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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-

tics discs were placed 30 mm 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 equals to 5 mm. 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, France) using the GN83 card for antibiotic susceptibility testing (AST).

2.8. Detection of Resistance Associated Genes

After extraction, DNA amplifications were done using sets of different primers targeting resistance genes against 3rd generation cephalosporins (Table 1), fluoroquinolones (Table 2) and carbapenems (Table 3). A reaction mixture of 25 µL was used in a mastermix containing 1 µl forward primer (0.2 µM), 1 µl reverse primer (0.2 µM), 11 µl pcr water, 11 µl pcr mix (QIAGEN) which includes *Taq* DNA Polymerase (2.5 units), PCR Buffer (1x), $MgCl_2$ (0.2 µM), and ultrapure dNTPs (200 µM),) followed by addition of 1 µl template DNA.

Table 1. Primers used for detection of 3rd Generation cephalosporins resistance genes.

Gene	Primer sequence	Expected size (bp)	Annealing Temp (˚C)	References
*bla*TEM	F-5'GCGGAACCCCTATTTG3' R-5'TCTAAAGTATATATGAGTAAACTTGGTCTGAC 3'	793	50	[26]
bla SHV	F-5'TTCGCCTGTGTATTATCTCCCTG 3' R-5'TTAGCGTTGCCAGTGYTCG 3'	854	50	[27]
bla OXA-1	F-5'ATGAAAAACACAATACATATCAACTTCGC 3' R-5'GTGTGTTTAGAATGGTGATCGCATT 3'	820	50	[28]
bla CTX-M	F-5'ATGTGCAGYACCAGTAARGTKATGGC 3' R-5'TGGGTRAARTARGTSACCAGAAYCAGCGG 3'	593	60	[29]

Table 2. 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	[30]
aac (6')-1b-cr2	F-5'-TTGCAATGCTGAATGGAGAG-3' R-5'CGTTTGGATCTTGGTGACCT-3'	482	55	[30]
*qnr*A	F-5'-ATAAAGTTTTTCAGCAAGAGG-3' R-5'-ATCCAGATCGGCAAAGGTTA-3'	624	55	[31]
*qnr*B	F-5'-GGMATHGAAATTCGCCACTGC-3' R-5'-TTTGCYGYYCGCCAGTCGAAC-3'	469	55	[31]
*qnr*S	F-5'-GCAAGTTCATTGAACAGGGT-3' R-5'-TCTAAACCGTCGAGTTCGGCG-3'	417	55	[31]
*par*C1 *par*C2	5'-ATGAGCGATATGGCAGAGCG-3 5'-TGACCGAGTTCGCTTAACAG-3	412	57	[31]
*par*E1 *par*E2	5'-GACCGAGCTGTTCCTTGTGG-3 5'-GCGTAACTGCATCGGGTTCA-3	272	55	[31]

Table 3. Primers used for detection of carbapenems resistance genes.

Gene	Primer sequence	Expected size (bp)	Annealing Temp (˚C)	References
*bla*KPC	F-5 TGTTGCTGAAGGAGTTGGGC'3' R-5' TGTTGCTGAAGGAGTTGGGC3'	863	61	[26]
*bla*NDM-1	F-5'GAGATTGCCGAGCGACTTG 3' R-5'CGAATGTCTGGCAGCACACTT 3'	591	61	[29]

Amplification conditions consisted of 30 cycles of 94˚C for 30 seconds, 55˚C for 30 seconds, and 72˚C for 30 seconds, with a final extension step of 72˚C for 10 min [30].

Gel electrophoresis of PCR products was carried out at a voltage of 100 V on a 1.5% agarose gel for 30 minutes and the DNA staining done using SYBR green dye.

2.9. 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 (0.2 µM), 11.5 µl PCR water, 11.5 µl PCR mix (QIAGEN, Germany), which includes *Taq* DNA Polymerase (2.5 Units), PCR Buffer, $MgCl_2$ (0.2 µM) and ultrapure dNTPs (200 µM) with 1 µl template DNA. Amplification conditions constituted; initial denaturation at 95˚C for 2 minutes, final denaturation for 30 seconds, annealing of primers at 40˚C for 30 seconds, initial extension at 65˚C for 5 minutes and final extension at 65˚C for 15 minutes. The amplified products were electrophoresed in 2% agarose gel stained using SYBR Green solution. 5 µl of loading dye was mixed with 10 µl of amplified PCR products. Gel electrophoresis was done on 100 V for 30 minutes and UV Tran illuminator was used to visualize the bands.

2.10. Data Management and Analysis

Participants' data was 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. Results

3.1. Demographic Characteristics of Children from Mukuru Slums

A total of 1171 children were recruited into this study comprising of 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' 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.

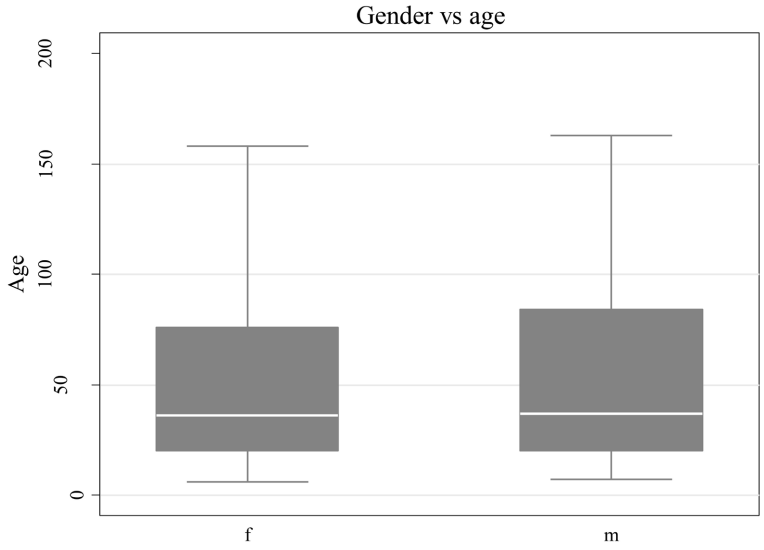

Figure 1. Distribution of children's age between genders; those colonized with *Klebsiella spp.*

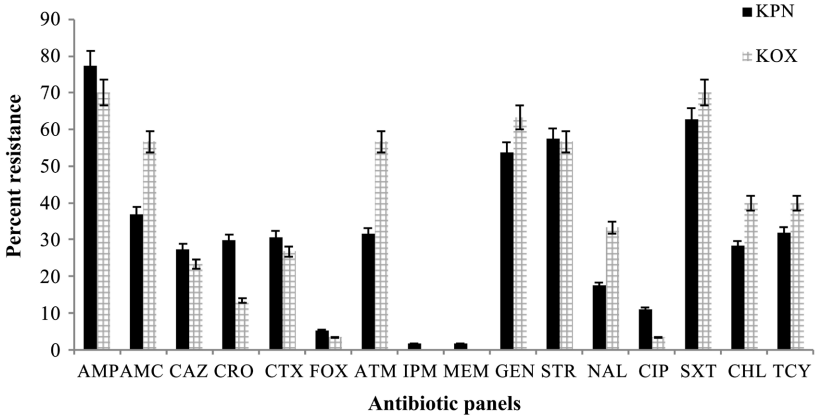

Figure 2. Resistance patterns of *Klebsiella pneumoniae* and Klebsiella *oxytoca* isolated from children and minors from Mukuru slums, Nairobi Kenya. Highest percentage resistance (with 5% margin of error) is observed for AMP with lowest resistance shown for IPM and MEM. Key: Ampicillin (AMP), Amoxicillin-Clavulanate 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.

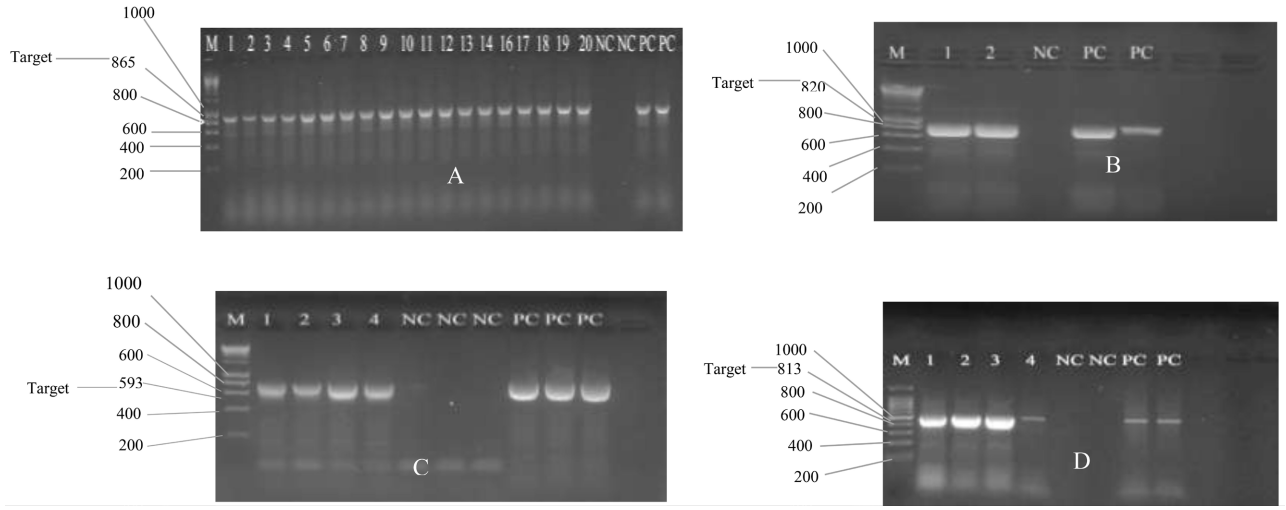

Figure 3. Gel photos for resistance genes to Beta-Lactam class of antibiotics. A: *bla* TEM gene (865 bp) B: *bla* OXA-1 gene (820 bp). C: *bla* CTX-M gene (593 bp). D: *bla* NDM gene (813 bp). M represents the Molecular ladder. NC represents Negative Control. PC represents the Positive Control.

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.

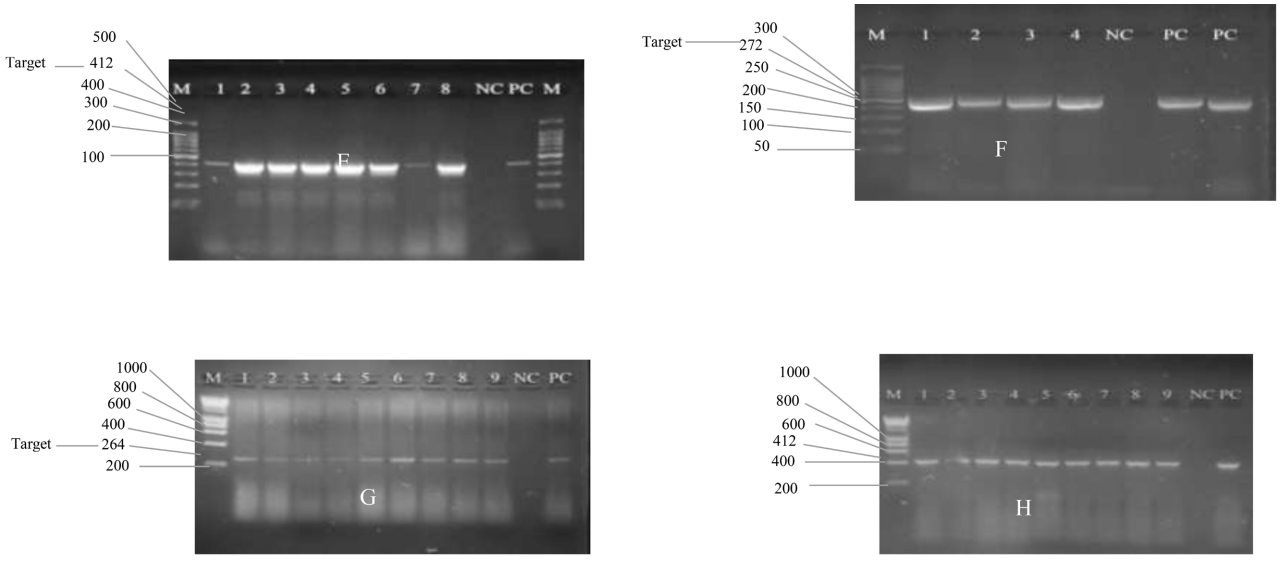

Figure 4. Gel photos for resistance genes to Quinolone and Fluoroquinolone class of antibiotics. E: *par* C gene (412 bp) F: *par* E gene (272 bp). G: *qnr*B gene (264 bp). H: *qnr*S gene (813 bp). M represents the Molecular ladder. NC represents Negative Control. PC represents the Positive Control.

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	*par*C, *par*E, *qnr*S	-
**2018	*bla* TEM-1, *bla* CTX-M, *bla* OXA-1	*par*C, *qnr*S	-
**1471	*bla* TEM-1, *bla* CTX-M, *bla* OXA-1	*par*C, *qnr*S	-
**1204	*bla* TEM-1, *bla* CTX-M, *bla* OXA-1	*par*C, *par*E, *qnr*B, *qnr*S	-
**2215	*bla* TEM-1, *bla* CTX-M, *bla* OXA-1	*par*C, *par*E, *qnr*B	-
**2548	*bla* TEM-1, *bla* CTX-M, *bla* OXA-1	*par*C, *par*E, *qnr*B	-
**2600	*bla* TEM-1, *bla* CTX-M, *bla* OXA-1	*par*C, *par*E, *qnr*B	-
***1588	*bla* TEM-1, *bla* SHV-1, *bla* CTX-M	*par*C, *par*E, *qnr*B	*bla* NDM
**2893	*bla* TEM-1, *bla* SHV-1, *bla* CTX-M, *bla* OXA-1	*par*C, *par*E, *qnr*S	-
**1882	*bla* TEM-1, *bla* SHV-1, *bla* CTX-M, *bla* OXA-1	*par*C, *par*E, *qnr*S	-
**2315	*bla* TEM-1, *bla* CTX-M, *bla* OXA-1	*par*C, *par*E, *qnr*S	-
**1989	*bla* TEM-1, *bla* SHV-1, *bla* CTX-M	*par*C, *par*E, *qnr*B, *qnr*S	-
**1484	*bla* TEM-1, *bla* CTX-M, *bla* OXA-1	*par*E, *qnr*B, *qnr*S	-
**2555	*bla* TEM-1, *bla* CTX-M, *bla* OXA-1	*par*E, *qnr*B, *qnr*S	-
**2968	*bla* TEM-1, *bla* SHV-1, *bla* CTX-M, *bla* OXA-1	*par*E, *qnr*S	-
**2499	*bla* TEM-1, *bla* SHV-1, *bla* CTX-M, *bla* OXA-1	*par*E, *qnr*S	-
**1678	*bla* TEM-1, *bla* SHV-1, *bla* CTX-M, *bla* OXA-1	*par*E, *qnr*B, *qnr*S	-
**1535	*bla* TEM-1, *bla* CTX-M, *bla* OXA-1	*par*E, *qnr*S	-
**1082	*bla* TEM-1, *bla* CTX-M, *bla* OXA-1	*par*E, *qnr*S	-
**1369	*bla* TEM-1, *bla* SHV-1, *bla* CTX-M, *bla* OXA-1	*par*E, *qnr*S	-
**1923	*bla* TEM-1, *bla* CTX-M, *bla* OXA-1	*par*E, *qnr*B	-
**2306	*bla* TEM-1, *bla* SHV-1, *bla* CTX-M, *bla* OXA-1	*par*E	-
**1581	*bla* TEM-1, *bla* CTX-M,	*par*C, *par*E	-
***1720	*bla* TEM-1, *bla* CTX-M, *bla* OXA-1	*par*C, *qnr*B	*bla* NDM
**2737	*bla* TEM-1, *bla* CTX-M, *bla* OXA-1	*par*E	-
**2472	*bla* TEM-1, *bla* SHV-1, *bla* CTX-M, *bla* OXA-1	*par*C	-
**2402	*bla* TEM-1, *bla* CTX-M, *bla* OXA-1	*par*C, *par*E, *qnr*B, *qnr*S	-
***2402	*bla* TEM-1, *bla* SHV-1, *bla* CTX-M	*par*C, *qnr*B, *qnr*S	*bla* NDM
**1214	*bla* TEM-1, *bla* SHV-1, *bla* CTX-M, *bla* OXA-1	*par*E, *qnr*B	-
**2642	*bla* TEM-1, *bla* SHV-1, *bla* CTX-M, *bla* OXA-1	*par*C, *qnr*B, *qnr*S	-
**2646	*bla* TEM-1, *bla* SHV-1, *bla* CTX-M	*par*C	-
**1287	*bla* TEM-1, *bla* SHV-1, *bla* CTX-M, *bla* OXA-1	*par*e	-
**2951	*bla* TEM-1, *bla* SHV-1, *bla* CTX-M	*par*e	-
**2343	*bla* TEM-1, *bla* SHV-1, *bla* CTX-M	*par*C, *qnr*B, *qnr*S	-
**1195	*bla* TEM-1, *bla* CTX-M	*qnr*B	-
**2382	*bla* TEM-1, *bla* SHV-1, *bla* CTX-M	*qnr*B	-
**1290	*bla* TEM-1, *bla* CTX-M	*qnr*B	-
**2207	*bla* TEM-1, *bla* SHV-1	*qnr*S	-

** 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.

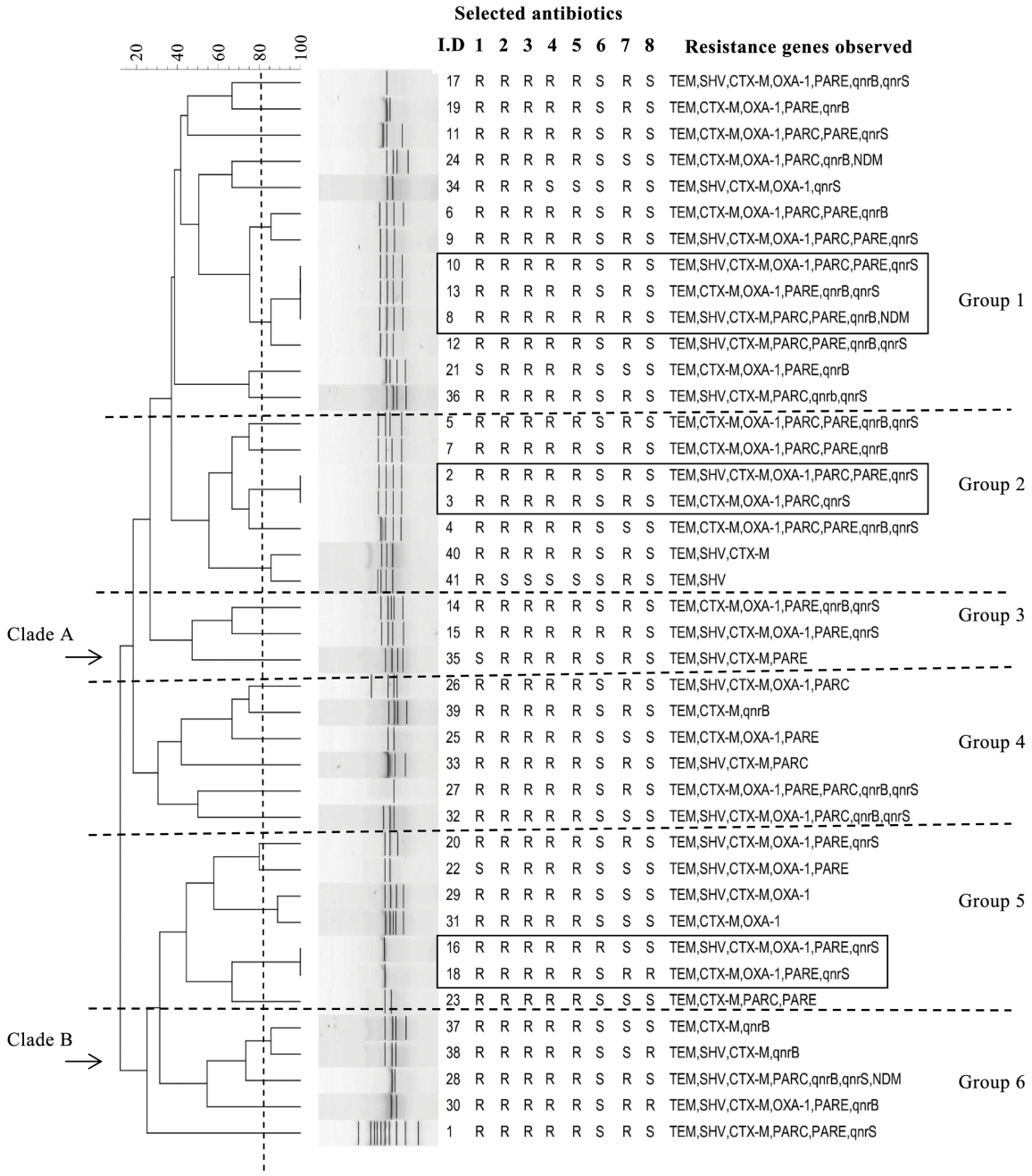

Figure 5. Phylogenetic relatedness of *Klebsiella spp* isolated from Children in Mukuru slum. Key: I.D: Isolate DNA Number. Antibiotic 1: Amoxicillin Clavulanate 2: Cefotaxime 3: Ceftazidime 4: Ceftriaxone 5: FEP 6: Amikacin 7: Ciprofloxacin 8: Meropenem.

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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