Scientific Research Publishing

Advances in Microbiology, 2019, 9, 507-524
http://www.scirp.org/journal/aim
ISSN Online: 2165-3410
ISSN Print: 2165-3402

Antimicrobial Profiles of Selected Gram-Negative Bacteria Recoverable from Sewage and Sludge from Juja and Kibera Informal Settlements of the Larger Nairobi Metropolis

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How to cite this paper: Maina, J., Ndung'u, P., Muigai, A., Onyango, H., Mukaya, J.K., Wambui, S., Judah, T., Kinyua, J., Muriuki, J., Chesenge, L., Kisoo, L., Thuku, R., Wachira, B., Bett, V., Gachuki, T. and Kiiru, J. (2019) Antimicrobial Profiles of Selected Gram-Negative Bacteria Recoverable from Sewage and Sludge from Juja and Kibera Informal Settlements of the Larger Nairobi Metropolis. *Advances in Microbiology*, **9**, 507-524.
https://doi.org/10.4236/aim.2019.96031

Received: May 2, 2019
Accepted: June 15, 2019
Published: June 18, 2019

Abstract

Africa has experienced rapid urban migration in the past two decades. New informal settlements continue to emerge and expand but the sanitation provision of facilities has not improved at the same pace and this poses a serious health concern to the public especially the urban poor. Open sewage systems and sludge-clogged drainage systems as well as soil contaminated with industrial and domestic wastes are possible sources of germs that probably cause clinical infections and epidemics. In this cross-sectional study, we recorded diverse genera of Gram-negative non-fastidious bacteria that included; *Escherichia coli* (23%), *Klebsiella spp* (21%), *Enterobacter spp* (19%), *Citrobacter spp* (10%), *Pseudomonas aeruginosa* (8%), *Proteus spp* (7%), *Salmonella* (3%), *Yersinia spp* (3%), *Shigella spp* (2%), *Morganella morganii* (2%), *Edwardisella spp* (1%), *Hafnia spp* (1%), *Serratia marcesence* (0.5%), and *Acinetobacter baumannii* (0.5%). Most of these isolates were resistant to ampicillin while imipenem and ciprofloxacin were the most effective antimicrobial agents. Resistance combination towards ampicillin, trimethoprim, sulfamethoxazole and streptomycin was also noted in recovered isolates (16%). An overall high antimicrobial resistance was recorded among isolates from slum as compared to those recovered from Juja, a middle-class settlement located at the edge of Nairobi metropolis. The prevalence of isolates with a combined resistance to 3rd generation cephalosporins (cefotaxime, ceftriaxone and ceftazidime), gentamicin and ciprofloxacin was the highest among *P. aeruginosa*

isolates (13%) but none of the *Yersinia* species and *Edwardisella tarda* exhibited this resistance. Carriage of bla_{TEM} (52%) was most prevalent in all bacteria species followed by $bla_{\text{CTX-M}}$ (20%), bla_{SHV} (18%) while bla_{OXA} (17%) was the least common. The phylogeny analysis revealed significant genetic similarity among strains belonging to *E. coli*, *K. pneumoniae*, *E. agglomerans* and *P. mirabilis strains* but less relatedness was noted among strains belonging to *C. freundii*. Further analysis showed possible clonal expansion of *E. agglomerans* and *K. pneumoniae* within the environmental ecosystems.

Keywords

Antimicrobial Resistance, Multiple-Drug Resistance (MDR), Extended Spectrum β-Lactamases (ESBL), Enterobacteriaceae, Sewage and Sludge

1. Introduction

The emergence and spread of antimicrobial resistance have become a global concern and affect our ability to combat severe infections. It is estimated that nearly 1 M deaths annually occur across the globe as a result of treatment failure due to antimicrobial resistance [1]. Notably, in resource-poor countries, especially those in Sub-Sahara Africa, cases of morbidity and mortality due to treatment failure are on the rise. The increase in infections caused by Gram-negative bacteria has also led to a surge in usage and over-reliance on antimicrobials for human and animal infection treatment and prophylaxis.

As a result of over-use and misuse of antibiotics in human and animal health, domestic waste is likely to be highly contaminated with fecal materials which in turn have bacteria that are resistant to multiple antimicrobials due to their possible clinical origin [2]. Previous studies have documented sludge and sewage effluents as major sources of antimicrobial resistant bacteria strains in the environmental compartment [3] [4].

Antimicrobial resistance in Gram-negative bacteria is on the rise which is partially attributed to the ease of spread and acquisition of resistance genes especially amongst the members of the family Enterobacteriaceae. Horizontal transfer of these resistance genes has largely been attributed to plasmid-borne integrons which are able to assemble resistance genes in cassettes [5]. Multiple-drug resistance (MDR) strains in sewage, sludge or soil can easily get into water bodies such as river and municipal water supply lines and end up as clinical strains responsible for various infections in humans and animals. This is even more plausible in informal settlements where water supply lines often pass through open sewers and where many illegal water connections happen. In such settings, most of street food-vending points are often situated next to open sewer and hand hygiene is hardly observed further increasing risk of acquisition and spread of MDR-strains. Therefore, foods sold in such environments can cause food borne infections and outbreaks. Some antimicrobial agents such as tetracycline

and quinolone have a long shelf-life in environmental compartments while others such as sulfonamides are water soluble and their persistence may create selection pressure that leads to emergence of resistant clones [6] [7]. Contamination and long-term exposure of soil to antimicrobial residuals due to livestock rearing and agricultural practices may therefore lead to resistance build-up and positively select for highly resistant strains [8].

In Kenya, like in most sub-Saharan countries, the role played by sewage, sludge and fecal contaminated soils as sources for isolates with MDR strains has not been investigated. This is even more important in informal settlements characterized by poor sanitation, limited access to clean water and over-congestion [9]. Such data is particularly important because it may find important applications in outbreaks management and in policy formulations to curb spread of AMR and for identification of hotspots that play a role as flush-points for outbreaks associated with MDR strains.

In this cross-sectional study, we determined the diversity of non-fastidious Gram-negative bacteria and their associated antimicrobial resistance patterns to common antimicrobials and the phylogenic relatedness of strains from different sites. The current study was conducted in Kibera informal settlement in Nairobi which is the second largest slum in Africa continent and in Juja town, a middle-class settlement at the edge of Nairobi metropolis.

2. Materials and Methodology

2.1. Study Design and Sample Collection

A convenient random sampling design was used in this cross-sectional study to obtain soil, sludge and sewage samples from sites near food vending point in Kibera informal settlements and Juja town, Figure 1 and Figure 2. A purposeful 100 samples each of sewage, sludge and soil were obtained across 13 villages of

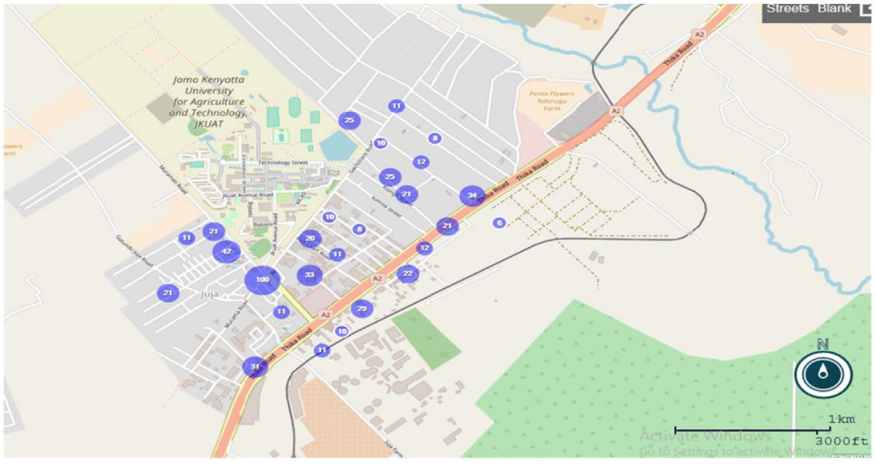

Figure 1. Distribution pattern of multiple drugs resistance Gram negative bacteria isolates from Juja metropolis. The spot map show the distribution pattern of Enterobacteriaceae isolates that were resistant to more than 3 antimicrobial agents belonging to different classes.

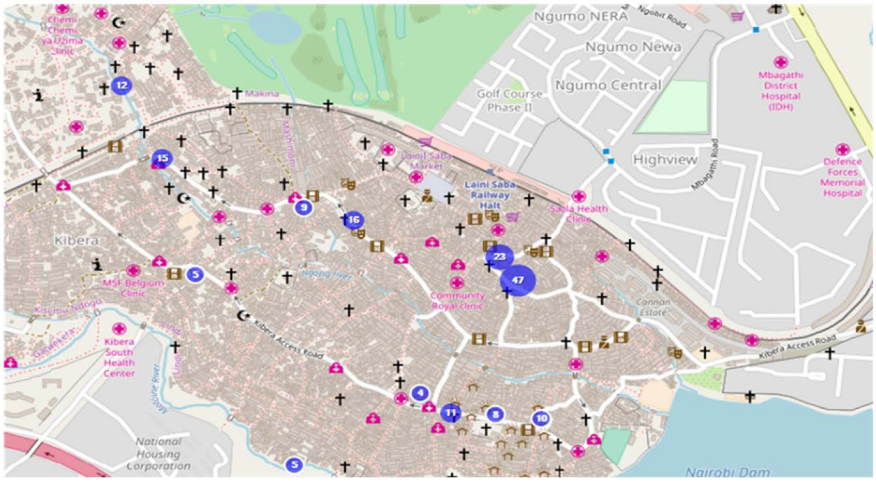

Figure 2. Distribution pattern of multiple drugs resistance Gram negative bacteria isolates from Kibera slums. The spot map shows the distribution pattern of Enterobacteriaceae isolates that were resistant to more than 3 antimicrobial agents belonging to different classes.

Kibera slum between July to December 2017. During that period, a similar set of samples were also obtained in Juja town. Approximately 30 ml of sewage and sludge sample was obtained by holding universal bottle at the base and plunging it below the surface of flowing sewer. Approximately 30 g of soil sample was also collected near the point of sewage and sludge collection site. The samples were then transported to laboratory within 2 hours for processing and culture.

2.2. Sample Processing

In order to isolates non-fastidious Gram-negative bacteria especially those belonging to family Enterobacteriaceae, approximately 1 mL of sewage or sludge sample was inoculated in 9 ml of buffered peptone water and alkaline peptone water, shaken for 1 hr in order to resuscitate any injured cells before plating directly onto oxoid™ MacConkey and Eosin Methylene Blue Agar (EMBA) and blood agar (BA) plates. From each plate, three distinct colonies of similar morphology were purified on EMBA and on tryptone soy agar (TSA). Pure isolates were identified through Gram-staining and a series of biochemical tests that included; triple sugar iron, lysine-indole motility, citrate utilization, methyl red voges proskauer test and urease test as described in the past [10].

2.3. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility profiles were performed using the disk diffusion method on Mueller Hinton medium. The antimicrobial panel used consisted of ampicillin (AMP, 10 µg), amoxicillin/clavulanic acid (AMC, 20/10 µg), cefoxitin (FOX, 30 µg), ceftriaxone (CRO, 30 µg), cefotaxime (CTX, 30 µg), ceftazidime (CAZ, 30 µg), cefepime (FEP, 30 µg), aztreonam (ATM, 30 µg), ciprofloxacin (CIP, 5 µg), nalidixic acid (NA, 30 µg), gentamicin (CN, 10 µg), streptomycin (S, 10 µg), imipenem (IPM, 10 µg), sulfamethoxazole (RL, 200 µg) and trimetho-

prim (W, 5.2 µg). *E. coli* ATCC 25922 reference strains was used for ensuring media quality and disc potency. Interpretation of antimicrobial susceptibility zones was done using the CLSI 2017 guidelines. Chi-square test was used for statistic analysis where a value of 0.05 or less was considered as indication of significant difference between test variables.

2.4. PCR Detection of *β*-Lactamase Genes

Extraction of DNA was done using the boiling method. This process entailed emulsifying bacterial colonies in 1000 µl molecular grade water. Cell lyses was then done by boiling the preparation at 95˚C on a thermal block for 10 - 15 min. Separation was the done through centrifugation at 14,000 rpm for 5 minutes. The supernatant containing DNA was then stored at −20˚C. Detection of genes that are frequently associated with resistance to important classes of ß-lactams such as bla_{TEM}, $bla_{CTX\text{-}M}$, bla_{SHV}, bla_{OXA} and class 1 integron was done using published primers, Table 1 [11] [12] [13]. The final volume in each PCR tube was 25 µl which included 10 µl of Qiagen master mix, 1 µl butane, 2 ul DNA, 10 µl PCR water and 2 µl forwards and reverse primer. Amplified PCR products were separated in 1.5% gel and banding patterns visualized under UV gel imager.

2.5. Finger Printing of Recovered Bacterial Isolates

Phylogenetic relatedness was determined using the $(GTG)_5$-PCR method using published strategies and primers indicated in Table 1. (13) PCR amplification of target DNA sequence was done at 40˚C annealing temperature. Amplified products were separated by running in 1% agarose gel for I hour. Visualization of banding patterns was done using a Gelmax® UV imager. Banding patterns were analyzed using bionumerics Gelcompar®2 software version 6.6 with the cluster

Table 1. PCR amplification primers used.

Target gene	Primer name	Primer sequence	Annealing T (˚C)	Product size	Reference
blaTEM	TEM-F	5'-GCGGAACCCCTATTTG-3'	50	964 bp	
		5'-TCTAAAGTATATATGAGTAAACTTGGTCTGAC-3'			12
blaCTX-M	CTX-M-F	5'-ATGTGCAGYACCAGTAARGTKATGGC-3'	60	593 bp	12
	CTX-M-R	5'-TGGGTRAARTARGTSACCAGAAYCAGCGG-3'			
blaSHV	SHV-F	5'-TTCGCCTGTGTATTATCTCCCTG-3'	50	854 bp	12
	SHV-R	5'-TTAGCGTTGCCAGTGYTCG-3'			
blaOXA	OXA-IF	5'-ATGAAAAACACAATACATATCAACTTCGC-3'	62	820 bp	12
	0XA-1R	5'-GTGTGTTTAGAATGGTGATCGCATT-3'			
***Int*1**	intM1_D	5'-GAAAGGTCTGGTCATACATG-3'	50	500bp	11
	intM1_U	5'-ACGAGCGCAAGGTTTCGGT-3''			
(GTG)5	(GTG)5	5'-GTGGTGGTGGTGGTG-3'	50	variable	13

Key: *bla*: *β*-lactamase gene, bp: molecular weight in base pair.

analysis done using the dice method based on UPGMA arithmetic mean. Randomly selected bacteria species from six most prevalent genera were analyzed for genetic relatedness. A correlation of ≥80% among bacterial species was considered as strong evidence of genetic relatedness among isolates as previously described [14].

2.6. Ethical Approval

Ethical approval prior to the study commence was obtained from scientific ethical review unit, Kenya medical research institute (SERU, KEMRI), approval number KEMRI/SERU/CMR/P00055/3514.

3. Results

3.1. Bacterial Isolates

A total of 348 Gram-negative isolates were obtained from sewage, 364 from sludge and 211 from soil, Table 2. A total of 14 non-fastidious bacteria genera were identified from these samples obtained from the slum (Kibera) and from middle class settings (Juja). Isolates obtained in this study were categorized in 4 groups; indicator organisms for AMR trends in human, animal and the environment [*Escherichia* (23%) and *Klebsiella* (21%)], genera of known Enteropathogens species [*Salmonella* (3%) and *Shigella* (2%)], genera associated with high intrinsic antmicrobial resistances [*A. baumannii* (0.5%), *P. aeruginosa* (8%), *M. morganii* (2%), *Sr. marcesence* (0.5%)] and other genera that are not significantly associated with diseases but significant as opportunistic infection pathogens such as [*Citrobacter spp* (10%), *Enterobacter spp* (19%), *Edwardisella spp* (1%), *Proteus spp* (7%), *Yersinia spp* (3%) and *Hafnia spp* (1%)].

Table 2. Bacterial diversity from sewage and sludge samples.

Gram negative bacteria diversity in environmental samples														
	Citrobacter spp	Enterobacter spp	E. tarda	E. coli	H. alvei	Klebsiella spp	M. morgannii	P.aeruginosa	A.baumannii	Proteus spp	Salmonella spp	Shigella spp	Yersinia spp	S. marcesence
Sewage														
Juja n = 139	17	28	0	38	0	31	0	10	0	10	0	0	5	0
Kibera n = 209	10	31	10	43	10	33	5	13	5	14	15	10	10	0
Sludge														
Juja n = 153	22	33	0	39	0	35	0	12	0	12	0	0	0	0
Kibera n = 211	25	42	0	32	0	41	10	18	0	15	12	6	10	0
Soil														
Juja n = 75	9	12	0	25	0	22	0	7	0	0	0	0	0	0
Kibera n = 136	10	30	0	36	0	30	0	10	0	15	0	0	0	5

Lctn: Location, **spp:** species, **n:** total number, ***E. tarda***: *Edwardisella tarda*, ***E. coli***: *Escherichia coli*, ***P. aeruginosa***: *Pseudomonas aeruginosa*, ***S. marcesence***: *Serratia marcesence.*

3.2. Spatial Difference in Antimicrobial Resistance Profiles

Isolates from slum sludge were overly more resistant to any tested antimicrobial agents. High resistance prevalence was recorded towards ampicillin (AMP 57%), trimethoprim (W 56%) and sulfamethoxazole (RL 55%) among sludge isolates recovered from the slum, Table 3. Soil isolates from Juja middle class setting were the least resistance to any tested antimicrobial agent. An overall high antimicrobial resistance was recorded in isolates from slum as compared to those recovered from Juja, a town located at the edge of Nairobi metropolis. Resistance towards cephalosporins was relatively higher among slum isolates with highest resistance values recorded against ceftriaxone (CRO 14%), cefotaxime (CTX 12%) and ceftazidime (CAZ 11%) respectively. Further analysis showed significant difference in cephalosporin resistance e.g. against ceftazidime resistance between the 2 study sites [OR: 0.31, CI 0.59, P: 0.00002)]. Isolates recovered from the slums area (Kibera) were more resistant to ciprofloxacin than those from obtained from the middle-class area where a significant difference was also noted (OR: 2.13, CI: 3.69, P: 0.006). Slum isolates were more resistance to aminoglycosides, with no significant difference been recorded [gentamicin (OR 1.26, CI 1.93, P: 0.28)]. Most isolates were resistant to ampicillin while imipenem and ciprofloxacin were the most effective antimicrobial agents.

3.3. Antimicrobial Resistance Patterns Based on Species

Among the 14 bacteria genera reported in this study, *P. aeruginosa* isolates were the most resistant and on average were resistant to at least 9 of the tested antimicrobial agents, Table 4. This species also had the highest prevalence of isolates (13%) with a combined resistance to 3rd generation cephalosporins (ceftriaxone 22%, cefotaxime 20%, and ceftazidime 15%), advanced classes of aminoglycosides (gentamicin 20%) and otherwise potent classes of quinolones (ciprofloxacin

Table 3. Antimicrobial resistance patterns of all bacterial isolates recovered from Juja and Kibera.

		Antimicrobial resistance %														
	n	AMP	CAZ	CTX	CRO	FEP	ATM	AMC	FOX	CN	S	CIP	NA	IPM	RL	W
Sewage																
Juja	139	50	8	10	11	5	14	11	22	10	19	5	18	0	42	39
Kibera	209	53	9	11	13	7	15	17	24	12	23	10	21	1	39	43
Sludge																
Juja	153	55	10	11	12	6	15	18	26	12	20	6	23	0	46	48
Kibera	211	57	11	12	14	7	19	19	28	14	27	12	26	0	55	56
Soil																
Juja	75	33	5	8	9	4	13	15	16	6	20	3	13	0	33	35
Kibera	136	40	7	9	10	6	10	15	15	10	22	7	22	0	40	44

AMP: Ampicillin, CTX: Cefotaxime, CRO: Ceftriaxone, FEP: Cefepime, ATM: Aztreonam, FOX: Cefoxitin, AMC: Amoxicillin-clavulanic acid, CN: Gentamicin, S: Streptomycin, CIP: Ciprofloxacin, NA: Nalidixic acid, IPM: Imipenem, RL: Sulfamethoxazole, W: Trimethoprim, n=total number bacteria isolates.

Table 4. Antimicrobial resistance profiles of sewage, sludge and soil samples.

	Antimicrobial resistance (%) in Gram negative bacteria															
	Study site	**n**	**AMP**	**CAZ**	**CTX**	**CRO**	**FEP**	**ATM**	**FOX**	**AMC**	**CN**	**S**	**CIP**	**NA**	**IPM**	**RL**	**W**
E. coli																	
	Juja	102	50	8	10	12	5	13	24	13	10	15	5	13	0	46	48
	Slum	111	58	9	12	13	7	14	29	15	14	29	9	15	0	52	55
K. pneumoniae																	
	Juja	88	53	9	11	14	7	15	26	14	13	26	6	24	0	49	50
	Slum	104	60	12	14	14	10	17	32	17	15	33	12	32	0	55	58
Salmonella ssp																	
	Juja	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Slum	27	44	7	11	11	4	11	19	15	15	22	4	33	0	48	44
Shigella spp																	
	Juja	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Slum	16	50	6	13	13	0	13	19	13	13	19	6	25	0	44	50
A. baumannii																	
	Juja	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Slum	5	40	20	20	20	0	20	40	20	20	40	20	40	0	40	40
P. aeruginosa																	
	Juja	29	52	14	17	21	10	31	41	31	17	41	10	45	0	62	45
	Slum	41	49	15	20	22	12	37	48	37	20	37	15	48	5	44	48
M. morganii																	
	Juja	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Slum	15	53	13	13	20	7	20	27	20	13	27	8	33	0	53	47
S. marcesence																	
	Juja	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Slum	5	40	0	20	20	0	20	20	20	0	20	0	20	0	40	20
Citrobacter spp																	
	Juja	49	43	6	8	10	2	12	16	12	8	12	2	16	0	0	0
	Slum	44	43	9	11	11	7	11	18	14	9	18	5	16	0	60	40
Enterobacter spp																	
	Juja	73	51	7	10	11	4	11	14	11	7	15	4	14	0	48	51
	Slum	103	48	10	12	13	5	14	19	13	11	13	5	13	0	50	50
E. tarda																	
	Slum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Juja	10	30	0	0	10	0	10	20	10	0	10	0	20	0	20	10
Proteus spp																	
	Slum	22	41	10	10	14	5	14	23	23	10	27	5	23	0	45	41
	Juja	44	55	9	11	14	7	16	25	25	14	30	9	30	0	52	50
H. alvei																	
	Juja	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Slum	10	40	0	10	10	0	10	10	20	0	20	0	20	0	50	40
Yersinia spp																	
	Juja	5	20	0	0	0	0	0	20	0	0	20	0	20	0	20	0
	slum	20	25	0	5	5	0	5	10	5	5	15	0	15	0	10	15

15%) We also recovered two *P. aeruginosa* isolates from slum sewage that were resistant to imipenem. However, none of the *Yersinia* species and *Edwardisella tarda* exhibited these resistance. The resistance prevalence for *Klebsiella* strains towards any class of antimicrobials was not significantly higher than that of *E. coli* strains (P: 0.36, CI: 1.88, OR: 1.22). Among known Enteropathogens, there was no difference in resistance profiles between *Shigella* and *Salmonella* strains (P: 1.25, C1:4.33, OR: 1.25).

3.4. PCR Detection of *β*-Lactamases Gene and Integron in Bacterial Isolates

A total of 106 selected isolates that were resistant to 3rd generation cephalosporins were screened for carriage of bla_{TEM}, $bla_{\text{CTX-M}}$, bla_{SHV}, bla_{OXA} and class 1 integron which has widely been reported in Gram-negative bacteria. Carriage of bla_{TEM} (52%) was most prevalent ESBL type followed by $bla_{\text{CTX-M}}$ (20%), bla_{SHV} (18%) while bla_{OXA} (17%) was the least common, Table 5. All isolates that carried these genes were resistant to ampicillin and at least one 3rd generation cephalosporin (ceftriaxone, cefotaxime and/or ceftazidime). Co-carriage of bla_{TEM}, $bla_{\text{CTX-M}}$, bla_{OXA} and *int*1 was prevalent in *P. aeruginosa* (13%) while bla_{SHV} was common in *Klebsiella* species.

3.5. Fingerprint Analysis

The phylogeny analysis revealed significant genetic similarity among strains belonging to *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter agglomerans*

Table 5. *β*-lactamases and integron detected in cephalosporin resistance bacteria.

Organism	Total screened	% b-lactamase and integron class 1 in Gram bacteria isolates.				
		*bla*TEM	*bla*CTX-M	*bla*SHV	*bla*OXA	*int*1
Citrobacter spp	n = 10	30	10	0	10	0
E. coli	n = 20	60	50	15	20	20
Proteus spp	n = 9	56	22	11	11	11
P.aeruginosa	n = 15	67	47	27	40	47
Salmonella spp	n = 3	33	33	0	0	0
Klebsiella spp	n = 20	60	20	50	30	25
Enterobacter spp	n = 20	30	10	10	0	0
Shigella spp	n = 2	0	0	0	0	0
Acinetobacter baumannii	n = 2	50	0	0	0	50
Morganella morgannii	n = 2	0	0	0	0	0
Serratia marcesence	n = 1	0	0	0	0	0
Edwardisella tarda	n = 1	0	0	0	0	0
Hafnia alvei	n = 1	0	0	0	0	0

and *Proteus mirabilis strains* but less relatedness was noted among strains belonging to *Citrobacter freundii*, Figures 3-7. Further analysis showed possible clonal expansion within the environmental ecosystems of *E. agglomerans* Figure 4, cluster 2), *Klebsiella pneumoniae* (Figure 5, cluster 2) and *E. coli* (Figure 8, cluster 1, 3). Such strains had identical resistance phenotypes and shared more than 80% genetic similarity. Other strains such as *Salmonella Typhi* showed a similarity of above 70% in terms of resistance patterns but differed significantly

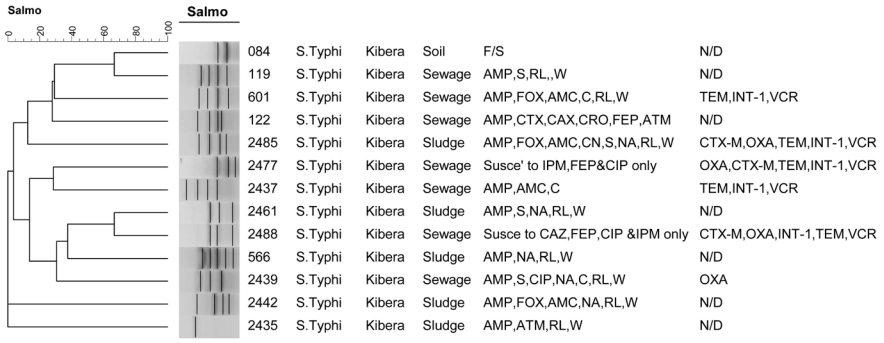

Figure 3. Cluster analysis of recover Salmonellatyphi from sewage, soil and sludge. The figure show phylogeny analysis of *Salmonella Typhi* isolates from sewage sludge and soil sample from Kibera slums. Description of various acronyms on this figure is as follows; *S. Typhi*: *Salmonella Typhi*, TEM: Temoneria *β*-lactamase, CTX-M: Cefotaxime Munich *β*-lactamase, SHV: Sulhydryl variant *β*-lactamase, *Int*-1: Integron, VCR: Variable cassette region, N/D: none of the screened genes were detected.

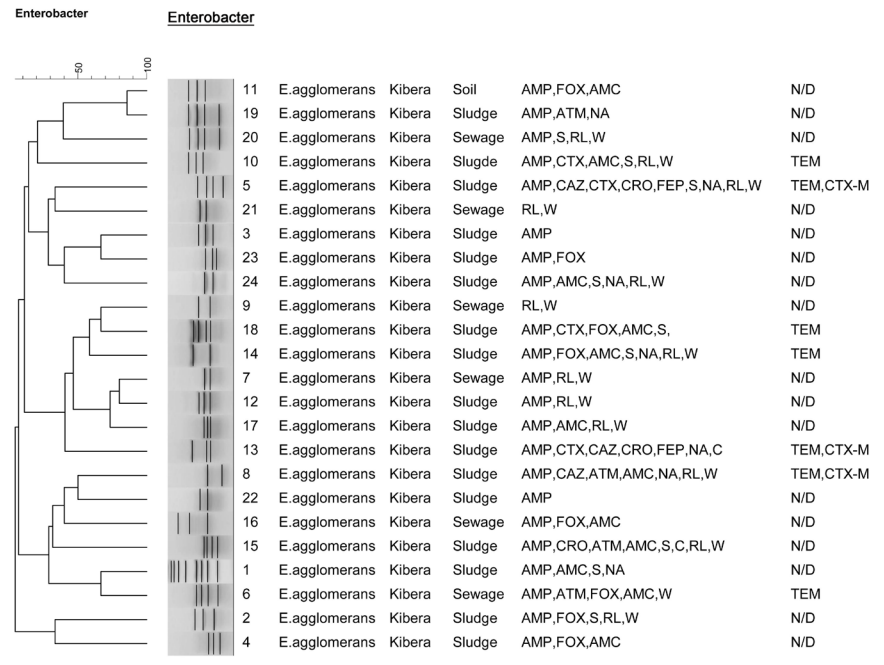

Figure 4. Cluster analysis of recover Enterobacter agglomerans from sewage, soil and sludge. The figure show phylogeny analysis *of Enterobacter agglomerans* isolates from sewage sludge and soil sample from Kibera slums. Description of various acronyms on this figure is as follows; *E. agglomerans*: *Enterobacter agglomerans*, N/D: none of the screened genes were detected.

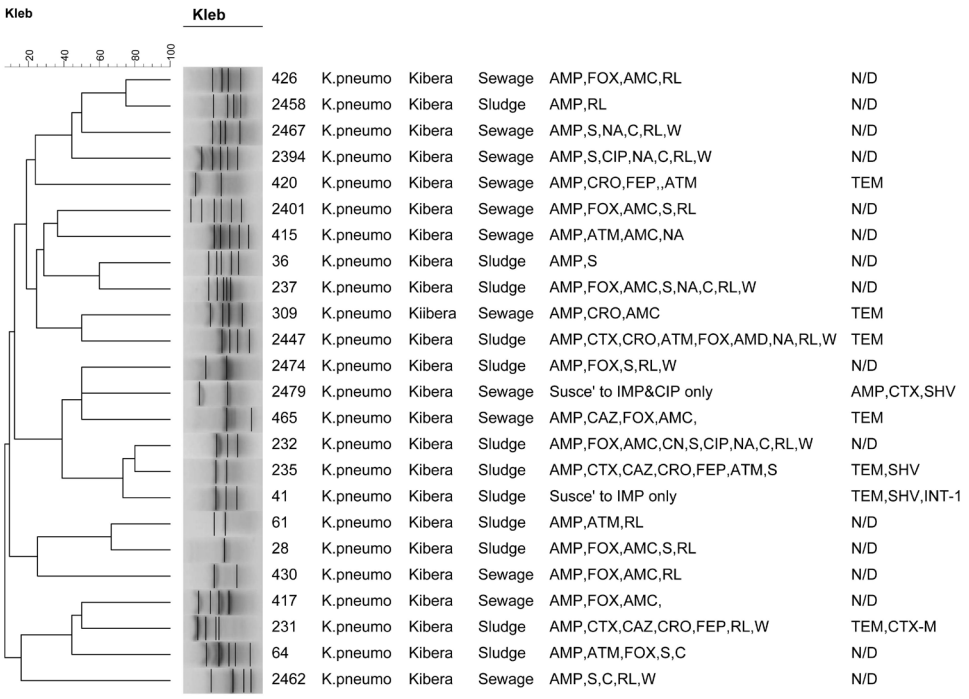

Figure 5. Cluster analysis of recover *Klebsiella pneumoniae* from sewage and sludge. The figure show phylogeny analysis of *Klebsiella pneumoniae* isolates from sewage sludge and soil sample from Kibera slums. Description of various acronyms on this figure is as follows; *K pneumo*: *Klebsiella pneumoniae*, *Int*-1: Integron, N/D: none of the targeted gens was detected.

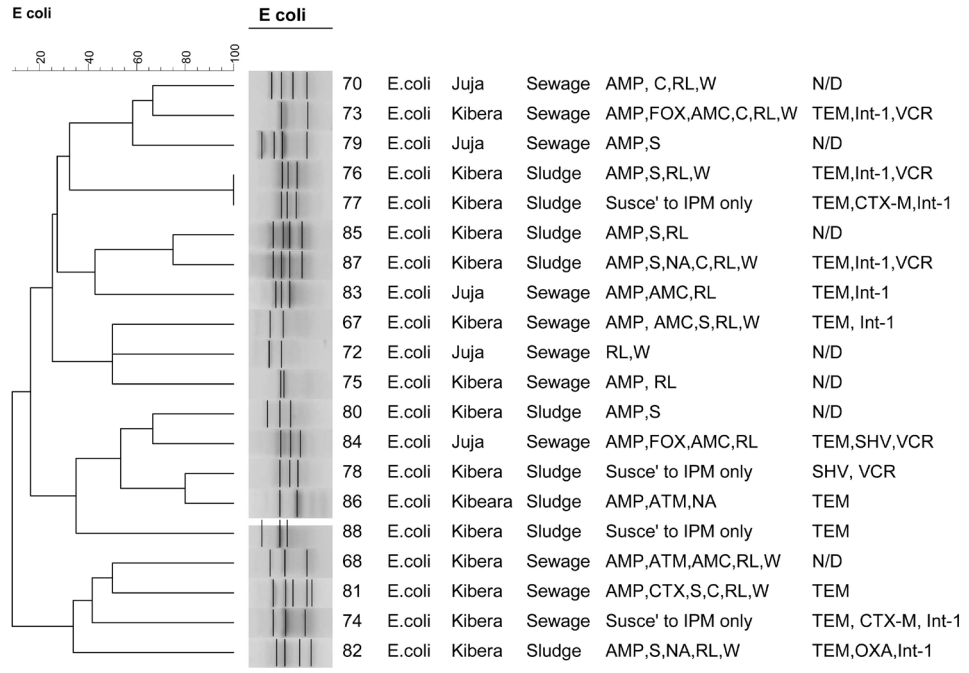

Figure 6. Cluster analysis of recover *Escherichia coli* from sewage and sludge. The figure show phylogeny analysis of *Escherichia coli* isolates from sewage sludge and soil sample from Kibera slum and Juja town. Description of various acronyms on on this figure is as follows; *E.coli*: *Escherichia coli*, *Int*-1: Integron, VCR: Variable cassette region, So: Soil, Se: sewage, Sl: Sludge, N/D: none of the targeted gens was detected.

Figure 7. Cluster analysis of recover Proteus mirabilis from soil, sludge and sewage samples. The figure show phylogeny analysis of *Proteus mirabilis* isolates from sewage sludge and soil sample from Kibera slums. Description of various acronyms on this figure is as follows; *P. mirabilis*: *Proteus mirabilis*, MDR: Multiple drug resistance, TEM: Temoneria β-lactamase, CTX-M: Cefotaxime Munich β-lactamase, SHV: Sulhydryl variant β-lactamase, *Int*: Integron, VCR: Variable cassette region.

Figure 8. Cluster analysis of recover *Citrobacter freundii* from sewage, soil and sludge. The figure show phylogeny analysis of *Citrobacter freundii* isolates from sewage sludge and soil sample from Kibera slums. Description of various acronyms on this figure is as follows; *C. freundii*: *Citrobacter freundii*, *Int*: Integron, N/D: none of the targeted genes were detected.

based on the banding patterns further indicating that different clones may have acquired similar sets of resistance determinants independent (**Figure 3**). Tight clustering of *E. coli* strains from slum (Kibera) and the middle class settlement (Juja town) was also noted; these isolates however had different resistance phenotypes suggesting independent evolution (**Figure 8**, cluster 1). Apparently, sample type and study site was not a key determinant in isolates clustering. These results call for application of high resolution typing of such strains using whole genome analysis strategies in future studies in order to identify the most stable clones that could significantly be related to emergence and spread of AMR.

4. Discussion

It is estimated that by the year 2050 the urban population will double. However, with poor urban planning especially in developing countries, high unemployment rate and poverty, informal settlements are increasingly becoming rampant [15]. Densely populated neighborhoods with poor sanitation infrastructure pose a serious health risk [16]. Previous independent observations have noted that raw and partially processed excreta leaks from domestic toilets in Kibera slums and find their way in the open sewer [17]. In order to reduce chances of release

of enteric bacteria and antimicrobial residues into the environment, there is a need for provision of improved basic sanitation such as clean toilets and proper drainage system in slums and urban centers.

In the current study we recorded a diverse group of non-fastidious Gram-negative bacteria isolates in environmental samples from slum and middleclass setting. Majority of genera isolated are those known to reside in human and animal gut such as *Escherichia*, *Klebsiella* and some are known Enteropathogens such as *Salmonella* and *Shigella* species. Although clinical reports of *Salmonella* and *Shigella* species is on declines, environment colonization still remain a possible source of typhoid, and *Shigellosis* pathogens. It is likely that human fecal and untreated domestic waste could be seeding bacteria of enteric origin into the environment and some of these may have clinical origins based on their resistance profiles. Our study underpins the role of environmental contamination as a driving factor for emergence of outbreaks some of which are caused by MDR strains. We also isolated MDR *P. aeruginosa* and *A. baumannii;* these organisms have previously been associated with wide range of difficult-to-treat infections [18]. The bacteria diversity recorded in this study is comparatively broader compared to those reported in a related study conducted in 2013 and analyzed the microbial content and resistance profiles of isolates obtained from the sewage-contaminated Nairobi river [19]. There is therefore a clear indication that ecosystem contamination with domestic wastes is on a gradual increase in contamination of the environment.

High antimicrobial resistances towards ampicillin (57%), trimethoprim (56%), cefoxitin (28%), sulfamethoxazole (27%) and nalidixic acid (26%) was recorded in isolates from slum. These findings strongly suggest that bacteria from the environment compartments are able accumulate many antimicrobial resistance determinants. The occurrence of such strains with resistance to a combination of antimicrobials raises concerns because if such strains are implicated in infections, available treatment options would be highly limited.

Our data show a higher prevalence of resistance to antimicrobials among isolates recovered from slum environment compartments compared to a previous study conducted in the same slum, Kibera in 2012 [20]. The former study that sought to characterize *E. coli*, *Salmonella* and *Shigella* species in water, soil, vegetables and meat reported and reported a resistance prevalence of 56.8% towards ampicillin against 57% reported here, 13.6% for streptomycin against 27%, 4.9% for nalidixic acid against 26% and 2.5% for gentamicin against 14% recorded in our study. Thus, the values from this previous study were generally lower than what we reported in the present study by a factor of greater than 10%. Although the reasons behind this apparent rise in resistance prevalence is not clear, previous study have reported that more than 80% of antibiotics consumed by humans and animals are excreted through urine and feces [21]. Therefore, a significant amount of active residues may end up in the sewage, sludge and other environment compartments thereby presenting a strong selection pressure that favor proliferation of MDR strains. Contamination of soil with enteric bacteria

and antimicrobial compounds comes from domestic and municipal waste and also from sewage sludge systems [22]. Significant amounts of antimicrobial residues belonging to more than 6 antimicrobial classes have previously been reported in sewage in Asia and Europe and some classes of antimicrobials such as tetracycline and chloramphenicol can persist in soil, sewage and sludge for decades [23]. The presence of such agents in the environment can consequently provide a strong selective pressure that preferentially allows MDR clones to spread [24]. Although our study did not assess presence of antimicrobial agents residues in the environment, our finds strongly underpins the need to such a study.

Higher levels of antimicrobial resistances than reported in our study with greater than 30% towards ceftazidime, cefotaxime, ceftriaxone, ciprofloxacin, aztreonam and gentamicin in *A. baumannii*, *E. coli*, *P. aeruginosa* and *K. pneumoniae* from blood, tracheal aspirates, wound, pus and urine samples have been documented in Kenya [25] [26]. Our study however show an increase in antimicrobial resistance in environmental compartments compared to a previous study conducted in Kakamega town in Kenya [27]. The former study reported *E. coli* from recovered from dumpsites, sludge and wastewater. We reported more 3% increase in resistance towards amoxicillin-clavulanic acid, gentamicin and ciprofloxacin in *Citrobacter species*, *Enterobacter spp.* and *Klebsiella spp.* compared to former study. Although the threat of antimicrobial resistance is more profound in clinical settings, our findings show environmental bacteria strains are increasingly becoming resistant and therefore should not be overlooked.

Carriage of bla_{TEM} (52%) was most prevalent in all bacteria species followed by $bla_{\text{CTX-M}}$ (20%), bla_{SHV} (18%) while bla_{OXA} (17%) was the least common *bla* gene. Co-carriage of bla_{TEM}, $bla_{\text{CTX-M}}$, bla_{OXA} and *int*1 was prevalent in *P. aeruginosa* (13%) while bla_{SHV} was common in *Klebsiella* species. Previous studies have shown that most bacteria that carry *bla* genes also carry integrons which are mostly harbored in mobile genetic elements such plasmids therefore suggesting spread and acquisition in bacteria community [28].

Most of our isolates had less than 70% similarity with distinct resistance phenotypes and genotypes suggesting very little evidence of clonal expansion in the environment. Tight clustering of ≥70 similarity matrix among some few *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter agglomerans* and *Proteus mirabili*s *strains* isolate however suggests independent acquisition of a similar set of resistance determinants among isolates with different profiles. Although we were not able to determine the cause of above mentioned resistance features, such phenomenon has previously been associated with carriage of genetic elements such as plasmids [29].

5. Conclusion

The high diversity of multiple-drugs resistance in sewage and sludge samples observed in this study pose a serious public health hazard in emanation of seri-

ous infection. Unregulated discharge of domestic waste highly polluted with fecal material on open sewer is a great risk to many residents of over-polluted Kibera informal settlements. There is therefore a dire need for proper sewage management to reduce or elimine the serious health risk posed by open sewer.

6. Study Limitations

Our study experienced a number of shortcomings that can be a basis for formulation of stronger studies in futures.

1) The broad range of bacteria genera reported in this study makes focused discussion challenging.

2) We were only able to screen for a few β-lactamases genes. Our study was also not able to determine the bases of resistance to other classes of antimicrobial agents such as aminoglycosides and fluoroquinolones.

3) We were unable to determine the content of detected *int*1 and whether they were born in mobile genetic elements such as plasmids.

4) Low resolution $(GTG)^5$ fingerprint method was used to establish bacteria phylogeny and genetic relatedness. Futures studies should apply high resolution methods such as whole genome sequencing and SNP typing which can shed more light resistance gene content and bacteria evolution in the environmental compartments.

Acknowledgements

The authors wish to send special thanks to KEMRI, CMR for allowing us to conduct this research study at the institution. We are also grateful to the CMR molecular biology laboratory staff for their relentless support and making this study a success. This work was partially funded by the Kenya National Research Fund (NRF).

Author Contributions

John Maina designed and wrote the research protocol for this study in addition to conducting lab work and the manuscript writing. Helen Onyango assisted in lab work and statistical analysis. John Kiiru assisted in designing the study and writing the manuscript. All authors read and approved final manuscript. Anne Muigai and Perpetual Ndung'u provided assist in protocol correction and amendments in study methodologies. Joel Mukaya, Susan Wambui, Terry Judah, Joyce Kinyua, Joystella Muriuki, Lynne Chesenge, Lydia Kisoo, Rebecca Thuku, Boniface Wachira, Vincent Bett and Thomas Gachuki offered laboratory technical support.

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Conflict of Interest

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

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