	-	1(5.88)	-	-	0	-	-	0	-	-	0	-	-
	VU	2(9.52)	-	-	2(11.77)	-	-	0	-	-	2(20)	-	-	0	-	-
Age category	0-50	17(80.95)	-	-	13(76.47)	-	-	0	-	-	6(60)	-	-	0	-	-
	51-100	2(9.52)	-	-	2(11.76)	-	-	1(100)	-	-	2(20)	-	-	0	-	-
	101-150	2(9.52)		-	2(11.76)	-	-	0	-	-	2(20)	-	-	0	-	-
	151-200	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-
Symptoms	D	13(61.90)	0.81	0.80	12(70.59)	2.05	0.35	1(100)	-	-	9(90)	9	0.55	0	-	-
	ND	8(38.10)	R	-	5(29.41)	R	-	-	-	-	1(10)	R	-	0	-	-

*Age category is in months *MN= Mukuru kwa Njenga village *MR= Mukuru kwa Reuben village * VU-Village unknown; These were persons who declined to provide their exact residence in Mukuru. kox= *K. oxytoca* kpn= *K. pneumoniae* OR-Odds Ratio P v- P value

Table 4.7 Frequency of resistance to Quinolone &fluoroquinolone class of drugs and common antibiotics in *Klebsiella oxytoca* isolated from Children in Mukuru slums

		Quinolone &fluoroquinolone			**Folate biosynthesis inhibitor**			**Phenicol**			**Tetracycline**			**Aminoglycosides**			**Beta-Lactamase Inhibitor**		
		N (%)	O.R	P.V	N (%)	O.R	P.V	N (%)	O.R	P.V	N (%)	O.R	P.V	N (%)	O.R	P.V	N (%)	O.R	P.V
Gender	Male	2(20)	0.37	0.28	9(42.86)	6	0.12	3(25)	0.52	0.43	4(33.33)	-	-	19(34.48)	-	-	8(47.06)	-	-
	Female	8(80)	R	-	12(57.14)	R	-	9(75)	R	-	8(66.67)	-	-	19(65.52)	-	-	9(52.94)	-	-
Residence	MN	9(90)	-	-	18(85.71)	-	-	12(100)	-	-	10(83.33)	-	-	26(89.60)	-	-	14(82.35)	-	-
	MR	0	-	-	1(4.76)	-	-	0	-	-	0	-	-	1(3.45)	-	-	1(3.33)	-	-
	VU	1(10)	-	-	2(9.58)	-	-	0	-	-	2(16.67)	-	-	2(6.90)	-	-	2(6.67)	-	-
Age category	0-50	9(90)	-	-	14(66.67)	-	-	9(75)	-	-	7(58.33)	-	-	22(75.86)	-	-	13(76.47)	-	-
	51-100	1(10)	-	-	2(9.52)	-	-	0	-	-	1(8.33)	-	-	2(6.90)	-	-	2(11.76)	-	-
	101-150	0	-	-	4(19.05)	-	-	2(16.67)	-	-	3(2.91)	-	-	4(13.79)	-	-	2(11.76)	-	-
	151-200	0	-	-	1(4.76)	-	-	1(8.33)	-	-	1(8.33)	-	-	1(3.45)	-	-	0	-	-
Symptoms	D	2(20)	0.04	0.002	19(90.48)	-	-	8(66.67)	1.27	0.75	11(91.67)	13.7	0.02	19(65.52)	-	-	14(82.35)	7.46	0.02
	ND	8(80)	R	-	2(9.52)	-	-	4(33.33)	R	-	1(8.33)	R	-	10(34.48)	-	-	3(17.65)	R	-

*Age category is in months *MN= Mukuru kwa Njenga village *MR= Mukuru kwa Reuben village * VU-Village unknown; These were persons who declined to provide their exact residence in Mukuru. kox= *K. oxytoca* kpn= *K. pneumoniae* OR-Odds Ratio P v- P value

4.5 Frequency of ESBL production in the isolated *Klebsiella spp*

The proportion of *K. pneumoniae* Extended Spectrum Beta Lactamase (ESBL) producing isolates was 22.74% (83/365). Out of these ESBLs, 11.23% (41/365) were resistant to at least one fluoroquinolone while 2.19% (8/365) were resistant to at least one carbapenem and at least one fluoroquinolone. Comparative analysis showed a significant likelihood with 60% more chance of isolating ESBLs among children aged between 0 – 50 months (OR=0.38; p=0.001) compared to children 51-100 months (OR=0.85; p=0.66). Again, although an association was observed for ESBLs and residence, this could not effectively be interpreted since a majority of the participants did not indicate their areas of residence. (Table 4.8). The prevalence of *K. oxytoca* ESBLs was 13.33% (4/30). Comparative analysis showed no difference in age category, gender, health facility, and symptoms (Table 4.8).

Table 4.8: Frequency of ESBL-producing *Klebsiella spp* isolated from children residing in Mukuru slums

Variables			N (%)	OD	P Value
		Female	40(48.19)	R	
	Kpn	Male	43(51.81)	0.96	0.87
		Female	2(50)	R	
Gender	Kox	Male	2(50)	2.25	0.46
		MN	43(51.81)	21.5	0.001
		MR	2(2.41)	R	-
		Village			
	Kpn	unknown	38(45.78)	-	-
		MN	3(75)	-	-
		MR	0	-	-
		Village			
Residence	Kox	unknown	1(25)	-	-
		1-50	34(40.96)	0.38	0.001
		51-100	28(33.73)	R	-
		101-150	17(20.48)	0.85	0.66
	Kpn	151-200	4(4.82)	-	-
		1-50	1(25)	-	-
		51-100	1(25)	-	-
		101-150	2(50)	-	-
Age category	Kox	151-200	0	-	-
		D	65(78.31)	4.84	0
	Kpn	ND	18(21.69)	R	
		D	4(100)	_	_
Symptoms	Kox	ND	0	_	_

KEY: *kpn= *K. pneumoniae* kox= *K. oxytoca* *D=diarrheic *ND=Non-Diarrheic *Age category is in months *MN= Mukuru kwa Njenga *MR= Mukuru kwa Reuben * Village unknown= Village information not provided in questionnaire

4.6 Resistance genes in the isolated *Klebsiella spp*

A total of 42/395(10.64%) isolates were examined for carriage of resistance genes. They were all from *K. pneumoniae* isolates. The *bla*TEM gene was the most common with all the 42 (100%) samples demonstrating the presence of this gene (Figure 4.5). The second gene identified was *bla*CTX-M, demonstrated in 40 (95.2%) of samples (Figure 4.5). It was followed closely by *bla*OXA, which was demonstrated in 28(66.67%) isolates (Figure 4.5) while *bla*SHV was demonstrated in 24 (57.14%) isolates (Figure 4.5). Among genes

conferring resistance to beta lactam class of antibiotics *bla*NDM demonstrated the least resistance in 3 (7.14%) Isolates (Figure 4.5).

Among genes conferring resistance to quinolones and fluoroquinolones *qnr*S was the most common, it was demonstrated in 23(54.14%) isolates (figure 4.6). It was followed closely by *par*C which demonstrated in 20(47.62%) isolates (figure 4.6). The low resistance was observed in *qnr*B which was demonstrated in 20(47.62% isolates (figure 4.6) while the least resistance was demonstrated by the *par*E, which was present in 16(38.09%) isolates (figure 4.6).

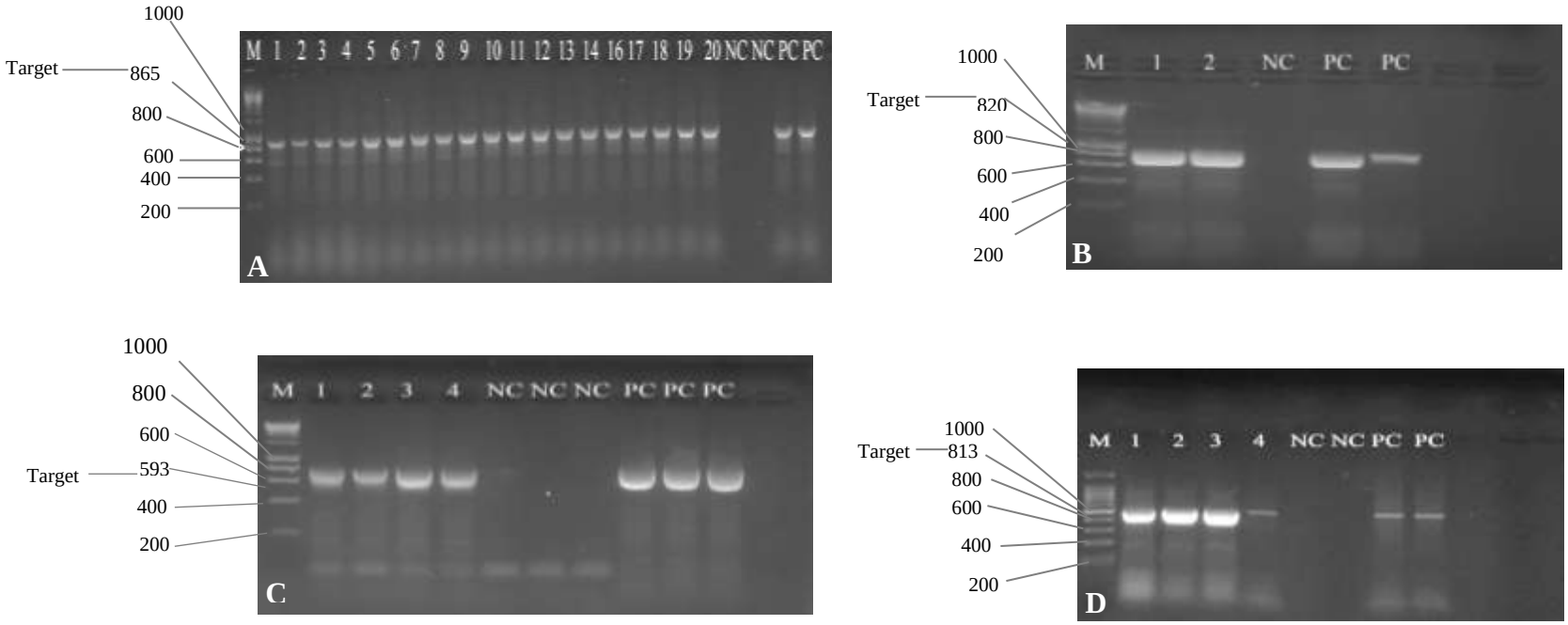


Figure 4.5: Gel photos for resistance genes to Beta-Lactam class of antibiotics.

A: bla TEM gene (865bp) B: bla OXA-1 gene (820bp). C: bla CTX-M gene (593bp). D: bla NDM gene (813bp).M represents the Molecular ladder. Numbers represent DNA numbers of test isolates. NC represents Negative Control (PCR water). PC represents the Positive Control (A=*Escherichia coli* NCTC 11560, B= *K. pneumoniae* ATCC 700603, C= *K. pneumoniae* NCTC 13465, D= *K. pneumoniae* NCTC 13443).

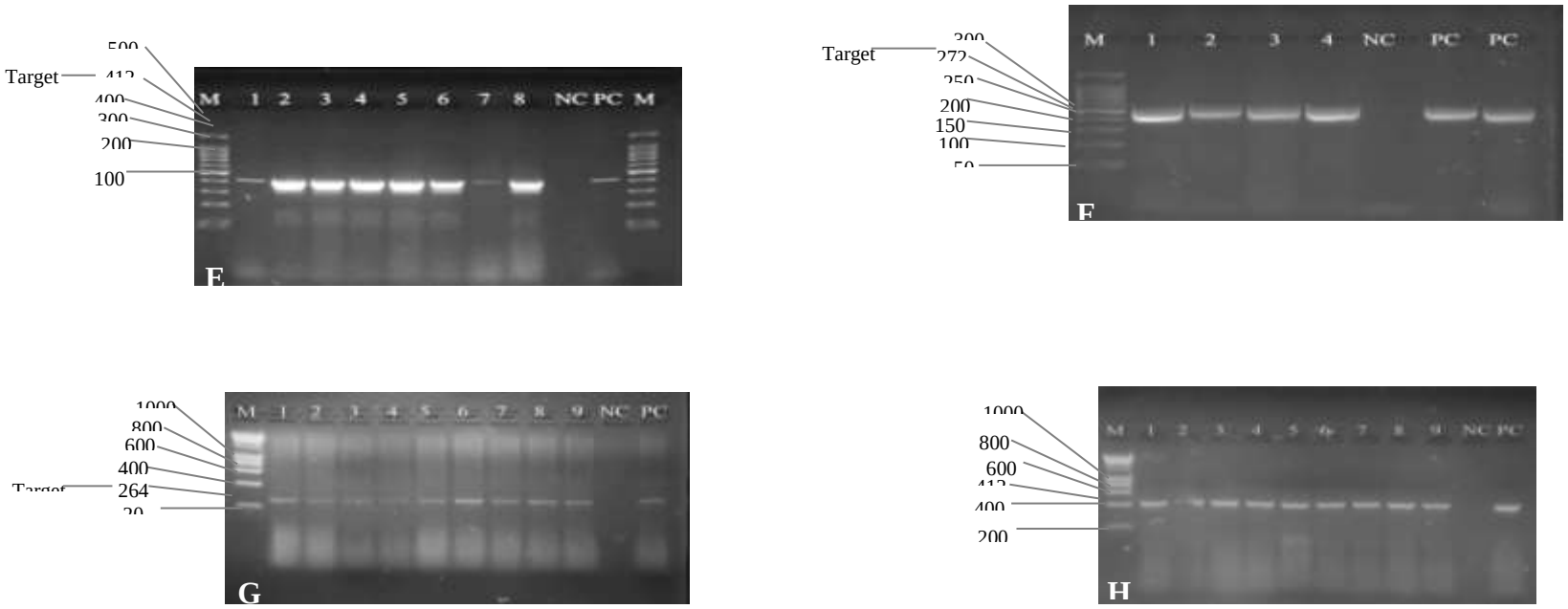


Figure 4.6: Gel photos for resistance genes to Quinolone and Fluoroquinolone class of antibiotics.

E: par C gene (412bp) F: par E gene (272bp). G: qnrB gene (264bp). H: qnrS gene (813bp).M represents the Molecular ladder. Numbers represent DNA numbers of test isolates NC represents Negative Control (PCR water). PC represents the Positive Control (E=*Citrobacter freundii* ATCC BAA 3038, F= *Staphylococcus aureus* ATCC BAA 3114, G= *K. pneumoniae* ATCC BAA-3066, H= *K. pneumoniae* ATCC BAA 3075).

Carriage of multiple genes bearing resistance to both 3rd generation cephalosporins and fluoroquinolones resistance antibiotics was observed in 90.48%) (38/42) isolates (Table 4.9) while carriage of 3rd generation cephalosporins and carbapenems resistance genes was observed in 7.14% (3/42) isolates. Carriage of resistance genes against three classes of drugs (Beta lactams, fluoroquinolones and Carbapenems) was only observed in 7.14% (3/42) *K. pneumoniae* isolates (Table 4.9).

Table 4.9: Carriage of multiple resistance genes in *K. pneumoniae* isolated from Children in Mukuru slums

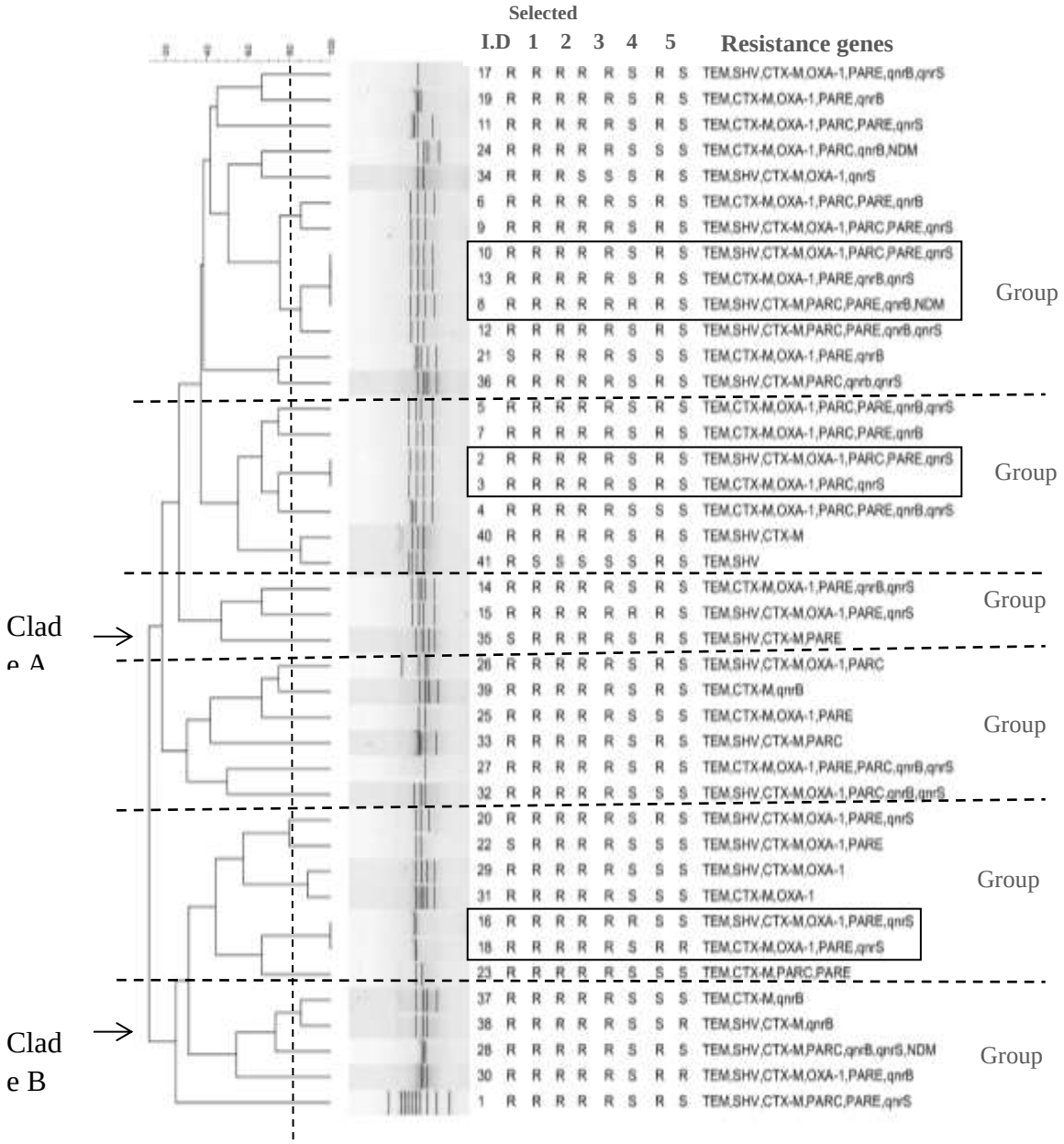
Isolate no.	3rd generation Cephalosporins resistance genes	Quinolone and Fluoroquinolones resistance genes	Carbapenems resistance genes
**1298	bla TEM-1,bla SHV-1,bla CTX-M	parC, parE, qnrS	-
**2018	bla TEM-1, bla CTX-M, bla OXA-1	parC, qnrS	-
**1471	bla TEM-1,bla CTX-M, bla OXA-1	parC, qnrS	-
**1204	bla TEM-1,bla CTX-M, bla OXA-1	parC, parE, qnrB, qnrS	-
**2215	bla TEM-1,bla CTX-M, bla OXA-1	parC, parE, qnrB	-
**2548	bla TEM-1,bla CTX-M, bla OXA-1	parC, parE, qnrB	-
**2600	bla TEM-1,bla CTX-M, bla OXA-1	parC, parE, qnrB	-
***1588	bla TEM-1,bla SHV-1,bla CTX-M	parC, parE, qnrB	bla NDM
**2893	bla TEM-1,bla SHV-1,bla CTX-M, bla OXA-1	parC,parE,qnrS	-
**1882	bla TEM-1,bla SHV-1,bla CTX-M, bla OXA-1	parC,parE,qnrS	-
**2315	bla TEM-1,bla CTX-M, bla OXA-1	parC,parE,qnrS	-
**1989	bla TEM-1,bla SHV-1,bla CTX-M	parC,parE,qnrB,qnrS	-
**1484	bla TEM-1,bla CTX-M, bla OXA-1	parE,qnrB,qnrS	-
**2555	bla TEM-1,bla CTX-M, bla OXA-1	parE,qnrB,qnrS	-
**2968	bla TEM-1,bla SHV-1,bla CTX-M, bla OXA-1	parE,qnrS	-
**2499	bla TEM-1,bla SHV-1,bla CTX-M, bla OXA-1	parE,qnrS	-
**1678	bla TEM-1,bla SHV-1,bla CTX-M, bla OXA-1	parE,qnrB,qnrS	-
**1535	bla TEM-1,bla CTX-M, bla OXA-1	parE,qnrS	-
**1082	bla TEM-1, bla CTX-M, bla OXA-1	parE,qnrS	-
**1369	bla TEM-1, bla SHV-1 ,bla CTX-M, bla OXA-1	parE,qnrS	-
**1923	bla TEM-1,bla CTX-M, bla OXA-1	parE,qnrB	-
**2306	bla TEM-1, bla SHV-1, bla CTX-M,bla OXA-1	parE	-
**1581	bla TEM-1 ,bla CTX-M,	parC,parE	-
***1720	bla TEM-1,bla CTX-M ,bla OXA-1	parC,qnrB	bla NDM
**2737	bla TEM-1, bla CTX-M, bla OXA-1	parE	-
**2472	bla TEM-1,bla SHV-1,bla CTX-M, bla OXA-1	parC	-
**2402	bla TEM-1, bla CTX-M, bla OXA-1	parC,parE,qnrB,qnrS	-
***2402	bla TEM-1, bla SHV-1, bla CTX-M	parC,qnrB,qnrS	bla NDM
**1214	bla TEM-1, bla SHV-1, bla CTX-M, bla OXA-1	parE,qnrB	-
**2642	bla TEM-1, bla SHV-1, bla CTX-M, bla OXA-1	parC,qnrB,qnrS	-
**2646	bla TEM-1, bla SHV-1, bla CTX-M	parC	-
**1287	bla TEM-1 , bla SHV-1, bla CTX-M, bla OXA-1	pare	-
**2951	bla TEM-1, bla SHV-1, bla CTX-M	pare	-
**2343	bla TEM-1, bla SHV-1, bla CTX-M	parC,qnrB,qnrS	-
**1195	bla TEM-1, bla CTX-M	qnrB	-
**2382	bla TEM-1, bla SHV-1, bla CTX-M	qnrB	-
**1290	bla TEM-1, bla CTX-M	qnrB	-

**2207	bla TEM-1, bla SHV-1	qnrS	-

** Is indicative of isolates that demonstrated resistance to two classes of antibiotics while *** is indicative of isolates that demonstrated resistance to three classes of antibiotics

4.7 Phylogenetic analysis of the isolated *Klebsiella spp*

The dendrogram was derived from the 42 *K. pneumoniae* ESBL producing isolates that were also resistant to fluoroquinolones and/or carbapenems. Within this dendrogram there were Clades (branch that includes a common ancestor and all of its descendants). Clustering groups (descendants in a clade at 40% similarity), Clustering sub groups (descendants in a clade at 100% similarity). Two clades designated A and B, 6 clustering groups designated group1-6 and 40 subgroups were recorded. Clade A includes group 1-4 while clade B includes group 5 and 6. There was 100% similarity index in Group 1, 2 and 5 as highlighted in the boxes. Of notice, was an outgroup observed in group 6 of clade B which showed a higher number of bands compared to the rest of the isolates group 6. Out of the 40 Sub groups 37.5% (15/42 showed >80% similarity index with the highest number observed in group 1 while, 62.5% (25/42) showed a similarity index <80% indicating that these 25 isolates were distantly related. Isolates that carried resistance genes to fluoroquinolones clustered tightly as observed in all the groups, while isolates that carried resistance genes to carbapenems were diverse and did not cluster together as observed in group 1 and 6. From the phylogenetic analysis therefore we state that 83.33% (35/42) of the isolates were diverse and hence disbanding the possibility of clonal spread of MDR strains (Figure 4.7).



Key: I.D: Isolate DNA Number. Antibiotic 1: Amoxicillin Clavulanate 2: Cefotaxime 3: Ceftazidime 4: Ceftriaxone 5: FEP 6: Amikacin 7: Ciprofloxacin 8: Meropenem. Highlighted boxes show isolates that demonstrated 100% similarity index.

Figure 4.7: Phylogenetic relatedness of *Klebsiella spp* isolated from Children in Mukuru slum

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 DISCUSSION

K. pneumoniae and K. *oxytoca* are ubiquitous and are found in various environments including mucosal membranes of humans where they colonize the gastrointestinal tract, the skin, and the nasopharyngeal. In the gastrointestinal tract, they occur as normal flora. However, when they cross the gastrointestinal mucosal membrane into other systems of the body, they become opportunistic pathogens, causing infections such as pneumonia, bloodstream infections, meningitis, and urinary tract infection.

This study reported a community prevalence of gastrointestinal *K. pneumoniae* of 31.16% (Table 4.2) and *K. oxytoca* of 2.56% (Table 4.2) among the slum-dwelling children. This prevalence noted in the community is higher than what has previously been reported among ICU patients (Gorrie *et al.,* 2017; Martin *et al.,* 2016) of 23% and 19%. No significant difference in isolation of *Klebsiella spp* was observed between males and females and, diarrheic and non-diarrheic children. However, a difference was noted among age categories where children between 101-150 months showed higher odds of *Klebsiella spp* colonization. This can be attributed to differences in dietary patterns and lifestyle. The high prevalence of *Klebsiella spp* in the community may not have a major impact on the children as the organisms do not cause infection in the gastrointestinal tract. However, these micro-organisms indicate the resistance genes circulating in Mukuru. These genes could be disseminated to other pathogens which poses a challenge in patient management (Janda *et al.,* 2006; Magill *et al.,* 2014; Shahab, 2017).

The proportion of MDR *Klebsiella* noted in this study (64.1%) (Chapter 4.4) is similar to a study done by Henson *et al.,* (2017) in Kilifi where the proportion of MDR was 63%, although the isolates were from invasive infections.

In contrast, a study conducted by Taitt *et al.,* (2017) in rural western Kenya showed a lower proportion of MDR *Klebsiella* of 36.7%. This contrast can be attributed to differences in economic, social, and environmental settings. In East Africa, the

proportion observed ranges from 80-95%, which is comparatively higher than that observed in this study (Stanley *et al.,* 2018; Tellevik *et al.,* 2016). While the prevalence of *K. oxytoca* was low at 2.56% among children in Mukuru, isolation of MDR *K. oxytoca* was high at 96.67% (29/30). Though not highly prevalent in children in Mukuru, it's alarming that nearly all the isolates of *K. oxytoca* are MDR. The latter implies that, if colonization by *K. oxytoca* proceeds to infection, the disease can record high treatment failures, particularly among immune-compromised persons. Additionally, *K. oxytoca* can transfer its resistance genes to other organisms including *K. pneumoniae* and other enteric bacterial pathogens, leading to a high burden of treatment failure. Unlike *K. pneumoniae*, horizontal transfer of genes in *K. oxytoca* is not well documented, although trends of the low prevalence of *K. oxytoca* with high isolation rates of MDR *K. oxytoca* have been documented in India (Singh *et al.,* 2016), among adults, and in Iran (Malekjamshidi *et al.,* 2020). In South Africa, MDR *K. oxytoca* prevalence ranged from 17.9% to 36%, although the isolates were from hospital-acquired infections (Fadare *et al.,* 2021; Vasaikar, *et al.,* 2017). The proportion of MDR *K. pneumoniae* observed in this community can be attributed to selective pressure for certain antibiotics and horizontal gene transfer through plasmids (Ruppé *et al.,* 2015). This indicates antibiotic use /misuse in Mukuru which contributes to the emergence and persistence of antibiotic resistance. The therapeutic use of different antibiotics for empirical and prophylactic management of gastrointestinal infections, which is rampant, in this slum community, cold also contribute to resistance. Additionally, ease of access to antibiotics as over-the-counter (OTC) medications, dispensing chemists record high purchase of relatively cheap antibiotics such as chloramphenicol, ampicillin, and co-trimaxazole may also lead to selective pressure for these antibiotics.

AMR determinants such as plasmids and insertion sequences containing multiple resistance genes can be present in these microorganisms. These determinants can transfer resistance genes in vitro (Hu *et al.,* 2020). Indeed, other studies describing the MDR patterns in Nairobi have described Mukuru slums as MDR hotspots (Kariuki *et al.,* 2019). The potential for aggravated transmission of MDR genes to vulnerable populations was demonstrated in this study due to the determination that there was no significant difference in the prevalence of MDR infections in the asymptomatic (non-

diarrheic) and the symptomatic (diarrheic) cases. The latter finding demonstrated that both the symptomatic and the asymptomatic play an equally significant role in the carriage of MDR.

Resistance patterns observed in 3rd generation cephalosporins can be attributed to the findings of the previous study done by Maina *et al.,* (2020) alluding to their widespread use and/or misuse in the health facilities in Kenya. The high frequency of the βeta-Lactams resistance genes of *bla* CTX-M, *bla* TEM, *bla* OXA-1, and *bla* SHV may be due to the presence of mobile genetic elements bearing these genes in this slum environment. Further, various studies in Africa (Katale *et al.,* 2020; Maina *et al.,* 2012; Mbelle *et al.,* 2020) have alluded to the fact that the high economic growth in recent years has led to ease of accessibility of βeta-Lactams over-the-counter leading to increased abuse and/or misuse of these antibiotics, hence the predominance of *bla* TEM and *bla* CTX-M genes in the environment. Of the 22.7% prevalent ESBL-producing *K. pneumoniae*, the rate of isolation was significantly higher in children below 50 months, potentially attributed to their underdeveloped immune system or possible nutritional deficiencies due to their residential environment. This ESBL prevalence however appears lower compared to other studies done in Kenya ranging between 44% (Henson *et al.,* 2017) and 71% (Maina *et al.,* 2012). It is however, noted that the prevalence of ESBL at 71% was established among *K. pneumoniae* isolated from urine samples.

Resistance to Quinolone and Fluoroquinolone from this study was generally low at 18.36% (Figure 4.2) and the predominant genes were *qnr*S, *qnr*B. Studies conducted in Africa noted similar genes in Morocco (Benaicha *et al.,* 2017), Egypt (Hamed *et al.,* 2018), and Tanzania with a prevalence ranging from 5-24%. In contrast, a study conducted in Togo reported qnrA as the most predominant qnr gene among *K. pneomoniae* (Salah *et al.,* 2019). The low prevalence observed can be attributed to low selective pressure for these antibiotics and horizontal gene transfer mediated by plasmids. Therefore, exacerbating fluoroquinolone resistance which is the choice of treatment for a variety of infections. In addition to PMQR, fluoroquinolone resistance can be mediated by chromosomal mutations, especially in DNA gyrase and topoisomerase encoding genes such *par*E and *par*C genes., which were also detected

during this study at 38% and 48% respectively Although these genes were observed in this study in relatively high proportions, the mutations can only be observed after performing DNA sequencing, which was a limitation in this study. Notably, isolates that carried *qnr* genes were all resistant to nalidixic acid, however, some isolates exhibited a partial reduction of ciprofloxacin efficacy to *K. pneumoniae* as opposed to conferring complete resistance to the antibiotic 0.25-0.5µg/mL). This indicates that *qnr* genes confer complete resistance to quinolones and partial resistance to fluoroquinolones. The low rate of resistance can be due to the low prescription of Ciprofloxacin and its high cost despite being widely available. Similar findings where *qnr*S and *qnr*B genes are most prevalent in Africa (Moumouni *et al.,* 2017; Salah *et al.,* 2019) have been documented.

Low resistance to carbapenems of 3.3% (Figure 4.2) was noted in this study. The prevalence of carbapenem resistance gene *bla* NDM-1 was also low at 7.1%. The low resistance can be due to their limited use and availability in the market in Kenya (Kivoto, 2016). Indeed, a similar study by Poirel *et al.,* (2011) conducted in Nairobi only observed one *bla* NDM positive isolate, with a similar study conducted in Kilifi, Kenya by Henson *et al.,* (2017) observed no *bla* NDM isolates.

The study in Kilifi however, documented a plasmid with a genetic architecture of a known *bla* NDM-carrying plasmid in a total of 25 isolates.

In this study *qnr* B and S genes were found to co-exist with *bla* CTX-M ESBLs. Co-carriage of ESBLs with fluoroquinolones can be attributed to the presence of plasmids containing a plethora of resistance determinants such as the *qnr* genes which encode for *qnr* protective proteins. According to literature, plasmid-mediated resistance to quinolone is often associated with ESBLs (Filippa *et al.,* 2013; Lagacé-Wiens *et al.,* 2007). Isolates that carried resistance genes to the 3 classes of drugs that were of interest to this study (3rd generation cephalosporins, fluoroquinolones, and carbapenems), were very low at 3/365(0.82%). The isolate that showed the highest rate of carriage of AMR determinants was as follows: *bla* TEM-1, *bla* SHV-1, *bla* CTX-M, *bla* NDM, *par*C, *par*E and *qnr*B. This coexistence of genes is uncommon but very worrisome as available options for treatment are extremely limited thus highlighting the dire effects of AMR on public health. By definition, carbapenem resistance also

fosters resistance to third-generation cephalosporins, and hence carbapenem resistance genes co-exist with ESBL encoding genes, a phenomenon that is well documented (Bi *et al.,* 2018; Hamzaoui *et al.,* 2018; Mathlouthi *et al.,* 2016; Messaoudi *et al.,* 2019).

Phylogenetic relatedness analysis showed a high number of <80% similarity index amounting to 62.5% (Figure 4.6), which is indicative of the high diversity among the isolates, ruling out the possibility of clonal spread of MDR strains. Isolates that showed >80% similarity index, amounting to 37.5% (Figure 4.6) were closely related. Those that showed a 100% similarity index were considered to be completely related and they amounted to 15% (Figure 4.6). The findings showed a high genetic diversity of *Klebsiella* strains circulating. Other studies conducted in Kenya showed that *K. pnemoniae* isolates fell into four or more phylogenetic lineages. (Henson *et al.,* 2017; Taitt *et al.,* 2017).

If colonization precedes infection, and there's high concordance between colonizing and infecting isolates (Martin *et al.,* 2016) then MDR *K. pneumoniae* such as those carrying AMR genes for 3rd generation cephalosporins (ESBLs), fluoroquinolones and or carbapenems pose a great risk to the community. Therefore, the identification of colonizing strains can inform patient care interventions.

5.2 LIMITATIONS

1. The study did not account for the polyclonal nature of *Klebsiella* spp as only one colony was picked from a single plate.
2. An exhaustive panel of resistance genes was not studied due to limited resources.
3. GTG 5' test used to determine the phylogenetic relatedness of *Klebsiella spp* has low resolution compared to tests such as whole genome sequencing which have high resolution.

5.3 CONCLUSIONS

A high proportion of MDR *K. pneumoniae* and MDR *K. oxytoca* observed among the participants is of public health concern.

Children in the <50 months age group showed the highest proportion of colonization with ESBL *K. pneumonia* and, therefore, they have the highest risk of infection which could result in dire outcomes including death.

Symptomatic (diarrheic) and asymptomatic (non-diarrheic) individuals play an equally significant role in the carriage and dissemination of MDR *Klebsiella*.

A high proportion of resistance genes against 3rd generation cephalosporins and fluoroquinolones demonstrated in the gastrointestinal tract of participants presents a threat to the community spread of MDR *Klebsiella*.

The low proportion of *Klebsiella* spp isolates carrying multiple resistance genes against 3rd generation cephalosporins, fluoroquinolones, and carbapenems combined shows that these classes of antibiotics are still effective as the choice of treatment for *Klebsiella spp.*

Phylogenetic relatedness analysis showed high diversity among *Klebsiella spp* isolated, which rules out the possibility of clonal spread of MDR strains.

5.4 RECOMMENDATIONS

There is a need for regular surveillance of AMR genes and mapping of MDR *Klebsiella* spp hotspots by researchers and the Ministry of health in Mukuru to facilitate potential interventions.

There is a need for researchers to strengthen the knowledge and evidence base of the gut resistome including transmission dynamics of MDR *Klebsiella* spp in the Mukuru community.

There is a need for the Ministry of Health to prioritize AMR intervention development focusing on key target populations such as asymptomatic individuals.

There is a need for health stakeholders to create awareness of antimicrobial resistance in the community, especially among those who are caregivers to children in the <50 months' age group.

There is a need for health stakeholders to properly implement various AMR policies such as the National Policy on Prevention and Containment of AMR.

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APPENDICES

Appendix I: Informed consent form (English)

STUDY TITLE: **Antimicrobial susceptibility and genetic basis of resistance of *klebsiella* spp isolated from diarrheic and non-diarrheic patients at outpatient health facilities in Mukuru informal settlement, Nairobi, Kenya.**

Principal investigator: Celestine Wanjiku Wairimu

Informed consent.

You are being invited to participate in this medical research study, whose objective is to identify the germs that are causing diarrhoea in children and how they are responding to commonly prescribed drugs. It is very important that you understand the following general principles that apply to all participants in our studies:

1) You and your child's participation is entirely voluntary;
2) You may withdraw from participation in this study or any part of this study at any time with no penalty or harm ;
3) After you read or listening as the investigator explain about this study, please ask any questions that will allow you to understand the study clearly.

What is important for you to know?

This is a non-invasive procedure where there is no pain. In this study, we need to study some of your child's faeces, the stool samples will be taken to the laboratory for

preparation and other tests. We will test for the presence of *Klebsiella* spp, antimicrobial susceptibility and genetic basis of resistance of *Klebsiella* spp.

In case a harmful germ has been detected will return to the hospital with these findings and seek medical intervention for your child. Your child's stool will be given a unique study number, all data collected will be kept confidential.

You and your family may not get any direct benefits from being in this study but what we find out will help us determine the best approach in management of *Klebsiella* infection in children in Kenya.

You can choose if you want to participate in this study or not. Participating in this study will not cost you or your family anything. You can also leave for any reason without any problem.

Who can participate in this study?

We can only include your child in this study if you give consent to participate, and if your child agrees to participate.

Questions about research

If you have any questions about this study, you may contact Celestine WanjikuWairimu Tel; +254715176048 during the study and in the future. If you have concerns about human rights, ethics and welfare issues you may contact the scientific and ethics review unit (SERU) at KEMRI P.O Box 54840-00200, Nairobi; telephone +254717719477, email address: seru@kemri.org.

INFORMED CONSENT AGREEMENT

I, Mr./Mrs./Miss _____, being a person aged 18 years and over and being the lawful/legal guardian of: (Child's name) ---------- voluntarily agree that my child may be included in the study which I have read or has been read to me. I have understood the implications and benefits of the study. I accept the tests to be carried out. I understand that I may withdraw him/her from the research at any time, for any reason, without any penalty or harm. All the above conditions have been explained to me in the _____ language in which I am fluent.

_____ Name of Child

_____ Age of child

_____ Parent's/Guardian's name

_____ Parent's/Guardian's signature

_____ Date

_____ Place

_____ Person Obtaining Consent

_____ Witness

Treatment Consent

If your child has bacteria pathogens, he/she can be offered treatment. The treatments are free. Is it okay for your child to receive treatment if he/she has *Klebsiella* infection?

_____ Yes

_____ No

_____ Parent's/Guardian's signature

OFFICIAL STAMP

Appendix II: Informed Consent Form (Swahili translation)

FOMU YA IDHINI

Uchunguzi huu ni juu ya: Utafiti juu ya dawa zinzofaa zaidi na msingi wa kigenetiki ya upinzani wa viini vya bacteria vilivyo patikana kutoka kwa wagonjwa na wasio kuwa wagonzwa wa kuharisha katika vituo vya Afya katika makaazi yasio rasmi Mukuru.

Wachunguzi wakuu: Celestine WanjikuWairimu

Maelezo kuhuzu ridhaa au ruhusa: Mwango anaombwa kushiriki kwenye utafiti huu wa uchunguzi unaoongozwa na mchunguzi ambao jina lake limeorodheswa hapo juu.

Ushiriki wako na wa mwanao ni wa kujitolea. Iwapo hutaki kushiriki, hutapata adhabu yoyote, mwanao atatibiwa na daktari au afisa wa uta bibu kwa njia ya kawaida. Unaweza kumwondoa mwanao katika mradi huu wakati wowote. Ukiamua kumwondoa mwanao kwenye mradi huu unapaswa kurudi kwa daktari wa mradi ili uweze kumwaarifu.

Unapaswa kusoma taarifa ifuatayo na uulize maswali yoyote kuhusu jambo lolote ambalo hujalifahamu kabisa kabla ya kuamua kushiriki au kutoshiriki. Utapewa fomu ili uweze kuweka.

Nini muhimu kujua: Huu ni utaratibu usiokuwa na maumivu yoyote. Kufanya uchunguzi huu, tutahitaji kinyesi cha mtoto wako. Kinyesi kitapelekwa kwenya maabara yetu kuandaliwa na kupiwma. Tutapima kama uko na viini vya bacteria na ni dawaa gani hususan inaweza kuuwa hivi viini vya bacteria. Mtoto wako akipatikana

na bacteria hawa tutarudi hapa hosipitali na majibu hayo ili aweze kupewa tiba. Mtoto wako atapewa nambari kwa ajili ya uchunguzi huu, jina la mtoto halitafichuliwa, tutatumia habari hii kujua juu ya wadudu hawa na jinsi ya kuwadhibiti tu.

Hakuna faida kamili kwako au familia yako utakaposhiriki katika uchunguzi huu ila matokeo ya uchunguzi huu yatasaidia kudhibiti magonjwa yanayoletwa na wadudu tumboni kwa watoto.

Unaweza amua kama utashiriki kwenye uchunguzi huu. Kushiriki kwako hakutakugharimu wewe au familia yako chochote, na waweza wacha kushiri wakati wowote.

Nani waweza shiriki kwenye uchunguzi huu? Twaweza shirikisha mtoto wako kwenya uchunguzi huu ikiwa utatoa idhinisho and kama mtoto atakubalikushiriki.

Maswali kuhusu uchunguzi

Ukiwa na swali lolote kuhusu uchunguzi huu, unawaeza kuwasiliana na Celestine WanjikuWairimu kupitia nambari za simu; +254715176048. Ikiwa una swali kuhusu haki za binadamu, maadili au shauri za maslahi, tafadhali wasiliana na kitengo cha marekebisho ya maadili ya kisayansi kule KEMRI kupitia sanduku la poster 54840-00200, Nairobi; nambari ya simu; +254717719477, barua pepe seru@kemri.org.

IDHINISHO

Mimi Bwana/Bi_____ nikiwa na miaka 18 au zaidi na nikiwa mzazi au mlezi halali wa (jina la mtoto)_____ na kubali kwa hiari mtoto wangu ajumuishwe kwenye uchunguzi huu ambao nimesoma au nimesomewa. Nimeilewa kiini na manufaa ya uchunguzi huu na ninakubabi uchunguzi huu uelndelee. Naelewa

kuwa ninaweza kumuondoa mtoto wangu kwenye uchunguzi huu wakati wowote bila faini au madhara yoyote. Nimeelezewa kanuni hizi zote kwenye lugha _____ninayo elewa vizuri

_____ Jina la mtoto-

_____ Miaka ya mtoto

_____ Jina la mzazi/mlezi

_____ Sahihi la mzazi/mlezi

_____ Tarehe

_____ Mahali

_____ Jina la anayepokea Idhini

_____ Jina la shahid

Idhinisho la kutibiwa

Ikiwa mtoto wako atapatikana na vimelea tumboni, anaweza tibiwa. Matibabu ni ya bure. Unakubali mtoto wako apewe matibabu ikiwa atapatikana na magonjwa haya?

_____ Ndio

_____ La

_____ Sahihi la mzazi/mlezi

STEMPU RASMI

Appendix III: Ethical training certificate

Appendix IV: Ethical approval letter

KENYA MEDICAL RESEARCH INSTITUTE

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KEMRI/RES/7/3/1 **February 25, 2019**

TO: **CELESTINE WANJIKU WAIRIMU**
PRINCIPAL INVESTIGATOR

THROUGH: **THE DIRECTOR, CMR**
NAIROBI

Dear Madam,

RE: **KEMRI/SERU/CMR/P00097/3796 *(RESUBMITTED II OF INITIAL SUBMISSION)*: ANTIMICROBIAL SUSCEPTIBILITY AND GENETIC BASIS OF RESISTANCE OF *KLEBSIELLA SPP* ISOLATED FROM DIARRHEIC AND NON-DIARRHEIC PATIENTS AT OUTPATIENT HEALTH FACILITIES IN MUKURU INFORMAL SETTLEMENTS**

Reference is made to your letter dated February 12, 2019. The KEMRI Scientific and Ethics Review Unit (SERU) acknowledges receipt of the revised study documents on February 13, 2019.

This is to inform you that the Committee notes that the following issues raised during the 282nd Joint Committee A, B and C meeting of the KEMRI Scientific and Ethics Review Unit (SERU) held on **December 11, 2018** have been adequately addressed.

Consequently, the study is **granted approval** for implementation effective this day, **February 25, 2019** for a period of one year. Please note that authorization to conduct this study will automatically expire on **February 24, 2020**. If you plan to continue data collection or analysis beyond this date, please submit an application for continuation approval by **January 13, 2020.**

You are required to submit any proposed changes to this study to SERU for review and the changes should not be initiated until a written approval from SERU is received. Please note that any unanticipated problems resulting from the implementation of this study should be brought to the attention of SERU and you should advice SERU when the study is completed or discontinued.

Yours faithfully,

ENOCK KEBENEI
ACTING HEAD
KEMRI SCIENTIFIC AND ETHICS REVIEW UNIT

Appendix V: *Klebsiella spp* biochemical testing summary

Table 1:Tube Biochemical Tests

Test	**Result**
Tripple Sugar Iron	Slant/Butt:Acid/Acid yellow/yellow Gas production- positive H_2S-negative
Sulphur Indole Motility	Suphur – negative Indole- *K. pneomoniae*-negative; *K. oxytoca*-positive Motility- Non-motile
Simmon Citrate	Citate positive
Methyl Red	Negative
Voges Proskauer	Positive
Urea	Postive

Table 2: Analytical Profile Index Biochemical Test

Test	**Result**	
	K. pneumoniae	***K. oxytoca***
ONPG(Ortho-nitro-phenyl-galactoside)	+	+
ADH (Arginine dihydrolase.)	-	-
LDC (Lysine decarboxylase.)	+	+
ODC (Ornithine decarboxylase)	-	-
CIT (Citrate.)	+	+
H2S (Hydrogen sulphide.)	-	-
URE (Urea.)	+	+

TDA (Tryptophane deaminase.)	-	-
IND (Indole.)	-	+
VP (Voges proskauer.)	+	+
GEL (Gelatin.)	-	-
GLU (Glucose.)	+	+
MAN (Mannitol.)	+	+
INO (Innositol.)	+	+
SOR (Sorbitol.)	+	+
RHA (Rhabinose.)	+	+
SAC (Sucrose.)	+	+
MEL (Melbiose.)	+	+
AMY (Amygdalin.)	+	+
ARA (Arabinose.)	+	+

Appendix VI: CTX-M gene sequence analysis

Chromatogram files were inspected using GENtle v.1.9.4, particularly the correspondence of bases with their peaks. Poor-quality peaks were trimmed. Additionally, amino acids were extracted to obtain their corresponding protein sequence. The resulting sequences (nucleotide and protein) were compared to those available in the National Centre for Biotechnology Information Database (NCBI) using the Basic Local Alignment Search Tool (BLAST) https://blast.ncbi.nlm.nih.gov. Blastn search tool was used to align the nucleotide sequences while Blastp was used to align the protein sequences. All four sample nucleotide sequences were identical, and they showed 100% similarity to *Klebsiella pneumoniae* strain 2020CK-00096, accession CP104659. Similarly, all four sample protein sequences were identical and showed 100% similarity to class A extended-spectrum beta-lactamase CTX-M-15 *Escherichia coli*, Sequence ID BCM94848.1.

Table 1: Summary of CTX-M gene sequence analysis.

Sample I.D	Variant	Similarity index	Nucleotide reference (Balstn)	Protein reference (Balstp)
1	blaCTX-M	100%	CP104659	BCM9484.1
2	blaCTX-M	100%	CP104659	BCM9484.1
3	blaCTX-M	100%	CP104659	BCM9484.1
4	blaCTX-M	100%	CP104659	BCM9484.1

Table 2: Analyzed Nucleotide and Protein Sequences

Nucleotide sequence (sample 1,2,3, and 4)
AGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGAC CTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATG TCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTG GCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACC GCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACC GAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCACT TCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAA GCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGG CAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTG GGTTGTGGGGGATAAAACCGGCAGCGGTGGCTATGGCACCACCAACGA TATCG
Protein sequence (Sample 1,2,3, and 4)
SEPNLLNQRVEIKKSDLVNYNPIAEKHVNGTMSLAELSAAALQYSDNVAM NKLIAHVGGPASVTAFARQLGDETFRLDRTEPTLNTAIPGDPRDTTSPRAM AQTLRNLTLGKALGDSQRAQLVTWMKGNTTGAASIQAGLPASWVVGDK TGSGGYGTTNDI

Appendix VII: Publication

Scientific Research Publishing

Advances in Microbiology, 2021, 11, 554-578
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Antimicrobial Susceptibility and Genetic Basis of Resistance of *Klebsiella spp* Isolated from Diarrheic and Non-Diarrheic Children at Health Facilities in Mukuru Informal Settlement, Nairobi, Kenya

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Abstract

Antimicrobial resistance (AMR) is a global threat to public health and particularly to children. This study aimed to determine the prevalence of multidrug resistance of fecal *Klebsiella spp* on selected beta lactam (3rd generation cephalosporins and carbapenems) and fluoroquinolone classes of drugs in four health facilities serving the slum communities of Nairobi city in Kenya. Additionally, determine the genetic basis for the multidrug resistance observed. A cross sectional laboratory based study was undertaken where a total of 1171 children below 16 years were selected, from whom stool samples were collected, tested and analyzed. 395 (33.73%) *Klebsiella spp* were isolated, consisting of 365 (92.4%) *Klebsiella pneumoniae* and 30 (7.6%) *Klebsiella oxytoca* were isolated. The proportion of multi-drug resistance (MDR) *K. pneumoniae* and MDR *K. oxytoca* was 64.1% (234/365) and 96.67% (29/30) respectively. Third generation cephalosporins, cefotaxime ceftriaxone and ceftazidime showed the highest resistance of 30.7%, 29.9% and 27.4% respectively, whereas carbapenems including imipenem and meropenem had the least resistance of 1.6%, each, to *K. pneumoniae*. A significant association was observed in diarrheic children (OR = 1.88; p = 0.01) and those below 50 months (OR = 0.43; p = 0.002) and carrying *K. pneumoniae* resistance to one or more third generation cephalosporins. Genes associated with resistance included *bla* TEM 100%, *bla* CTX-M 95.2%, *bla* SHV 57.1%, *bla* OXA-1 66.7%,

*qnr*S 54.1%, *qnr*B 47.6% and *bla* NDM 7.1%. In conclusion, there's need for more effective infection control measures, antimicrobial stewardship to reduce emergence of antimicrobial resistance, improved drinking water, sanitation and hygiene (WASH) practices.

Keywords

Klebsiella, Antimicrobial Resistance, Carriage, Community, Children, Slums, Kenya

1. Introduction

The global burden of AMR is increasing alarmingly and the United Nations (UN) General Assembly AMR report estimates that resistance will be responsible for approximately 10 million deaths by 2050 [1], most of which will occur in poor resource setting, mainly, the Sub-Saharan Africa [1]. In the United States of America, for example, it is estimated that more than 2 million people are infected with AMR organisms, annually, with approximately 23,000 deaths [2]. Main causes in the USA are mainly misuse and/or abuse of antibiotics, use of antibiotics in agriculture and increased income [3] whereas in the developing countries, the situation is aggravated due to poor implementation of infection control measures and the availability of counterfeit or low quality drugs [4]. In developing countries, the data is limited due to inadequate surveillance and hence likely to be significantly higher than in developed countries.

Klebsiella spp are common intestinal commensals that obtain, accumulate, and disseminate a variety of antibiotics resistance genes such as *bla* KPC [5] [6]. Therefore, they serve as a significant reservoir for resistance in the intestinal tract [5] [6] and subsequently increase the risk of nosocomial and community acquired resistant infections [7]. *In vivo* dissemination of AMR genes from intestinal *Klebsiella spp* to other bacterial species has been documented [8] [9] [10] [11]. In addition, *Klebsiella spp* cause diarrheal disease and a myriad of extraintestinal infections especially in severely ill patients [12] [13]. Apart from diarrheal patients [14] [15] [16] multidrug resistant *Klebsiella spp* has also been documented in apparently healthy patients including children [17] [18].

Multi drug resistance in slums areas ensures faster spread due to high density of humans and livestock living in close proximity, frequent antibiotic misuse and insufficient drinking water, drainage and sanitation infrastructure. These settlements therefore serve as hotspots for AMR transmission [19] [20].

Reports on the emergence and global spread of multidrug-resistant (MDR) and hypervirulent clones of *Klebsiella spp* especially *K. pneumoniae* have been increasing in both nosocomial and community-acquired infections [13] [21]. As a result, the treatment of *Klebsiella spp* infections has become more difficult with the available options being restricted.

Various mechanisms have been implicated in antibiotic resistance including mutation of chromosomal genes and the production of β-lactamases enzymes such as extended-spectrum β-lactamases (ESBLs), cephalosporinases, and carbapenemases [22]. Genes encoding for these enzymes are mostly carried on mobile genetic elements such as conjugative plasmids, integrons, transposons and insertion sequences.

They not only bear resistance genes but also virulence genes which intensify the ability of an organism to colonize and create infection within the host [22].

Colonization precedes infection in pathogenicity of disease [13], therefore understanding colonization dynamics provides a basis for identification of colonized patients and potential establishment of intervention protocols to prevent subsequent infection.

2. Materials and Methods

2.1. Study Site

Mukuru slum is one of the largest urban settlements in Nairobi. It is located in Nairobi east which has a population of approximately 700,000 people [23]. Mukuru is densely populated and made of temporary structures mostly corrugated metal sheets. Basic services and infrastructure are providing adequate sanitation and clean water. In addition to poverty, a number of factors associated with informal settlements such as overcrowding, substandard housing, unclean and insufficient quantities of water and inadequate sanitation contribute to a high incidence of infectious diseases and increased mortality among children. The immunization coverage for childhood vaccination ranges from 40% - 84.9% which is below the WHO recommended rate [24]. Based on unpublished data Mukuru has approximately 5 public schools and 5 health facilities. The collection sites included; Mbagathi hospital (MB), Missionaries of Mary Mukuru kwa Njenga clinic (MMM), Mukuru kwa Reuben clinic (MR) and Municipal city council (MCC).

2.2. Study Design

This was a cross sectional laboratory based study which utilized purposive sampling method.

2.3. Study Population

Study participants were children and minors under the age of 16 years. Children below 5 years are vulnerable to a myriad of infections due to their under developed immunity while children above 5 years are exposed to lifestyle and behavioral risk factors such as eating habits and WASH challenges and hence included in this study. Included in the study were children and minors below the age of 16 years and who must have been residing in Mukuru slums for at least 3 months prior to the study. For diarrheic cases, participants must have presented with episodes of loose or watery diarrhoea within the last three days.

2.4. Ethical Consideration

The study protocol was approved by the Kenya Medical Research Institute Scientific and Ethics Review Unit (SERU) Reference number: KEMRI/RES/7/3/1.

2.5. Sample Collection and Specimen Processing

Participants were recruited purposively during regular hospital visits and stool samples collected before initiation of treatment. Up to 5 grams of stool samples were collected from the participants and transported to the Salmonella surveillance unit I (SASU I) laboratory in the Center for Microbiology Research (CMR) of the Kenya Medical Research Institute (KEMRI) at 4˚C in Carry Blair transport media. The samples were then enriched in Selenite fecal broth (Oxoid, UK) and incubated for 24 hours. Microbial culture was done on MacConkey Agar (Oxoid) where suspected *Klebsiella spp* appeared pink in color with a mucoid texture. Biochemical tests for identification involved tests on Triple sugar iron (TSI) (Oxoid, UK), Urea test (Oxoid, UK), Sulphur indole motility (SIM) (Oxoid, UK), Methyl red (Sigma aldrich, USA), Voges-proskauer (Sigma aldrich, USA) and Citrate utilization test (Oxoid).

2.6. Antibiotic Sensitivity Testing

Kirby-Bauer disc diffusion technique was used on the *Klebsiella spp* isolates [25]. *E. coli* ATCC 25922 quality control strains was used as the test quality control organism. A panel of antibiotic disks for Ampicillin (AMP, 10 µg), Cefotaxime (CTX 30 µg), Ceftriaxone (CRO 30 µg), Ceftazidime (CAZ 30 µg), Cefoxitin (FOX 30 µg), Imipenem (IPM 10 µg, Meropenem (MEM 10 µg), and Amoxicillin-Clavulanate acid (AMC 30 µg) was used on the first plate. This facilitates the observation of a synergistic zone that typically forms when a cephalosporin antimicrobial combines with a Beta-Lactamase inhibitor.

The second plate had: Gentamicin (CN 10 µg), Ciprofloxacin (CIP 5 µg), Nalidixic acid (NA 30 µg), Chloramphenicol (C 30 µg), Streptomycin (STR 30 µg) Trimethoprim Sulfamethoxazole: (SXT 25 µg), Tetracycline (TE 30 µg) and Aztreonam (ATM 30 µg). All discs were obtained from Oxoid, UK.

All the plates were incubated at 37˚C for 18 hours, inhibition zones measured and interpreted according to Clinical Laboratory Standard Institute (CLSI) 2020, guidelines. The standard control strain *E. coli* (ATCC-25922) was used to assure testing performance of the potency of antibiotics discs and the quality of the media.

2.7. Phenotypic Screening for ESBL-Producing *K.pneumoniae*

The double disk synergy method was used to detect ESBL-producing *K. pneumoniae* where 4 antibiotics discs were used including Cefotaxime (CTX) (BD), Cefotaxime/Clavulanic acid (CTX/CLA) (BD, USA), Ceftazidime (CAZ) (BD, USA) and Ceftazidime/Clavulanic acid (CAZ/CLA) (BD, USA). These antibio-

(50.56%) males and 579 (49.44%) females. Distribution of participants among 1 - 50, 51 - 100, 101 - 150 and 151 - 200 age categories (in months) was as follows; 576 (49.19%), 364 (31.08%), 138 (11.79%) and 93 (7.94%) respectively. Diarrheic children were 514 (43.89%) while non-diarrheic children were 656 (56.02%). Distribution between resident villages namely; Mukuru kwa Njenga village (MN) and Mukuru kwa Reuben village (MR) was 413 (35.27%) and 196 (16.74%) respectively. 562 (47.99%) children' guardians did not provide their exact residence in Mukuru (Table 4).

3.2. Prevalence of *Klebsiella spp* Isolated in Children from Mukuru Slums

Of the 1171 participants recruited in the study, prevalence of *Klebsiella* spp carriage was 33.7% (395/1171). Prevalence of *K. pneumoniae* was established at 31.2% (365/1171) while that of *K. oxytoca* was at 2.6% (30/1171). Within *Klebsiella* spp therefore children were significantly 12 times more likely to be colonized with *K. pneumoniae* (OR 12.2; p = 0.0001). Although a significant association was statistically derived between *Klebsiella* intestinal carriage and the residential area, this association could not clearly be concluded due to the number of participants whose villages were not captured (Table 4) (Figure 1). Further, no significant association was observed between carriage and presentation type (OR 1.2; p = 1.3). All other correlates of carriage included age and gender (Table 5).

3.3. Antibiotic Resistance Patterns of *K. pneumoniae* and *K. oxytoca*

K. pneumoniae showed highest resistance to ampicillin at 77.5% moderate resistance to one of the most commonly prescribed amoxicillin/clavulanic acid at 37% with low or close to no resistance for imipenem and meropenem each recording percentage resistance of 1.6% (Figure 2). Generally, *K. pneumoniae* showed high resistance to 3rd generation cephalosporins (cefotaxime, ceftriaxone and ceftazidime) compared to fluoroquinolones (nalidixic acid and ciprofloxacin). The least possible resistance from *K. pneumoniae* was shown for cephamycin (cefoxitin) and carbapenems (imipenem and meropenem).

A similar trend was shown for *K. oxytoca* that again showed high resistance to ampicillin at 70% with resistance to the most commonly empirically prescribed amoxicillin/clavulanic acid also being relatively high at 56.7%. Moderate resistance was observed for Nalidixic acid and cefotaxime at 33.3% and 26.7% respectively, with low resistance observed for Ciprofloxacin and cefoxitin both at 3.3%. No resistance was observed to the carbapenems (imipenem and meropenem) by *K. oxytoca*.

3.4. Prevalence of Multidrug Resistant (MDR) *K. pneumoniae and K. oxytoca* and Their Resistance Patterns across Different Antibiotic Panels

Multidrug resistance (MDR) was defined as an isolate non-susceptible to at least

one agent in three or more antibiotic categories/classes [32]. The prevalence of MDR *K. pneumoniae* in the population was 20.75%. (243/1171) while that of *K. oxytoca* was 2.47% (29/1171). Among the isolates, MDR *Klebsiella pneumoniae* was 64% while MDR *K. oxytoca* was 96.7% (29/30).

Table 4. Demographic characteristics of study participants'.

Variable		Frequency (n)	Percentage (%)
Gender	Male	592	50.56%
	Female	579	49.44%
*Age category	1 - 50	576	49.19%
	51 - 100	364	31.08%
	101 - 150	138	11.79%
	151 - 200	93	7.94%
Residence	*MN	413	35.27%
	*MR	196	16.74%
	*Village unknown	562	47.99%
Symptoms	Diarrheic	514	43.89%
	Non diarrheic	656	56.02%

*Age category is in months *MN = Mukuru kwa Njenga village *MR = Mukuru kwa Reuben village * Village unknown = Village information not provided in questionnaire.

Table 5. Prevalence of *Klebsiella spp* in Children from Mukuru slum (n = 1171).

Variable		Frequency (n)	Percentage (%)	O.R	P value
Serotype	*K. pneumoniae*	365	31.16%	12.17	0.0001
	K. oxytoca	30	2.56%	R	
Gender	Male	202	17.25%	1.05	0.07116
	Female	193	16.48%	R	
*Age category	1 - 50	238	60.25%	2.7	0.001
	51 - 100	88	22.28%	R	
	101 - 150	59	14.94%	0.67	0.0001
	151 - 200	10	2.53%	0.11	
Residence	*MN	135	11.52%	0.78	0.0001
	*MR	88	7.51%	0.51	0.0732
	*Village unknown	172	14.68%	R	
Symptoms	Diarrheic	216	18.45%	1.21	0.1285
	Non diarrheic	179	15.29%	R	

*Age category is in months *MN = Mukuru kwa Njenga village *MR = Mukuru kwa Reuben village * Village unknown = Village information not provided in questionnaire.

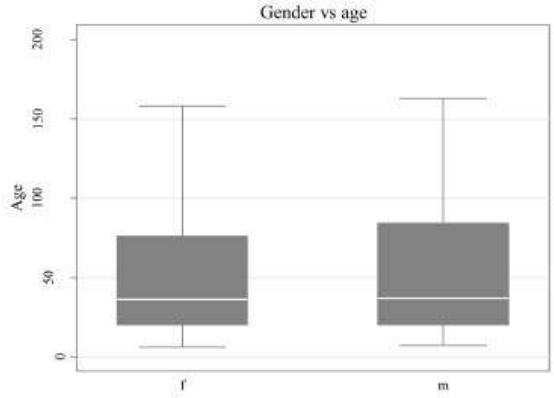

Figure 1. Distribution of children's age between genders; those colonized with *Klebsiella spp.*

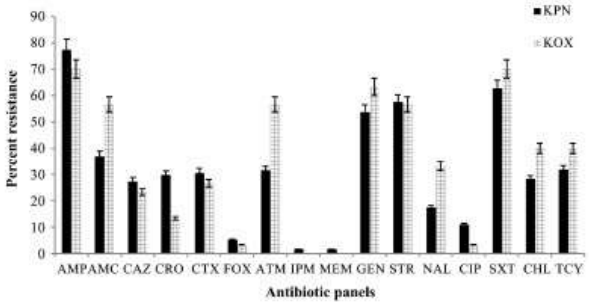

Figure 2. Resistance patterns of *Klebsiella pneumoniae* and Klebsiella *oxytoca* isolated from children and minors from Mukuru slums, Nairobi Kenya. Highest percentage resistance (with 5% margin of error) is observed for AMP with lowest resistance shown for IPM and MEM. Key: Ampicillin (AMP), Amoxicillin-Clavulanate acid (AMC), Ceftazidime (CAZ), Ceftriaxone (CRO), Cefotaxime (CTX), Cefoxitin (FOX), Aztreonam (ATM), Imipenen (IPM), Meropenem (MEM), Gentamicin (GEN), Streptomycin (STR), Nalidixic acid (NA), Ciprofloxacin (CIP), Trimethoprim Sulphamethoxazole: (SXT), Chloramphenicol (CHL) and Tetracycline (TCY). KOX = *K. oxytoca* KPN = *K. pneumoniae.*

K. pneumoniae multidrug resistance was high accounting for 77.5% (283/365) of samples exposed to Penicillin, 73.7% (269/365 among Aminoglycosides and 62.7% (229/365 among Folate biosynthesis inhibitor. Beta lactam inhibitor combination, Tetracycline and Monobactam showed rate to resistance of 37% (135/365, 31.8% (116/365) and 31.5% (115/365) respectively. Third generation cephalosporins recorded rate to resistance of 30.9% (113/365) while Quinolone and Fluoroquinolone 18.4% (67/365). Less resistance rate was demonstrated against Cephamycin at 5.2% (19/365 and Carbapenem 3.3% (12/365 (Table 6).

Table 6. Multidrug Resistance frequency of *K. pneumoniae* (n = 365) and *K. oxytoca* (n = 30) to various classes of antibiotics.

Class of antibiotics	*K. pneumoniae* n (%)	*K. oxytoca* n (%)
Penicillin	283 (77.5)	21 (70)
Beta-Lactam Inhibitor	135 (37)	17 (56.7)
Monobactam	115 (31.5)	17 (56.5)
Cephamycin	19 (5.2)	1 (3.3)
Third generation cephalosporins	113 (30.9)	10 (33.3)
Quinolone and Fluoroquinolone	67 (18.36)	10 (33.3)
Folate biosynthesis Inhibitor	229 (62.7)	21 (70)
Phenicol	103 (28.2)	12 (40)
Tetracyline	116 (31.8)	12 (40)
Aminoglycosides	269 (73.7)	29 (96.7)
Carbapenems	12 (3.3)	0

Multidrug resistance for *K. oxytoca* was the highest against Aminoglycosides at 96% (29/30), Penicillin and Folate Biosynthesis Inhibitor each at 70% (21/30). The rate of resistance to monobactam and Beta-Lactam Inhibitor were each 57% (17/30). *K. oxytoca* showed minimal resistance to the 3rd generation cephalosporins, Quinolones and Fluoroquinolones, each group standing at 33.3% (10/30), with no resistance recorded against Carbapenems (Table 6).

There was a significant difference in resistance to monobactam (OR = 0.56; p = 0.02), third generation cephalosporins (OR = 1.88; p = 0.01), aminoglycosides (OR = 3.6; p = 0.00) and beta lactam inhibitor (OR = 1.54 p = 0.05) observed in *K. pneumoniae* isolated from diarrheic children. This means that diarrheic children have a higher chance of colonization with *K. pneumoniae* resistant to the antibiotics stated above. There was a significant difference noted in resistance to third generation cephalosporins, among *K. pneumoniae* isolates obtained from children between 1 and 50 months (OR = 0.43; p = 0.002). Children in in this age group have higher odds of carrying *K. pneumoniae* resistant to third generation cephalosporins. Additionally, a significant difference was observed in resistance to phenicol (OR = 1.81; p = 0.02), tetracycline (OR = 3.14; p = 0.00), aminoglycosides (OR = 4.35; p = 0.000) and folate biosynthesis inhibitor (OR = 3.6; p = 0.000) among *K. pneumoniae* isolates obtained from children residing in Mukuru kwa Njenga village. Male children (OR = 4.69; p = 0.05) showed a higher chance of colonization with *K. pneumonia* resistant to carbapenems (Tables 7-10).

There was no significant difference in resistance to cephamycin from isolates obtained from participants among the various age categories, gender, resident villages and symptoms. There was no significant difference in resistance to third generation cephalosporins among isolates obtained from various resident villages and gender. In addition, no significant difference in resistance to quinolone and fluoroquinolone among isolates obtained from children among various age categories, resident villages and gender. With regard to carbapenems resistance,

no significant difference was observed among isolates obtained from children among various age categories, resident villages and symptoms (Tables 7-10).

Table 7. Frequency of resistance to Beta Lactam class of drugs in *Klebsiella pneumoniae* isolated from Children in Mukuru slums.

		Penicillin			Mono-bactam			Cephamycin			3rd Gen cephalosporins			Carbapenems		
		N (%)	O.R	P.V	N (%)	O.R	P.V	N (%)	O.R	P.V	N (%)	O.R	P.V	N (%)	O.R	P.V
Gender	Male	151 (53.36)	1.14	0.592	70 (61.90)	0.80	0.64	9 (47.37)	0.80	0.64	60 (53.10)	1.03	0.89	10 (10.33)	4.69	0.05
	Female	132 (46.64)	R	-	45 (39.2)	R	-	10 (52.63)	R	-	53 (46.90)	R	-	2 (16.67)	R	-
Residence	MN	102 (36.04)	-	-	25 (21.43)	0.56	0.09	11 (57.89)	-	-	60 (53.10)	0.48	0.05	4 (33.33)	1.19	0.77
	MR	65 (22.97)	-	-	31 (27.38)	-	-	0	-	-	38 (33.63)	0.93	0.86	0	-	-
	VU	116 (40.99)	-	-	59 (51.19)	-	-	8 (42.11)	-	-	15 (13.27)	R	-	8 (66.67)	-	-
Age category	0 - 50	164 (57.95)	0.59	0.12	43 (37.39)	0.80	0.62	11 (57.89)	2.21	0.31	53 (46.90)	0.43	0.002	7 (58.33)	1.37	0.28
	51 - 100	71 (25.09)	R	-	54 (46.96)	R	-	2 (10.53)	R	-	36 (31.36)	R	-	2 (16.67)	R	-
	101 - 150	43 (15.19)	0.56	0.18	13 (11.30)	-	-	4 (21.05)	3.09	0.20	20 (17.70)	0.72	0.35	3 (25)	2.2	0.37
	151 - 200	5 (1.77)	0.23	0.04	5 (4.35)	-	-	2 (10.53)	11.71	0.02	4 (3.54)	1.06	0.93	0	-	-
Symptoms	D	148 (52.30)	0.74	0.23	49 (42.86)	0.56	0.02	13 (68.42)	1.91	0.20	73 (64.30)	1.88	0.01	12 (100)	-	-
	ND	135 (47.70)	R	-	66 (57.14)	R	-	6 (31.56)	R	-	40 (35.40)	R	-	-	-	-

*Age category is in months *MN = Mukuru kwa Njenga village *MR = Mukuru kwa Reuben village * VU-Village unknown; These were persons who declined to provide their exact residence in Mukuru. kox = *K. oxytoca;* kpn = *K. pneumonia;* OR-Odds Ratio; P v-P value.

Table 8. Frequency of resistance to Quinolone & fluoroquinolone class of drugs and commonly antibiotics in *Klebsiella pneumoniae* isolated from Children in Mukuru slums.

		Quinolone &fluoroquinolone			Folate biosythesis inhibitor			Phenicol			Tetracyline			Aminoglycosides			Beta-Lactamase Inhibitor		
		N (%)	O.R	P.V	N (%)	O.R	P.V	N (%)	O.R	P.V	N (%)	O.R	P.V	N (%)	O.R	P.V	N (%)	O.R	P.V
Gender	Male	33 (49.25)	0.84	0.54	122 (53.28)	1.07	0.73	59 (57.28)	1.3	0.26	56 (48.28)	0.77	0.26	134 (19.81)	0.65	0.07	69 (51.11)	0.91	0.66
	Female	34 (50.75)	R	-	107 (46.72)	R	-	44 (42.72)	R	-	60 (51.70)	R	-	135 (50.19)	R	-	66 (48.89)	R	-
Residence	MN	26 (38.81)	1.67	0.07	86 (37.50)	-	-	40 (38.83)	1.81	0.02	54 (46.55)	3.14	0.00	97 (36.06)	4.35	0.00	61 (45.19)	3.2	0.00
	MR	9 (13.43)	R	-	44 (19.21)	-	-	19 (18.45)	R	-	13 (11.21)	R	-	52 (19.33)	R	-	21 (15.56)	R	-
	VU	32 (47.76)	-	-	99 (43.23)	-	-	44 (42.72)	-	-	49 (42.24)	-	-	120 (44.61)	-	-	53 (39.20)	-	-
Age category	0 - 50	38 (56.72)	0.78	0.46	131 (57.21)	0.62	0.09	64 (62.14)	0.95	0.84	66 (56.90)	0.98	0.97	156 (57.99)	1.19	0.55	73 (54.07)	0.925	0.773
	51 - 100	18 (26.87)	R	-	60 (26.20)	R	-	26 (25.24)	R	-	26 (22.41)	R	-	58 (21.56)	R	-	30 (22.22)	R	-
	101 - 150	7 (10.45)	0.51	0.20	34 (14.85)	0.59	0.15	10 (9.71)	0.47	0.08	19 (16.38)	1.11	0.77	46 (17.10)	1.87	0.13	27 (20.00)	1.62	0.17
	151 - 200	4 (5.95)	2.9	0.14	4 (1.75)	0.32	0.11	3 (2.91)	1.12	0.88	5 (4.31)	2.79	0.15	9 (3.35)	-	-	5 (3.70)	2.25	0.25
Symptoms	D	33 (49.25)	1.67	0.07	123 (53.71)	0.97	0.89	53 (51.5)	0.86	0.54	70 (60.34)	1.46	0.09	167 (62.08)	3.6	0.00	82 (60.74)	1.54	0.05
	ND	34 (50.75)	R	-	106 (46.29)	R	-	50 (48.5)	R	-	46 (39.66)	R	-	102 (37.92)	R	-	53 (39.26)	R	-

*Age category is in months *MN = Mukuru kwa Njenga village *MR = Mukuru kwa Reuben village * VU-Village unknown; These were persons who declined to provide their exact residence in Mukuru. kox = *K. oxytoca;* kpn = *K. pneumonia;* OR-Odds Ratio; P v-P value.

Table 9. Frequency of resistance to Beta Lactam class of drugs in *Klebsiella oxytoca* isolated from Children in Mukuru slums.

		Penicillin			Mono-bactam			Cephamycin			3rd Gen cephalosporins			Carbapenems		
		N (%)	O.R	P.V	N (%)	O.R	P.V	N (%)	O.R	P.V	N (%)	O.R	P.V	N (%)	O.R	P.V
Gender	Male	13 (61.90)	2.15	0.40	6 (35.29)	1.22	0.79	1 (100)	-	-	4 (40.00)	1.55	0.58	0	-	-
	Female	8 (38.10)	R	-	11 (64.71)	R	-	0	-	-	6 (60.00)	R	-	0	-	-
Residence	MN	18 (85.71)	-	-	14 (82.35)	-	-	1 (100)	-	-	8 (80)	-	-	0	-	-
	MR	1 (4.70)	-	-	1 (5.88)	-	-	0	-	-	0	-	-	0	-	-
	VU	2 (9.52)	-	-	2 (11.77)	-	-	0	-	-	2 (20)	-	-	0	-	-
Age category	0 - 50	17 (80.95)	-	-	13 (76.47)	-	-	0	-	-	6 (60)	-	-	0	-	-
	51 - 100	2 (9.52)	-	-	2 (11.76)	-	-	1 (100)	-	-	2 (20)	-	-	0	-	-
	101 - 150	2 (9.52)		-	2 (11.76)	-	-	0	-	-	2 (20)	-	-	0	-	-
	151 - 200	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-
Symptoms	D	13 (61.90)	0.81	0.80	12 (70.59)	2.05	0.35	1 (100)	-	-	9 (90)	9	0.55	0	-	-
	ND	8 (38.10)	R	-	5 (29.41)	R	-	-	-	-	1 (10)	R	-	0	-	-

*Age category is in months *MN = Mukuru kwa Njenga village *MR = Mukuru kwa Reuben village * VU-Village unknown; These were persons who declined to provide their exact residence in Mukuru. kox = *K. oxytoca;* kpn = *K. pneumonia;* OR-Odds Ratio; P v-P value.

Table 10. Frequency of resistance to Quinolone & fluoroquinolone class of drugs and commonly antibiotics in *Klebsiella oxytoca* isolated from Children in Mukuru slums.

		Quinolone &fluoroquinolone			Folate biosythesis inhibitor			Phenicol			Tetracyline			Aminoglycosides			Beta-Lactamase Inhibitor		
		N (%)	O.R	P.V	N (%)	O.R	P.V	N (%)	O.R	P.V	N (%)	O.R	P.V	N (%)	O.R	P.V	N (%)	O.R	P.V
Gender	Male	2 (20)	0.37	0.28	9 (42.86)	6	0.12	3 (25)	0.52	0.43	4 (33.33)	-	-	19 (34.48)	-	-	8 (47.06)	-	-
	Female	8 (80)	R	-	12 (57.14)	R	-	9 (75)	R	-	8 (66.67)	-	-	19 (65.52)	-	-	9 (52.94)	-	-
Residence	MN	9 (90)	-	-	18 (85.71)	-	-	12 (100)	-	-	10 (83.33)	-	-	26 (89.60)	-	-	14 (82.35)	-	-
	MR	0	-	-	1 (4.76)	-	-	0	-	-	0	-	-	1 (3.45)	-	-	1 (3.33)	-	-
	VU	1 (10)	-	-	2 (9.58)	-	-	0	-	-	2 (16.67)	-	-	2 (6.90)	-	-	2 (6.67)	-	-
Age category	0 - 50	9 (90)	-	-	14 (66.67)	-	-	9 (75)	-	-	7 (58.33)	-	-	22 (75.86)	-	-	13 (76.47)	-	-
	51 - 100	1 (10)	-	-	2 (9.52)	-	-	0	-	-	1 (8.33)	-	-	2 (6.90)	-	-	2 (11.76)	-	-
	101 - 150	0	-	-	4 (19.05)	-	-	2 (16.67)	-	-	3 (2.91)	-	-	4 (13.79)	-	-	2 (11.76)	-	-
	151 - 200	0	-	-	1 (4.76)	-	-	1 (8.33)	-	-	1 (8.33)	-	-	1 (3.45)	-	-	0	-	-
Symptoms	D	2 (20)	0.04	0.002	19 (90.48)	-	-	8 (66.67)	1.27	0.75	11 (91.67)	13.7	0.02	19 (65.52)	-	-	14 (82.35)	7.46	0.02
	ND	8 (80)	R	-	2 (9.52)	-	-	4 (33.33)	R	-	1 (8.33)	R	-	10 (34.48)	-	-	3 (17.65)	R	-

*Age category is in months *MN = Mukuru kwa Njenga village *MR = Mukuru kwa Reuben village * VU-Village unknown; These were persons who declined to provide their exact residence in Mukuru. kox = *K. oxytoca;* kpn = *K. pneumonia;* OR-Odds Ratio; P v-P value.

3.5. Frequency of ESBL Production in the Isolated *Klebsiella spp*

The proportion of *K. pneumoniae* Extended Spectrum Beta Lactamase (ESBL) producing isolates was 22.74% (83/365). Out of these ESBLs, 11.50% (42/365) were resistant to at least one fluoroquinolone; while 2.19% (8/365) were resistant

to at least one carbapenem and to at least one fluoroquinolone. Comparative analysis showed a significant likelihood with 60% more chance of isolating ESBLs among children aged between 0 - 50 months (OR = 0.38; p = 0.001) compared to children 51 - 100 months (OR = 0.85; p = 0.66). Again, although an association was observed for ESBLs and residence, this could not effectively be interpreted since majority of the participants did not indicate their areas of residence (Table 11). The prevalence of *K. oxytoca* ESBLs was 13.33% (4/30). Comparative analysis showed no difference in age category, gender, health facility and symptoms (Table 11).

3.6. Resistance Genes in the Isolated *Klebsiella spp*

A total of 42/395 (10.64%) isolates were examined for carriage of resistance genes. They were all from *K. pneumoniae* isolates. The *bla* TEM gene was the most common with all the 42 (100%) samples demonstrating the presence of this gene (Figure 3). The second gene identified was *bla* CTX-M, demonstrated in 40 (95.2%) of samples (Figure 3). It was followed closely by *bla*OXA.

Table 11. Frequency of ESBL producing *Klebsiella spp* isolated from children residing in Mukuru slums.

Variables			N (%)	OD	P Value
Gender	Kpn	Female	40 (48.19)	R	
		Male	43 (51.81)	0.96	0.87
	Kox	Female	2 (50)	R	
		Male	2 (50)	2.25	0.46
Residence	Kpn	MN	43 (51.81)	21.5	0.001
		MR	2 (2.41)	R	-
		Village unknown	38 (45.78)	-	-
	Kox	MN	3 (75)	-	-
		MR	0	-	-
		Village unknown	1 (25)	-	-
Age category	Kpn	1 - 50	34 (40.96)	0.38	0.001
		51 - 100	28 (33.73)	R	-
		101 - 150	17 (20.48)	0.85	0.66
		151 - 200	4 (4.82)	-	-
	Kox	1 - 50	1 (25)	-	-
		51 - 100	1 (25)	-	-
		101 - 150	2 (50)	-	-
		151 - 200	0	-	-
Symptoms	Kpn	D	65 (78.31)	4.84	0
		ND	18 (21.69)	R	
	Kox	D	4 (100)	_	_
		ND	0	_	_

KEY: *kpn = *K. pneumoniae* kox = *K. oxytoca* *D = diarrheic *ND = Non-Diarrheic *Age category is in months *MN = Mukuru kwa Njenga *MR = Mukuru kwa Reuben * Village unknown = Village information not provided in questionnaire.

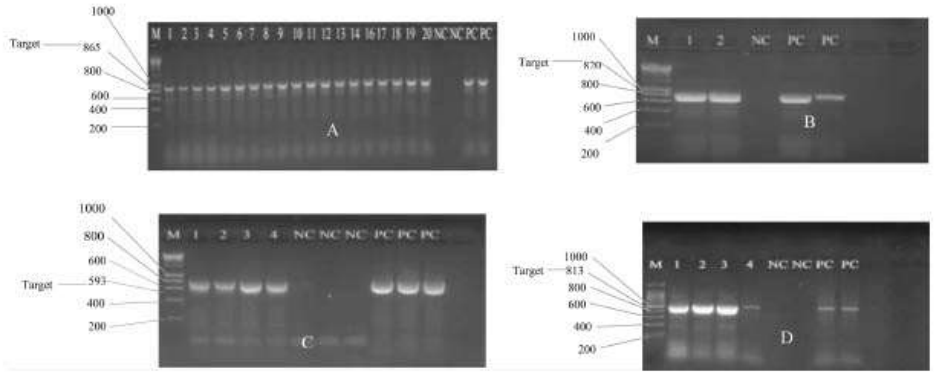

Figure 3. Gel photos for resistance genes to Beta-Lactam class of antibiotics. A: *bla* TEM gene (865 bp) B: *bla* OXA-1 gene (820 bp). C: *bla* CTX-M gene (593 bp). D: *bla* NDM gene (813 bp). M represents the Molecular ladder. NC represents Negative Control. PC represents the Positive Control.

Which was demonstrated in 28 (66.67%) isolates (Figure 3) while *bla* SHV was demonstrated in 24 (57.14%) isolates (Figure 3). Among genes conferring resistance to beta lactam class of antibiotics *bla* NDM demonstrated the least resistance in 3 (7.14%) Isolates (Figure 3).

Among genes conferring resistance to quinolones and fluoroquinolones *qnr*S was the most common, it was demonstrated in 23 (54.14%) isolates (Figure 4). It was followed closely by *par*C which demonstrated in 20 (47.62%) isolates (Figure 4). The low resistance was observed in *qnr*B which was demonstrated in 20 (47.62% isolates Figure 4 while the least resistance was demonstrated by the *par*E, which was present in 16 (38.09%) isolates (Figure 5).

Carriage of multiple genes bearing resistance to both 3rd generation cephalosporins and fluoroquinolones resistance antibiotics was observed in 90.48%) (38/42) isolates (Table 12) while carriage of 3rd generation cephalosporins and carbapenems resistance genes was observed in 7.14% (3/42) isolates. Carriage of resistance genes against three classes of drugs (Beta lactams, fluoroquinolones and Carbapenems) was only observed in 7.14% (3/42) *K. pneumoniae* isolates (Table 12).

3.7. Phylogenetic Analysis of the Isolated *Klebsiella spp*

The dendrogram was derived from the 42 *K. pneumoniae* ESBL producing isolates that were also resistant to fluoroquinolones and/or carbapenems. Within this dendrogram there were Clades (branch that includes a common ancestor and all of its descendants), Clustering groups (descendants in a clade at 40% similarity), Clustering sub groups (descendants in a clade at 100% similarity). Two clades designated A and B, 6 clustering groups designated group1 - 6 and 40 subgroups were recorded. Clade A includes group 1 - 4 while clade B includes group 5 and 6. There was 100% similarity index in Group 1, 2 and 5 as highlighted in the boxes. Of notice, was an outgroup observed in group 6 of clade B which showed a higher number of bands compared to the rest of the isolates

group 6. Out of the 40 Sub groups 37.5% (15/42 showed >80% similarity index with the highest number observed in group 1 while, 62.5% (25/42) showed a similarity index < 80% indicating that these 25 isolates were distantly related. Isolates that carried resistance genes to fluoroquinolones clustered tightly as observed in all the groups, while isolates that carried resistance genes to carbapenems were diverse and did not cluster together as observed in group 1 and 6. From the phylogenetic analysis therefore we state that 83.33% (35/42) of the isolates were diverse and hence disbanding the possibility of clonal spread of MDR strains (Figure 5).

4. Discussion

In this study we report a community prevalence of gastrointestinal *K. pneumoniae* of 31.16% and of *K. oxytoca* of 2.56% among the slum dwelling children. This prevalence noted in the community is higher than what has previously reported among ICU patients [13] [33] of 23% and 19%. *K. pneumoniae* and K. *oxytoca* are ubiquitous in nature and are found in various environments including mucosal membranes of humans where they colonize the gastrointestinal tract, the skin and the nasopharyngeal. In the gastrointestinal tract, they occur as normal flora. However, when they cross the gastrointestinal mucosal membrane into other systems of the body, they become opportunistic pathogens, causing infections such as pneumonia, bloodstream infections, meningitis and urinary tract infection. The high prevalence of *Klebsiella spp* in the community may not have a major impact on the children as the organisms do not cause infection in the gastrointestinal tract. However, these micro-organisms indicate the resistance genes circulating in Mukuru. These genes could be disseminated to other pathogens which pose a challenge in patient management.

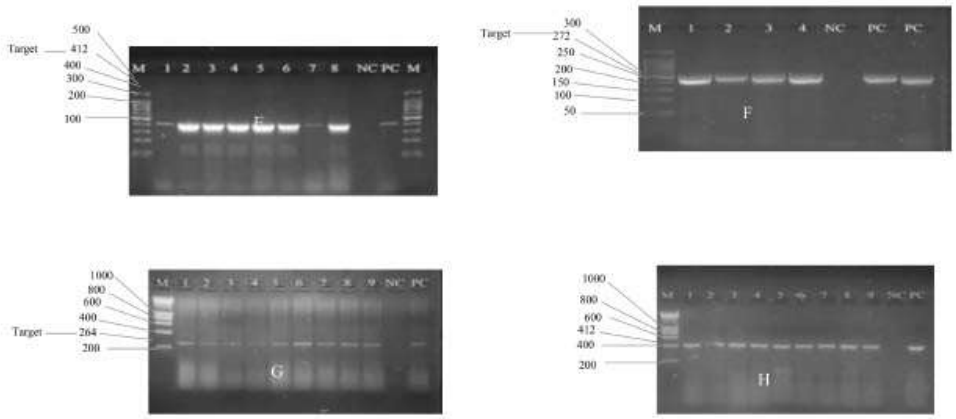

Figure 4. Gel photos for resistance genes to Quinolone and Fluoroquinolone class of antibiotics. E: *par* C gene (412 bp) F: *par* E gene (272 bp). G: *qnr*B gene (264 bp). H: *qnr*S gene (813 bp). M represents the Molecular ladder. NC represents Negative Control. PC represents the Positive Control.

Table 12. Carriage of multiple resistance genes in *K. pneumoniae* isolated from Children in Mukuru slums.

Isolate no.	3rd generation Cephalosporins resistance genes	Quinolone and Fluoroquinolones resistance genes	Carbapenems resistance genes
**1298	*bla* TEM-1, *bla* SHV-1, *bla* CTX-M	*parC*, *parE*, *qnrS*	-
**2018	*bla* TEM-1, *bla* CTX-M, *bla* OXA-1	*parC*, *qnrS*	-
**1471	*bla* TEM-1, *bla* CTX-M, *bla* OXA-1	*parC*, *qnrS*	-
**1204	*bla* TEM-1, *bla* CTX-M, *bla* OXA-1	*parC*, *parE*, *qnrB*, *qnrS*	-
**2215	*bla* TEM-1, *bla* CTX-M, *bla* OXA-1	*parC*, *parE*, *qnrB*	-
**2548	*bla* TEM-1, *bla* CTX-M, *bla* OXA-1	*parC*, *parE*, *qnrB*	-
**2600	*bla* TEM-1, *bla* CTX-M, *bla* OXA-1	*parC*, *parE*, *qnrB*	-
***1588	*bla* TEM-1, *bla* SHV-1, *bla* CTX-M	*parC*, *parE*, *qnrB*	*bla* NDM
**2893	*bla* TEM-1, *bla* SHV-1, *bla* CTX-M, *bla* OXA-1	*parC*, *parE*, *qnrS*	-
**1882	*bla* TEM-1, *bla* SHV-1, *bla* CTX-M, *bla* OXA-1	*parC*, *parE*, *qnrS*	-
**2315	*bla* TEM-1, *bla* CTX-M, *bla* OXA-1	*parC*, *parE*, *qnrS*	-
**1989	*bla* TEM-1, *bla* SHV-1, *bla* CTX-M	*parC*, *parE*, *qnrB*, *qnrS*	-
**1484	*bla* TEM-1, *bla* CTX-M, *bla* OXA-1	*parE*, *qnrB*, *qnrS*	-
**2555	*bla* TEM-1, *bla* CTX-M, *bla* OXA-1	*parE*, *qnrB*, *qnrS*	-
**2968	*bla* TEM-1, *bla* SHV-1, *bla* CTX-M, *bla* OXA-1	*parE*, *qnrS*	-
**2499	*bla* TEM-1, *bla* SHV-1, *bla* CTX-M, *bla* OXA-1	*parE*, *qnrS*	-
**1678	*bla* TEM-1, *bla* SHV-1, *bla* CTX-M, *bla* OXA-1	*parE*, *qnrB*, *qnrS*	-
**1535	*bla* TEM-1, *bla* CTX-M, *bla* OXA-1	*parE*, *qnrS*	-
**1082	*bla* TEM-1, *bla* CTX-M, *bla* OXA-1	*parE*, *qnrS*	-
**1369	*bla* TEM-1, *bla* SHV-1, *bla* CTX-M, *bla* OXA-1	*parE*, *qnrS*	-
**1923	*bla* TEM-1, *bla* CTX-M, *bla* OXA-1	*parE*, *qnrB*	-
**2306	*bla* TEM-1, *bla* SHV-1, *bla* CTX-M, *bla* OXA-1	*parE*	-
**1581	*bla* TEM-1, *bla* CTX-M,	*parC*, *parE*	-
***1720	*bla* TEM-1, *bla* CTX-M, *bla* OXA-1	*parC*, *qnrB*	*bla* NDM
**2737	*bla* TEM-1, *bla* CTX-M, *bla* OXA-1	*parE*	-
**2472	*bla* TEM-1, *bla* SHV-1, *bla* CTX-M, *bla* OXA-1	*parC*	-
**2402	*bla* TEM-1, *bla* CTX-M, *bla* OXA-1	*parC*, *parE*, *qnrB*, *qnrS*	-
***2402	*bla* TEM-1, *bla* SHV-1, *bla* CTX-M	*parC*, *qnrB*, *qnrS*	*bla* NDM
**1214	*bla* TEM-1, *bla* SHV-1, *bla* CTX-M, *bla* OXA-1	*parE*, *qnrB*	-
**2642	*bla* TEM-1, *bla* SHV-1, *bla* CTX-M, *bla* OXA-1	*parC*, *qnrB*, *qnrS*	-
**2646	*bla* TEM-1, *bla* SHV-1, *bla* CTX-M	*parC*	-
**1287	*bla* TEM-1, *bla* SHV-1, *bla* CTX-M, *bla* OXA-1	*pare*	-
**2951	*bla* TEM-1, *bla* SHV-1, *bla* CTX-M	*pare*	-
**2343	*bla* TEM-1, *bla* SHV-1, *bla* CTX-M	*parC*, *qnrB*, *qnrS*	-
**1195	*bla* TEM-1, *bla* CTX-M	*qnrB*	-
**2382	*bla* TEM-1, *bla* SHV-1, *bla* CTX-M	*qnrB*	-
**1290	*bla* TEM-1, *bla* CTX-M	*qnrB*	-
**2207	*bla* TEM-1, *bla* SHV-1	*qnrS*	-

** Is indicative of isolates that demonstrated resistance to two classes of antibiotics while *** is indicative of isolates that demonstrated resistance to three classes of antibiotics.

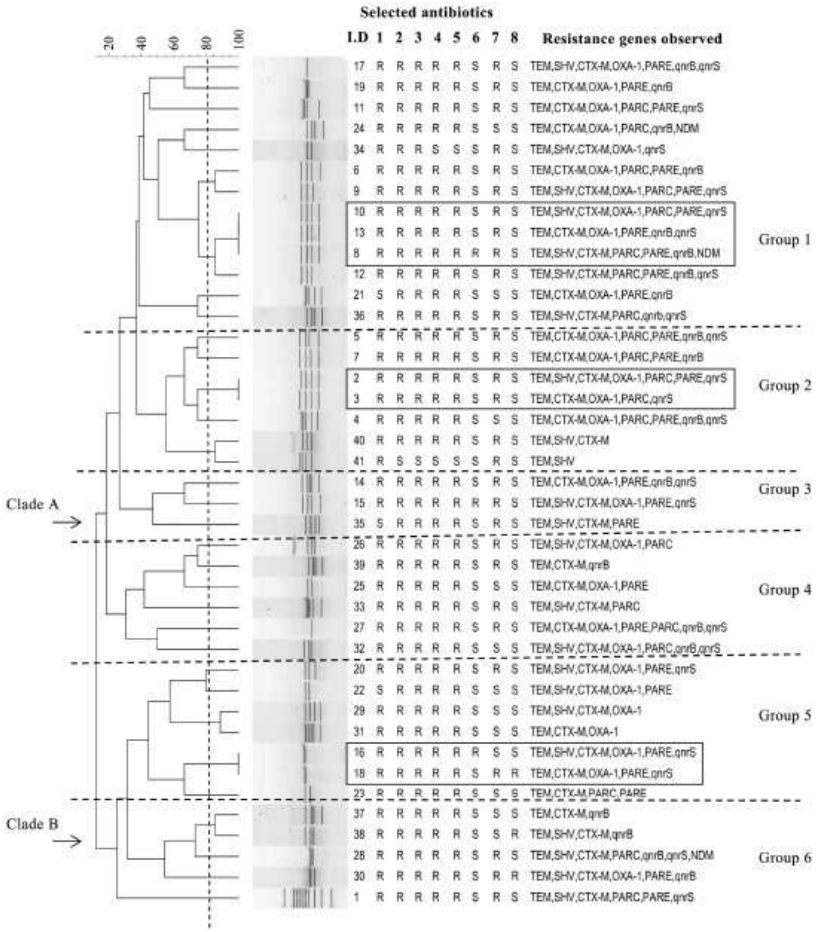

Figure 5. Phylogenetic relatedness of *Klebsiella spp* isolated from Children in Mukuru slum. Key: I.D: Isolate DNA Number. Antibiotic 1: Amoxicillin Clavulanate 2: Cefotaxime 3: Ceftazidime 4: Ceftriaxone 5: FEP 6: Amikacin 7: Ciprofloxacin 8: Meropenem.

The proportion of MDR *K. pneumoniae* observed in this community can be attributed to selective pressure for certain antibiotics [34]. This indicates antibiotic use/misuse in Mukuru which contributes to emergence and persistence of antibiotic resistance. The therapeutic use of different antibiotics for empirical and prophylactic management of gastrointestinal infections is rampant, in this slum community. Indeed, it has been established that due to high burden of pathogens causing gastrointestinal infections, uncontrolled use of antibiotics to the

communities contribute to selective pressure leading to resistance [35]. For example, due to poverty in this slum community and ease of access of antibiotics as over the counter (OTC) medications, dispensing chemists record high purchase of relatively cheap antibiotics such as chloramphenicol, ampicillin and co-trimaxazole. Due to high burden of HIV infections in the community we assessed there is also a high rate of empiric use of antibiotics such as trimethoprim sulfamethoxazole and gentamicin for treatment of gastrointestinal infections. Use of such drugs as first line for treatment of enteric infections or for prophylactic management for prevention of HIV opportunistic infections has been described as a major driver of antibiotic resistance [36]. AMR determinants such as plasmids and insertion sequences containing multiple resistance genes can be present in these microorganisms. These determinants have the ability to transfer resistance genes in vitro [37]. Indeed, other studies describing the MDR patterns in Nairobi have described Mukuru slums as MDR hotspots [38]. The potential for aggravated transmission of MDR genes to the vulnerable populations was demonstrated in this study due to the determination that there was no significant difference in the prevalence of MDR infections in the asymptomatic (non-diarrheic) and the symptomatic (diarrheic) cases. The latter finding demonstrated that both the symptomatic and the asymptomatic play an equally significant role in the carriage of MDR. The proportion of MDR *Klebsiella* noted in this study (64.1%) is similar to a study done [39] in Kilifi where the proportion of MDR was 63%, although the isolates were from invasive infections. In contrast, a study conducted [40] in rural western Kenya showed a lower proportion MDR *Klebsiella* of 36.7%. This contrast can be attributed to differences in economic, social and environmental settings. In East Africa, the proportion observed ranges from 80% - 95%, which is comparatively higher than that observed in this study [41] [42]. While the prevalence of *K. oxytoca* was low at 2.56% among children in Mukuru, isolation of MDR *K. oxytoca* was high at 96.67% (29/30). Though not highly prevalent from children in Mukuru, it's alarming that nearly all the isolates of *K. oxytoca* are MDR. The latter implies that, if colonization by *K. oxytoca* proceeds infection, the disease can record high treatment failures particularly among the immune compromised persons. Additionally, *K. oxytoca* can transfer its resistance genes to other organisms including *K. pneumoniae* and other enteric bacterial pathogens, leading to a high burden of treatment failure. Unlike *K. pneumoniae*, horizontal transfer of genes in *K. oxytoca* is not well documented, although trends of low prevalence of *K. oxytoca* with high isolation rates of MDR *K. oxytoca* have been documented in India [43], among adults, and in Iran [44]. Similar studies data are scarce in Africa.

Resistance patterns observed in 3rd generation cephalosporins can be attributed to their widespread use and/or misuse in the health facilities in Kenya [45]. The high frequency of the βeta-Lactams resistance genes of *bla* CTX-M, *bla* TEM, *bla* OXA-1 and *bla* SHV may be due to the presence of mobile genetic elements bearing these genes in this slum environment. Further, various studies in Africa [46] [47] [48] have alluded to the fact that the high economic growth in

the recent years has led to ease of accessibility of βeta-Lactams over the counter leading to increased abuse and/or misuse of these antibiotics, hence the predominance of *bla* TEM and *bla* CTX-M genes in the environment.

Of the 22.7% prevalent ESBL producing *K. pneumoniae*, the rate of isolation was significantly higher in children below 50 months, potentially attributed to their underdeveloped immune system or possible nutritional deficiencies due to their residential environment. This ESBL prevalence however appears lower compared to other studies done in Kenya ranging between 44% [39] and 71% [46]. It is however noted that the prevalence of ESBL at 71% was established among *K. pneumoniae* isolated from urine samples. Resistance to Quinolone and Fluoroquinolone from this study was generally low at 18.36%, indicating low selective pressure for these antibiotics. The predominant *qnr* genes (which is plasmid mediated; PMQR) were *qnr*S, *qnr*B, indicating possible horizontal transfer of these genes can occur to other organisms including pathogens. Therefore, exacerbating fluoroquinolone resistance which is the choice of treatment for a variety of infections. In addition to PMQR, fluoroquinolone resistance can be mediated by chromosomal mutations especially in DNA gyrase and topoisomerase encoding genes such *par*E and *par*C genes., which were also detected during this study at 38% and 48% respectively Although these genes were observed in this study in relatively high proportions, the mutations can only be observed after performing DNA sequencing, which was a limitation in this study. Notably, isolates that carried *qnr* genes were all resistant to nalidixic acid, however some isolates exhibited partial reduction of ciprofloxacin efficacy to *K. pneumoniae* as opposed conferring complete resistance to the antibiotic (0.25 - 0.5 μg/mL). This indicates that *qnr* genes confer complete resistance to quinolones and partial resistance to fluoroquinolones. The low rate of resistance can be due to the low prescription of Ciprofloxacin and its high cost despite being widely available. Similar findings where *qnr*S and *qnr*B genes have been found to be most prevalent in Africa [49] [50] have been documented. Low resistance to carbapenems of 3.3% was noted in this study. The prevalence of carbapenem resistance gene *bla* NDM-1 was also low at 7.1%. The low resistance can be due to their limited use and availability in the market in Kenya [51]. Indeed, a similar study (Poirel *et al.*, 2010) conducted in Nairobi only observed one *bla* NDM positive isolate, with similar study conducted in Kilifi, Kenya [39] observed no *bla* NDM isolates. The study in Kilifi however, documented a plasmid with a genetic architecture of a known *bla* NDM carrying plasmid in a total of 25 isolates.

In this study *qnr* B and S genes were found to co-exist with *bla* CTX-M ESBLs. Co-carriage of ESBLs with fluoroquinolones can be attributed to the presence of plasmids containing a plethora of resistance determinants such as the *qnr* genes which encode for *qnr* protective proteins. According to literature, plasmid mediated resistance to quinolone is often associated with ESBLs [52] [53]. Isolates that carried resistance genes to the 3 classes of drugs that were of interest to this study (3rd generation cephalosporins, fluoroquinolones and carbapenemes), were

very low at 3/365 (0.82%). The isolate that showed the highest rate of carriage of AMR determinants was as follows: *bla* TEM-1, *bla* SHV-1, *bla* CTX-M, *bla* NDM, *par*C, *par*E and *qnr*B. This coexistence of genes is uncommon but very worrisome as available options for treatment are extremely limited thus highlighting the dire effects of AMR on public health. By definition, carbapenem resistance also fosters resistance to third generation cephalosporins and hence carbapenem resistance genes co-exist with ESBL encoding genes, a phenomenon that is well documented [54] [55] [56] [57].

Phylogenetic relatedness analysis showed a high number of <80% similarity index amounting to 62.5%, which is indicative of the high diversity among the isolates, ruling out the possibility of clonal spread of MDR strains. Isolates that showed >80% similarity index, amounting to 37.5% were closely related. Those that showed 100% similarity index were considered completely related and amounted to 15%. The findings showed a high genetic diversity of *Klebsiella* strains circulating. Other studies conducted in Kenya have also observed high genetic diversity among *K. pnemoniae* isolates [39] [40].

If colonization precedes infection, and there's high concordance between colonizing and infecting isolates [13] then MDR *K. pneumoniae* such as those carrying AMR genes for 3rd generation cephalosporins (ESBLs), fluoroquinolones and or carbapenems pose a great risk to the community. Therefore, identification of colonizing strains can inform on patient care interventions. Indeed, multidrug resistance is a problem in Mukuru slums and there is urgent need curb this menace. Various measures can be taken to reduce the emergence and spread of resistance. Creating awareness on antibiotic resistance and how it affects their well-being; Improvement of sanitation, provision of clean water and treatment of sewage waste; Antibiotic stewardship that allows for prudent use of antibiotics; Prioritization of research on antibiotics alternatives and development of AMR diagnostic tools [58].

5. Conclusion

The high proportion of MDR *K. pneumoniae* and MDR *K. oxytoca* and the carriage rates of resistance genes observed in the gastrointestinal tract of participants present a threat to community spread of MDR resistant *Klebsiella*. It accentuates the need for more effective infection control measures, proper implementation of public health policies, prioritization of AMR intervention development, surveillance of AMR circulating genes and mapping of MDR *Klebsiella spp* especially in the informal settlements. It also shows empirically that the gut is an important reservoir of a plethora of resistance genes especially in asymptomatic individuals who can disseminate to the vulnerable persons in the community. Such asymptomatic individuals provide key target populations for intervention. More studies are required therefore to further understand the gut resistome and transmission dynamics of AMR genes in informal settlements of low resource countries.

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Conflicts of Interest

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

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