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Research Article

Epidemiological Analysis and Diversity of Extended Spectrum B-Lactamases from *Escherichia coli* and *Klebsiella* Species Feacal Isolates in Nairobi, Kenya

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Abstract

The World Health Organization has declared antimicrobial resistance a public health threat. Known multidrug resistant strains including *Escherichia coli* and *Klebsiella* species have notably emerged as a major health threat, especially with their resistance to a number of antimicrobial agents available. This has been associated with high mortality and morbidity rates attributed to infections believed to have been caused by either *E. coli* or *Klebsiella* species. This study will unravel the prevalence of *E. coli* and *Klebsiella* species, known reservoirs of resistant genes including Bla genes. Despite the threat these organisms possess to the general public, there was need to avail community based data on its prevalence in both symptomatic and asymptomatic individuals and also determine the drug sensitivity profiles as well as the resistance genes present in isolated bacterial agents. The study conducted in Kibera and Dagoretti areas in Nairobi County targeted the outpatients who presented with arrange of stomach related discomfort and had visited either Mbagathi or Mutuini hospitals. These patients would be identified and requested to partake in the study by voluntarily consenting. Follow-up visits were done to have those who shared residential with the already recruited outpatients to take part in the study too as the asymptomatic group. Stool samples were collected and taken to the Microbiology section of Mbagathi district hospital for culture, Biochemical testing and AST profile analysis then later taken to KEMRI CMR for AMR genes analysis via PCR. Stool samples were cultured directly on MacConkey agar and sub-cultured on TSA agar for purification. Standard Biochemical analysis consisting of IMViC was carried out followed by subjecting the predetermined isolates to a number of antimicrobial agents to test for susceptibility testing. Positively identified potential resistance isolates were subjected to PCR to in presence of respective primers to determine presence of ESBL and KPC genes. Sixty-nine percent (69.8%) of the total study participants had target isolates with Kibera predominantly recording about 67.7%. Contacts participants noted an 80% prevalence. *E. coli* was the pre-dominant species (50%) but *Klebsiella* species noted high resistance of over 95%. Resistance was determined at 65% with high resistance being

reported in 71% and 63% Trimethoprim/sulphamethoxazole and tetracycline respectively. Notably low resistance was noted in IPM (15%) and Cephalosporin (20%). Most isolates exhibited resistance to more than three antimicrobial agents. Forty four percent of phenotypically resistant isolates had ESBL with OXA being pre-dominant (65%). High prevalence of infection noted in Kibera may be attributed to overcrowding and low level of socio-economic factors including lack of safe drinking water, lack of proper disposable systems, drainage systems and toilets and compromised living standards. Despite low prevalence of these bacteria in Dagoretti, high resistance rate was notably recorded attributing resistance to livestock that are kept almost in every residents. Data from the study will assist in further reference to determine the prevalence which will enlighten the policy makers, health officers including clinician, doctors and pharmacists to only prescribe antimicrobial to diagnosed infections to reduce chances of drug resistance. The general public will also get awareness and education on the dangers of over-counter prescription. More data to determine the role played by domesticated livestock in infections will be critical in the fight against AMR.

Keywords: *E. coli; Klebsiella*; Kenya

Introduction

Background

Enterobacteriaceae is a bacterial family including known pathogens, such as *Salmonella, Shigella, Klebsiella*, and *E. coli* species. The members of this family are bacilli (rod-shaped), facultative anaerobes, which ferment sugars to produce lactic acid and various other end products. They are 1-5 μm in length and they are Gram-negative. Most have many flagella used to move about, but a few genera are non-motile. They do not form spores. Most members of *Enterobacteriaceae* have fimbriae involved in the adhesion of the bacterial cells to their hosts. Epidemiologically, most member makes up normal part of the gut flora found in the intestines of humans and other animals, while others are found in water or soil, or are parasites on a variety of different animals and plants [1].

In the recent past, resistance in *Enterobacteriaceae* is on the increase globally. This increase is believed to have been attributed to the increased prevalence of extended-spectrum β-lactamase (ESBL) producing *Enterobacteriaceae* () which has also been characterized by the increase in use of last resort antimicrobial drugs including third generation cephalosporins and carbapenems Enterobacteriaceae have been found to harbor several antibiotic resistance genes. Bacteria such as *E. coli* and *Klebsiella* species are the two known community indicators for presence of multidrug resistant strains [2]. The two have resistant genes which confer resistance to a broad spectrum of antimicrobial panel. These genes include ESBL genes like *blaTEM, blaOXA blaSHV,* and *blaCTX-M* for *E. coli* and *KPC* for *Klebsiella* species. These genes can be transmitted across species through mobile genetic elements like integron or conjugative plasmids [3]. *E. coli* and *Klebsiella* species are also believed to contribute in causing gastroenteritis infection [4,5] respectively.

Locally, there is limited data on the prevalence of co-infection with *E. coli and Klebsiella* species despite high burden of infections being attributed to it. This is mainly because the majority of available findings are lone hospital-based having focused mainly on diagnosis and treatment as opposed to screening of both symptomatic and asymptomatic individuals, determining possibility of co-infections, and also try to look at possible sources of infection. There is also limited data on the presence of multidrug resistance genes in the general public. *E. coli* and *Klebsiella* are believed to withstand the high acidic level found in the stomach characterized by limited availability of nutrients and frequent gastric emptiness [6,7]. Likewise, despite the increase in stomach inflammation being attributed to members of *Enterobacteraceae* family, currently, there is scarce data on prevalence of infection in community-based research and AMR patterns locally. Additionally, there is also lack of data on co-existence of the three species in Kenya mostly due to the fact that majority of studies tent to focus on individual species at a time, or else, they focus mainly on diagnosis and treatment therapy as opposed to risk factors and sources of infection. AMR strains may easily transfer resistance genes to other pathogenic gut microbes including *H. pylori* strains as discussed by [8], which may thus make it difficult for treatment, prevention/ control and also management of infection believed to have been brought by such bacterial agents. This poses threat to the public

health and calls for more research to come up with evidence based findings.

The current study will adopt a community-based approach with an aim of screening for occurrence, antimicrobial resistance trend in *E. coli* and *Klebsiella* species infection and risk factors attributed. This study made use of molecular techniques beside serology and microbiological techniques. The spread of *E. coli* and *Klebsiella* species may be associated with densely populated areas [9], lack of access to clean water, contaminated environment, and presence of domesticated animals [10]. Screening using and diagnosis using both microbiological, biochemical and molecular analysis will help understand underlying risk factors for infection and effective prevention measures across species. Findings from this study will be able to influence some change in policy making and create public awareness across the communities. This will have a positive impact on Global Development Program and economic sustainable goal which resonates with the Governments' vision 2030.

Material and Methods

Research design

This was a cross-sectional study where a section of the population were screened between October 2020 and February 2021. Area of residence, clinical symptoms and signs and previous history were determining factors for recruitment. An informed questionnaire was filled from respective patients after patients have consented or signed assent in the case of children.

Study population

This study was conducted in two regions in Nairobi County: a low-income area (Kibera informal settlement) and a middle-income area (Dagoretti area). Both regions border each other in the South-west of Nairobi, sharing same geographical factors, and the majority of their residents frequent the same hospitals. This study recruited patients from Mbagathi district and Mutu-ini sub-district hospitals. These are government-run hospitals and represent the main health care facilities in these regions. The hospitals provide health care services to both local residents as well as patients from the neighboring regions within Nairobi County.

Sample size determination

A convenient sample size was collected from patients consented. Fecal matter was collected from recruited patients in sterile stool caps and immediately taken to the hospital microbiology section where analysis were done. A follow-up of upto 3 first degree relatives and upto 3 domesticated animals were also be screened from each resident occupied by primary case.

Sampling strategy

Physicians and clinicians at the facility randomly identified outpatients of all ages who were presenting signs and symptoms of an enteric infection and referred them to the recruiting Research Assistant (RA) in a separate room within the hospital. Targeting outpatients of all ages was intentional to bring out a clear picture of the main possible risk factors for enteric infection. Symptoms and signs used by clinicians for to select cases included clinical history of acid reflux, abdominal pain, dyspepsia, heartburn, vomiting, bloating, flatulence, burning sensation and lack of appetite. Only potential participants who meet the study inclusion criteria and were willing to participate in this study were recruited. Samples were collected with a consent and/or assent form both adults and children patients. Immediate relatives were also recruited to this study at the follow-up stage and were screened for the carriage of *E. coli* and *Klebsiella* species. Outpatients who participated in the study were referred to as cases while those who stayed with them at home were referred to as contacts.

Sampling techniques

A trained registered laboratory technologist obtained stool samples from the participants (cases) and taken to microbiology laboratory in Mbagathi District Hospital within two hours. Collection of stool samples from infants (contacts) was collected in plastic wraps that would be spread over seats of toilet used by children who took part in the study. Plastic wraps were also used in lining of infant or toddler diapers to direct urine to run into the diaper and not into the wrap. Stool was prevented to come in contact with the inside of disposable diapers because the lining which often has antibacterial properties would interfere with the test results. All stool samples from immediate relatives (contacts) were collected in Carry Blair, double packed in leak proof zip lock bags with absorbent, and transported in a cooler box with ice packs.

Sample processing

Isolation and identification of bacteria

All stool samples collected were directly cultured on MacConkey's agar. Isolated colonies that fermented lactose (pink

colonies) were sub-cultured on TSA. Doughnut shaped pink and mucoid moist pink colonies were presumptively considered to be *E. coli* and *Klebsiella* species, respectively. Conventional biochemical tests (IMViC) were used to confirm *E. coli* and *Klebsiella* species. A single colony of rapid fermentation (pink colour) was randomly picked from the plate for each original sample and tested for indole and acid production, and oxidase activity. Indole-positive, methyl red positive, oxidase-negative strains were presumptively considered as E. coli. Mucoid moist pink colonies that turned positive for both Voges and Citrate were confirmed to be *Klebsiella*. Quality of the isolation of these bacteria was controlled by *E. coli* ATCC 25922, *and Klebsiella* species ATCC KPN 700603 and KPN 13883 as positive control. Characteristically identified colonies on MacConkey agar were sub-cultured on TSA for purification. After 18-24 hours, a few colonies were picked for identification by Biochemical reagents including Indole, Methyl red, Voges proskauer and Citrate (IMViC) and subsequent susceptibility testing on MHA using various antibiotic drug discs.

Biochemical tests IMViC and AST profiles

Four Biochemical tests were done, these included Indole, Methyl red, Vokes proskauer and Citrate. Expected results were *E. coli* (++--) and *Klebsiella species* (--+).

Bacterial Isolate	`Biochemical	Positive
E. coli	Indole Motility	Red
	Methyl red	Red
Klebsiella	Voges proskauer	Brown
	Citrate	Blue
Urease	Urease test	Pink
	Catalase test	Bubbles
	Oxidase	Indigo

Table 1: Biomedical Identification.

Antimicrobial susceptibility testing (AST) was performed by disc diffusion technique (Kirby-Bauer) following Clinical Laboratory Standard Institute (CLSI-2019) guidelines. Briefly, stocked isolates were revived on Mueller Hinton agar (MHA) and incubated for 18hours at 37 C. Isolates were emulsified in normal saline to attain 0.5 McFarland standard concentration before inoculating the MHA plates. Antimicrobial discs were then introduced then inoculated plates left for 15 minutes to stick before incubation for 18-24 hours. The zones of inhibition were measured and results interpreted.as susceptible (S), intermediate (I) or resistant (R). Antibiotics used included Ampicillins (30 mg) (AMP), Beta-Lactams including Amoxicillin/Clavulanate (30 mg) (AMC), Cefepime/Cephalosporin/Cephems which included Cephalosporin second generation like Cefuroxime (30 mg) CXM, third generation Cephalosporin like Cefotaxime (30 mg) (CTX), Ceftriaxone (30 mg) (CRO) and Ceftazidime (30 mg) (CAZ) and forth generation Cephalosporin which included Cefepime (30 mg) (FEP), Monobactam like Aztreonam (30 mg) (ATM), Imipenem (30 mg) (IMP), Tetracycline (30 mg) (TET), Quinolones like Nalidixic Acid (30 mg) (NA) and Ciprofloxacin (30 mg) (CIP), Folate Pathways Antagonists like Trimethoprim-Sulphamethoxazole (30 mg).

Phynotypic confirmation of ESBL and Carbapenemases

The main characteristics of ESBL mediated resistance observed were determined as follows, resistance to amoxiclav, AMC, second-generation cephalosporin, CXM and one or several third generation cephalosporins, CAZ, CTX, CRO and fourth-generation cephalosporins FEP or monobactam, ATM. Confirmation was done by formation of a synergy between these antibiotics and β-lactamase inhibitors, particularly clavulanate. Isolates resistant to Carbapenems were as well considered to be carbapenemase producers [11].

Molecular identification

DNA Extraction and Polymerase Chain Reaction

Phynotypically identified ESBL and carbapenem isolates were revived in readiness for molecular analysis and identification. DNA was extracted by boiling method as discussed in [12]. Clean DNA samples were kept in cryotubes and temporarily stored at -20^{o} awaiting amplification. Several ESBL and Carbapenems primers including TEM, SHV, CTX-M-15 and OXA, KPC respectively.

1ul of sample DNA, 12 ul of PCR water, 12 ul of pre-aliquoted Qiagen master-mix and 1 ul of forward reverse primer of each gene tested. The mixture was subjected to Conventional PCR for product amplification. The products were later subjected to gel

	Antibiotic classes	Antimicrobial agent	Disc code	Sensitive	Intermediate	Resistant
1	Ampicillins	Ampicilli	AMP-30	>17	14-16	<13
2	B-lactams	Amoxicillin/Clavulanate	AMC-30	>18	14-17	<13
3	Cephalosporin	Cefuroxime	CXM-30	>18	15-17	<14
		Cefotaxime	CTX-30	>26	23-25	<22
		Ceftriaxone	CRO-30	>23	20-22	<19
		Ceftazidime	CAZ-30	>21	18-20	<17
		Cefepime	FEP-30	>25	19-24	<18
4	Monobactam	Aztreonam	ATM-30	>21	18-20	<17
5	Carbapenem	Imipenem	IPM-30	>23	20-22	<19
6	Tetracycline	Tetracycline	TET-10	>15	14-15	<11
7	Quinolones	Nalidixic Acid	NA-30	>19	14-18	<13
		Ciprofloxacin	CIP-30	>26	22-25	<21
8	Folate pathways Antagonists	Trimethoprim-Sulphamethoxazole	TMP/RL-30	>16	15-16	<10

Table 2: Antimicrobial breakpoints categorized into Sensitive (S), Intermediate (I) and Resistant (R) according to Clinical Laboratory Standard Institute (CLSI-2019).

electrophoresis for bands formation. All runs were done alongside ATCC samples as positive controls and blanks as negative controls. A 1.5% gel was used for all runs and observation made by UV reader.

Target genes	Primer name	Oligonucleotide Sequence 5'3'	Product size (Base pairs)	Annealing Temp (0C)	References
BlaTEM	*blaTEM-F*	GCGGAACCCCTATTTG	940	62	(Singh., *et al.* 2019)
BlaTEM	*BlaTEM-R*	TCTAAAGTATATATGAGT AAACTTGGTCTGAC			
BlaSHV	*BlaSHV-F*	TTCGCCTGTGATTATCTCCCTG	750	58	(Fils., *et al.* 2019)
BlaSHV	*BlaSHV-R*	TTAGCGTTTGCCAGTGYCG			
BlaCTX-M	*blaCTX-M-F*	ATGTGCAGYACCAG TAARGTKATGGC	899	60	(Amer., *et al.* 2019)
BlaCTX-M	*blaCTX-M-R*	TGGGTRAARTARGTSA CCAGAAYCAGCGG			
BlaOXA	*BlaOXA-F*	GGCACCAGATTCA ACTTTCAAG	882	57	(Fils., *et al.* 2019)
BlaOXA	*BlaOXA-R*	GACCCCAAGTTT CCTGTAAGTG			
BlaKPC	*blaKPC-F*	CGTTGACGCCCAATCC	600	52	(Singh., *et al.* 2019)
BlaKPC	*BlaKPC-R*	ACCGCTGGCAGCTGG			

Table 3: Identification of Targeted Resistant Genes.

Thermocycler GeneAmpR PCR System 9700 (version 3:12) from Applied Bioscience, was used for amplification. The conditions used for thermocycling are as shown in table below.

PCR stages	*TEM*	*SHV*	*CTX-M*	*OXA*
Initial Denaturation	95*5min	94*5min	94*5min	94*5min
Final Denaturation	95*1min	94*30sec	94*45sec	94*30sec
Annealing	52*1min	51*1min	58*45sec	51*1min
	30cycles	30cycles	30cycles	30cycles
Initial Extension	72*2min	72*1min	72*45sec	72*1min
Final Extension	72*5min	72*10min	72*10min	72*10min
Holding	4	4		4
Basepairs	999	851	599	821

Table 4: PCR Conditions.

Approximately 5µl of PCR products were loaded onto horizontal 1.5% w/v agarose gel and molecular size marker (Invitrogen, UK) and electrophoresed at 100V for 45minutes. The gels were stained with SYBR dye (7.5 µl). The DNA bands were then visualized with a UV trans-illuminator (UVP Inc.), and the images were taken using black and white Polaroid film.

Results

Carriage of *E. coli* and *Klebsiella* species in Kibera and Dagoretti

The study recruited 409 people 328 were infected by either *E. coli* or *Klebsiella* species or both. Out of the total infection 246 (75%) were *E. coli* isolates, and 160 (48%) isolates were *Klebsiella* species. *E. coli* was the predominant isolate. Seventy-eight isolates (23.8%) were co-infections.

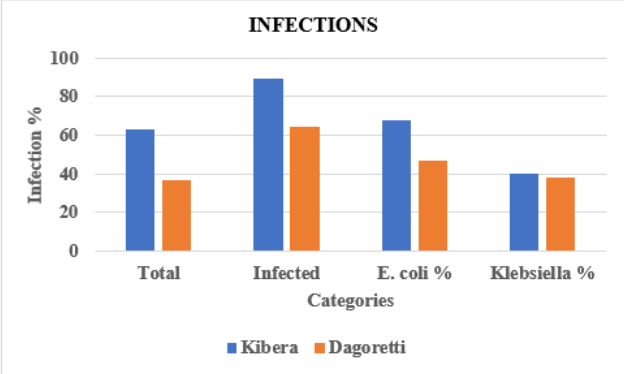

Figure a: General Carriage of *H. pylori*, E. coli and *Klebsiella*.

Carriage of *E. coli* and *Klebsiella* in Kibera vs. Dagoretti

	Total		Infected	%	*E. coli*	%	*Klebsiella*	%
Kibera	258	63.1	231	89.5	175	67.8	103	39.9
Dagoretti	151	36.9	97	64.2	71	47	57	37.8

Table a: Carriage of *E. coli* and *Klebsiella* in Kibera vs. Dagoretti.

Two hundred and fifty-eight (63.1%) participants were obtained from Kibera settlement; of these, 231 (89.5%) were infected. The rest 151 (36.9%) were from Dagoretti of which 97 (64.2%) were infected. *E. coli* isolates made upto 67.8% of Kibera isolates and 47% of isolates from Kibera. From Dagoretti, 39.9% isolates were *E. coli* while *Klebsiella* was 37.8%.

Proportion of infection between patients and contacts

There was notably high prevalence of infection among contacts 45.2% (185) when compared to prevalence among patients 38.1% (156). Of the isolates obtained, high carriage was also noted in *H. pylori*, 62% and *E. coli,* 63% among close contacts as opposed to

infection in *Klebsiella* species which was dominant among the cases (75%). High percentile of non-infected isolates was noted among Dagoretti isolates when compared to Kibera isolates which had competitive positive-negative rates.

	Total samples	%	Total infected		Patients recruited	%	Patients infected	%	Contacts recruited	%	Contacts infected	%
Kibera	258	63.1	231	89.5	119	46.1	102	44.1	139	60.2	129	55.8
Dagoretti	151	36.9	97	64.2	79	52.3	41	51.9	72	74.2	56	57.7
	409	100	328	80.2	198	48.4	143	43.6	211	51.6	185	56.4

Table b: Carriage of *E. coli* and *Klebsiella* in Patients vs. Contacts.

Carriage of respective isolates in Patients vs. in Contacts

Infection was predominant in contacts, 56.6% compared to patients, and 43.6%. Infection was high in both patients (51.9%) and contacts (57.7%) from Dagoretti than Kibera patients (44.1%) and contacts (55.8%). Table carriage in Patients vs. Contacts per bacterial isolates.

	Patients	%	Infected	%	*E. coli*	%	*Klebsiella*	%
Kibera	119	100	102	85.7	67	65.7	35	34.3
Dagoretti	79	100	41	51.9	32	78.1	9	22
Total	198	100	143	72.2	99	69.2	44	30.8
	Contacts	**%**	**Infected**	**%**	***E. coli***	**%**	***Klebsiella***	**%**
Kibera	139	100	129	92.8	103	74.1	37	28.7
Dagoretti	72	100	56	77.8	34	60.7	11	19.6
Total	211	100	185	87.7	137	74.1	48	26

Table c: Carriage of *E. coli* and *Klebsiella* in Patients vs. in Contacts.

Biochemical tests results

Out of the 328 isolates that turned positive, 231 were positive by both Indole and Methyl red tests (confirmed *E. coli* isolates), while 160 isolates turned positive by both Voges proskuer and citrate methods (confirmed K*lebsiella* isolates).

Antimicrobial resistance profiles for *E. coli* and *Klebsiella*

Overall resistance observed in isolates was 34.14% with 8 out of 13 antimicrobial agents exhibiting resistance from the bacterial isolates. The range of resistance was between 15% and 71% with Imipenem (a carbapenem) recording low resistance and RL, Trimethoprim/sulphathomexazole, a folate acid pathways antagonists, recording the highest percentage of resistance (71%) respectively. Cefepime FEP (a fourth generation Cephalosporin) also recorded slightly low resistance at 19.3%. High resistance was also noted in tetracycline (63%), aztreonam, quinolones and penicillin. Resistance in third generation cephalosporin was significantly high especially in CTX. CTX recorded 5% more than cefuroxime (CXM), a 2nd generation cephalosporin, which had 25%. All the 3rd generation cephalosporin tested recorded resistance in more than 20% isolates in all categories which in itself is a threat to the users. Resistance in fourth generation cephalosporin was at 19.3% and that of carbapenem (Imipenem) a broad spectrum antimicrobial agent was 14.8%. Resistance profiles were represented in percentages out of the total isolate subjected, while the remaining percentages of each drug disk panel were

undetermined, either because they fall off during incubation or just weren't introduced for some reasons. They included 6(0.02%) of AMC, IPM, NA, 7(0.02%) of CAZ, CTX, ATM, 9(0.03%) of CXM, CIP, RL, 10(0.04%) of CRO, 11(0.04%) of TE, 18(0.06%) of AMP, 31(0.1%) of FEP.

Antimicrobial Sensitivity test profiles for *Escherichia coli*

E. coli isolates accounted for 65.7% of the total resistance. Resistance profiles was determined by isolates being resistant towards Ampicillin (AMP), an Inhibitor (AMC) and Cephalosporins (CTX, CAZ, CXM, CRO and FEP which form the 2nd, 3rd and 4th generation cephalosporins) with formation of zone of inhibition. 48.3% (146 of 302) of *E. coli* isolates subjected to antimicrobial testing exhibited resistance hence potential ESBL producers.

	AMC	AMP	CXM	CRO	CAZ	CTX	FEP	NA	CIP	IPM	ATM	RL	TET	TTL	Ave.	TET
Kibera	62	63	44	36	29	37	19	69	58	17	67	78	69	647	50	69
Dagoretti	56	66	41	28	25	34	20	63	38	15	72	82	67	607	47	67
Patients	66	69	46	40	30	38	21	72	62	20	65	76	72	677	52	72
Contacts	52	60	39	24	24	33	18	60	35	12	74	84	64	579	45	64
Total	118	129	85	64	54	71	39	132	96	32	139	160	136	1254	96	136
Average	59	65	43	32	26	36	20	66	48	16	70	80	68	627	48	68

Table d: Antimicrobial Sensitivity profiles of *E. coli* isolates in various Antimicrobial agents.

Sensitivity profiles of *Escherichia coli* in Kibera vs. Dagoretti

Overall resistance was high in Kibera as compared to Dagoretti isolates. High resistance generally was noted in Ampicillin (AMP) 63%, Beta-Lactams AMC (62%) and Quinolones, Nalidixic, NA (69%) and Ciprofloxacin, CI (58%). Average resistance in 3rd Generation Cephalosporin was 32%, 26% and 36% respectively for CRO, CAZ and CTX. Resistance in CTX was predominant in both Kibera. Resistance in AMP (66 vs. 63), ATM (72 vs. 67) and RL (82 vs.78) was recorded in Dagoretti when compared to resistance in Kibera.

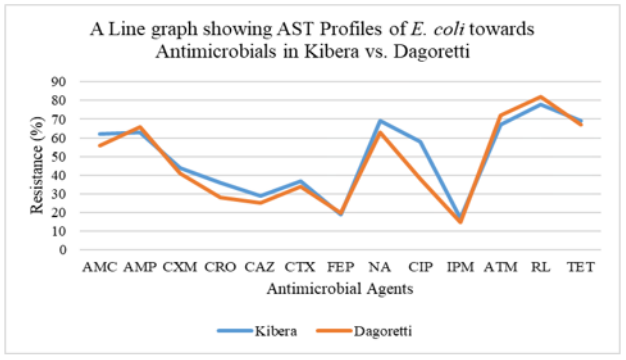

Figure 1: Antimicrobial Sensitivity profiles of *E. coli* Isolates to Antimicrobial agents in Kibera vs. in Dagoretti.

Antimicrobial Sensitivity profiles of *E. coli* in Patients vs. in Contacts

There was higher resistance in patients, (52.1%) than in contacts, (44.5%). Resistance in contacts was in most commonly used antimicrobial agents including RL (84%) and ATM (74). Patients reported very high resistance recorded AMC in both patients (66%) and contacts (52%) in (Figure). Significant resistance observed in all 3rd generation cephalosporin in both subjects, Patients vs. Contacts, CTX (46 vs. 39), CRO (40 vs. 24), CAZ (30 vs. 24).

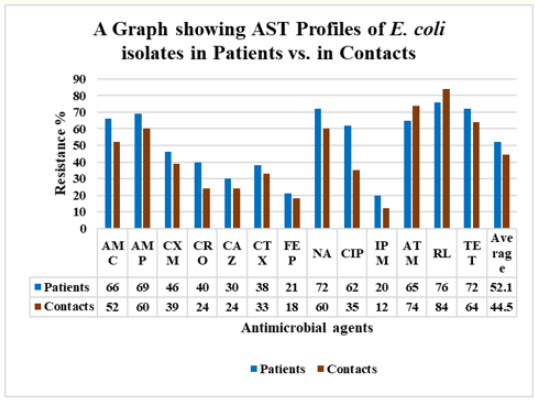

Figure 2: AST profiles of *E. coli* isolates in Patients vs. in Contacts.

Antimicrobial sensitivity profiles of *Klebsiella* isolates

Sixty-eight out of 160 (42.5%) *Klebsiella* isolates were resistant to antimicrobial agents. This made upto 31.1% of the total resistance recorded. Generally, there was high resistance towards commonly used antimicrobial agents as compared to broad spectrum. These included, Sulfamethoxazole, Tetracycline and Nalidixic acid with average resistance of (71%, 59% and 51% respectively). Resistance in Carbapenem (Imipenem, IMP) was alarming (19%) given the strength of the drug and was determinant for a potential *Klebsiella* resistant isolate. (Table 5)

	AMC	AMP	CXM	CRO	CAZ	CTX	FEP	NA	CIP	IPM	ATM	RL	TET	Total	Ave.
Kibera	30	35	25	23	27	21	17	59	33	17	32	78	69	466	36
Dagoretti	26	30	22	25	17	20	22	42	29	21	48	64	67	433	33
Patients	23	35	26	30	28	27	19	47	38	17	35	70	60	455	35
Contacts	33	40	21	17	16	14	20	54	24	21	45	72	58	435	34
Total	56	75	47	47	44	41	39	101	62	38	80	142	118	890	69
Average	28	38	24	24	22	21	20	51	31	19	40	71	59	445	34

Table 5: Antimicrobial sensitivity profiles of *Klebsiella* isolates.

Sensitivity profiles of *Klebsiella* in Kibera vs. in Dagoretti

There was generally a slightly high resistance (35.9%) in isolates obtained from Kibera as compared to those obtained from Dagoretti (33.3%). Resistant in Imipenem, a determinant for potential *Klebsiella* resistant isolate was high in isolates obtained in Dagoretti (21%) when compared to resistance in isolates from Kibera (17%). Overall high resistance was recorded in RL (71%), TET (59%) and NA (51%).

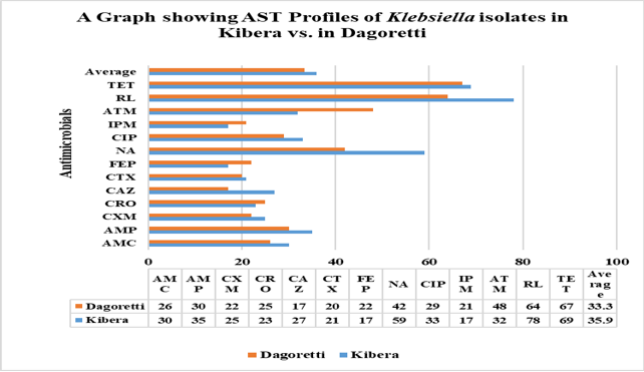

Figure 3: AST Profiles of *Klebsiella* Isolates in Patients vs. in Contacts.

Sensitivity profiles of *Klebsiella* species in Patients vs. in Contacts

Patients showed slightly high resistance in general (35%) when compared to resistance in Contacts (33.5%). However, Contacts isolates exhibited high resistance (21%) from Imipenem, a Carbapenem that shows potential resistance isolates while resistance towards patients isolates was 17%. There was also notably higher resistance towards Ciprofloxacin in patients than in contacts (38% vs. 24%) while high resistance was observed towards ATM in contacts than in patients (45% vs. 35%).

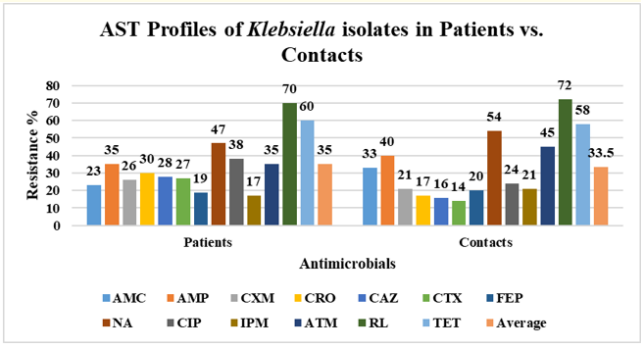

Figure 4: Molecular Identification of Resistance genes.

Antimicrobial resistance genes determination

Four *H. pylori* isolates conferred resistance towards Amoxicillin and Metronidazole. Resistance in Metronidazole antimicrobials was predominant in *H. pylori* with three isolates confirming resistance when tested using both rapid kit culture and confirmed by biochemical analysis and PCR. However, resistance towards *E, coli* and *Klebsiella* was alarming with 53% presenting double ghost zone, a characteristic phenotypic appearance for Extended Spectrum Beta-lactamases (ESBL) while 63% were resistant to Imipenem which is a phenotypically for isolates with resistance genes to Carbapenems. Eighteen samples [18] were subjected to ESBL gene testing by PCR. From these samples, 4 samples, (22%), and 8 samples, (44%) showed presence of *blaTEM* and *blaCTX-M* respectively. *BlaOXA* was reported in more than a half of the potentially positive *klebsiella* isolates. No sample reported *blaSHV* genes and KPC genes. Two samples (11%) had both *TEM* and *CTX-M* genes. From the 18 isolates, 10 (56%) were *E. coli* while 8 (44%) isolates were *Klebsiella* species isolates. Eight *E. coli* isolates (80%) had atleast one resistance gene or both. Twenty five 25% of *Klebsiella* species isolates had resistance genes. *CTX-M* genes were predominant resistance genes in *E. coli* isolates.

Sample	Isolates	Antibiotic agents	Resistance genes	Site
MDH059	*E. coli*	AMP, AMC, CXM, CTX, CAZ, CRO	*blaTEM, blaCTX-M*	Kibera
MSH050	*E. coli, Klebsiella*	AMP, AMC, CXM, CTX, CAZ, CRO, IMP	*blaTEM, blaOXA, blaCTX-M*	Kibera
MDH07	*E. coli*	AMP, AMC, CXM, CTX, CAZ, CRO	*blaOXA, blaTEM, blaCTX-M*	Kibera
MDH036	*Klebsiella*	AMP, AMC, CXM, CTX, CAZ, CRO	*blaOXA, blaTEM, blaCTX-M*	Kibera
MDH063	*E.coli, Klebsiella,*	AMP, AMC, CXM, CTX, CAZ, CRO, IMP	*blaOXA, blaTEM, blaCTX-M*	Kibera
MDH030	*E.coli, Klebsiella,*	AMP, AMC, CXM, CTX, CAZ, CRO, IMP	*BlaOXA blaTEM, blaCTX-M*	Kibera
MDH043	*E. coli*	AMP, AMC, CXM, CTX, CAZ, CRO	*blaTEM, blaCTX-M*	Kibera
MDH053	*E. coli, Klebsiella*	AMP, AMC, CTX, CRO, CAZ, CXM, IMP	*blaTEM, blaCTX-M, blaOXA*	Kibera
MDH041	*E. coli*	AMP, AMC, CRO, CAZ, FEP	*blaTEM, blaCTX-M*	Kibera
MSH035	*E. coli*	AMP, AMC, CXM, CTX, CAZ, CRO	*blaTEM, blaCTX-M*	Dagoretti
MSH056	*E. coli*	AMP, AMC, CXM, CRO, CTX, CAZ	*blaTEM, blaCTX-M*	Dagoretti
MSH021	*E. coli*	AMP, AMC, CAZ, CXM, CTX, CRO,	*blaOXA, blaTEM, blaCTX-M*	Dagoretti
MSH008	*Klebsiella*	AMP, AMC, FEP CXM, IMP, CAZ, CRO	*blaOXA, blaTEM, blaCTX-M*	Dagoretti
MSH031	*Klebsiella*	AMP, AMC, CXM, CAZ, IMP	*blaOXA, blaTEM, blaCTX-M*	Dagoretti

Table 6: Resistance genes identified by PCR.

Visualized gel images

BlaTEM

About 22% of the isolates were potential ESBL contained TEM genes believed to degrade third generation Cephalosporin including CTX, CAZ and CRO antimicrobial agents (Singh., *et al*. 2019).

BlaCTX-M-15

Forty four (44%) of the suspected ESBL isolates noted presence of blactx-m-15 which are isolates believed to exhibit plasmid mediated resistance which may affect the antimicrobial activity of penicillin and cephalosporin.

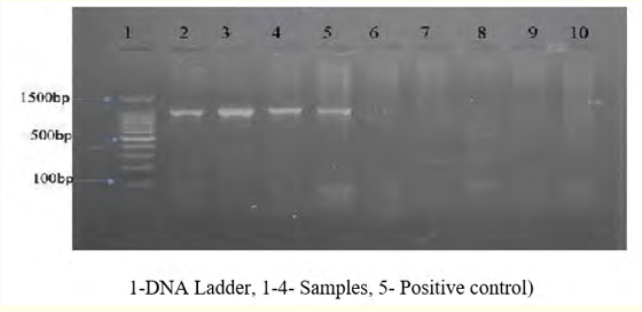

Figure 5: *E. coli* gene *BlaTEM* conferring resistance against Cephalosporin.

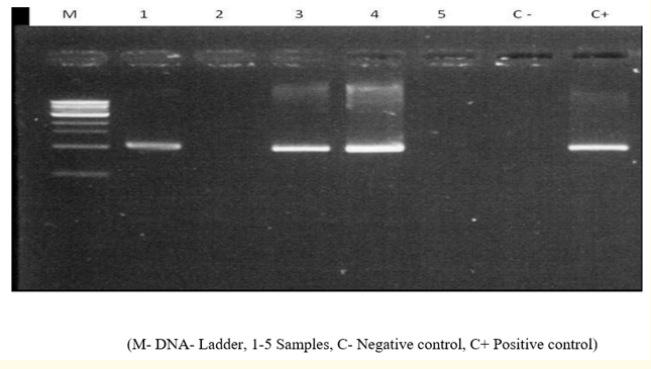

Figure 6: *E. coli* gene *BlaCTX-M-15* conferring resistance against Cephalosporin.

BlaOXA (E. coli and Klebsiella)

More than a half of the isolates that exhibited possible ESBL carriage has *blaOXA* genes which are predominant among the Carbapenem resistant bacteria hence was found in isolates that had shown resistance towards Imipenem (Fils., *et al*. 2019).

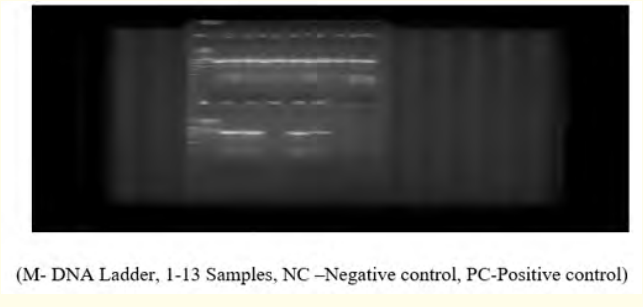

Figure 7: *BlaOXA* conferring resistance against Carbapenems.

Discussion and Conclusion

This was a comparative study in Kenyan urban which aimed at determining prevalence of known multidrug resistant strain between a slum setting and a middle class settlement area, determine the rate of resistance between cases (symptomatic group) and their close contacts which was believed to be asymptomatic group and determine prevalence between *E. coli* and *Klebsiella* species. High infection in people from Kibera slum was attributed to a number of factors including socio-economic factors like overcrowding, cultural factors especially primitive life style and also environmental factors like presence of open sewer lines, compromised sanitation and hygiene. This findings concurs with other research findings including [12-14], that associated slum setting with increased risk to infection. Although *E. coli* was predominant it is treated as a normal flora which inhabit unnoticed widely.

Some urease-producing bacteria such as *Klebsiella* species [15,16] are thought to cause stomach discomfort through bacteria-host-bacteria interactions. The journal [17]Tennessee. Subjects 15 days to 11 years of age, who presented with diarrhea and/or vomiting, were enrolled. Stool specimens were processed for detection of DEC using multiplex polymerase chain reaction. From December 1, 2011, to June 30, 2012, a total of 79 (38% also noted *E. coli* and *Klebsiella species'* association with gastroenteritis placing *E. coli* as the main cause of gastroenteritis in infants. However, colonization and co-infections are also affected by other multiple factors such as environmental and host factors. Our finding also noted aspect of co-infections between enteric several enteric species and *H. pylori* bacteria hence need for prescribed medication to reduce chances of administering a single antibiotic in scenario with multi-infection. The study also noted cases with potential cross infection especially between house hold members. This subscribes to findings by [18] with a Europe based study which noted that prolonged use of antibiotic-lased veterinary medicine in treating or preventing infections in food animals may result in lessening future efficacy of such antibiotics in both treating emerging bacterial cells. This according to Philip, led to banning several antibiotic growth promoters in these animals.

There was high prevalence of infection noted among close contacts who were asymptomatic when compared to cases who presented with symptoms and signs for stomach infection. This finding calls for need to roll-out mass screening to unravel

infection rate and reduce possibility of spread of bacterial species to unsuspecting individuals. In another findings [19] noted 45.2% of infection attributed to stomach discomfort among symptomatic individuals, this study reported 66.1% infection in the same group. This records an average of above 50% possibility of having infection attributed to either *E. coli* or *Klebsiella* species if one has stomach discomfort but there is need for proper diagnosis before administration of any medication.

Enteric isolates demonstrated great resistance towards folic pathways antagonists like Trimethoprim/sulfamethoxazole (RL), in Tetracycline, Ampicicillin, and Aztreonam. Folates and Tetracycline were considered for testing because of its extensive administration and use in most hospital pharmacies and chemists despite having been found to have high resistance in *E. coli* use strains in uncomplicated cystitis according to [20], but is believed to be effective in UTIs. Trimethoprim also is a better option in treatment of acute infections as a third line drug in place of fluoroquinolones and nitrofurantoin. An overall resistance of 33.58% (436.6/13 is a threat to the communities given the fact that most of the isolates came from asymptomatic individuals. This indicates fast spread of these genes across population without signals. Cephalosporin retained high resistance rates throughout the tests without significant changes. Among the third generation agents, CTX demonstrated greater resistance as compared to CAZ and CRO. Fourth generation cephalosporin cefepime is rapidly gaining resistance while second generation is showing some decreased resistance percentages opposed to the trend witnessed in a pooled study summarized by Mengya wang., *et al*. in 2020.

Carbapenemes also demonstrated significant percentages of non-efficacy towards both *E. coli* and *Klebsiella* species isolates. Carbapenemes resistance enterebacteriaceae has been found to be a present in the US being associated with adverse clinical and economic outcomes [21], this was attributed to antibiotic use and the length of hospital stay. Despite Dagoretti recording less cases of infection compared to Kibera, most of the isolates from Dagoretti demonstrated great resistance towards most classes of antimicrobial agents, likewise, Dagoretti area produced more isolates from domesticated animals than isolates from Kibera animals, this may be presumption to why greater resistance was noted in Dagoretti, an area with characteristically high numbers of animals as compared to Kibera settlement. *E. coli* isolates recorded resistance to most classes of antimicrobial agents than the *Klebsiella* species isolates. Less resistance was noted in FEP and Imipenem (IMP), however, being a Carbapenem, IMP was considered a stronger antibiotic as compared to Cephalosporin. Animal isolates had greater resistance than human isolates, this also demonstrated high chances of cross infection across domesticated animals and their human hosts. Likewise, *Klebsiella* species recorded high resistance to antibiotic agents if compared to *E. coli* isolates.

Resistance genes for ESBL and Carbapenemes were tested concurrently. When testing for antimicrobial agents used for ESBL detection, *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 was provided as a supplemental QC strain. Use of more than 1 3rd generation Cephalosporin was to improve the sensitivity of ESBL detection. Also, testing necessitates testing using cefotaxime and ceftazidime alone and in combination clavulanate in order to conclude ESBL genes production. Resistance in all the three 3rd class generation Cephalosporin was significant having been slightly above 20%. Resistance in these class and Aztreonam represents present of ESBLs. This shows present of ESBL production in more than 20% of isolates obtained.

In conclusion, most studies have weakly convincing fact of determining prevalence conclusion based on culture hence encouraging researchers to vouch for need for further diagnostic techniques especially molecular analysis. Overcrowded areas should be considered a possible home for most infections. Antimicrobial prescription should be based on diagnosed infections to reduce chances of mis-prescription which may lead to cases of drug resistance. The use of antimicrobial is an aspect that require all stakeholders to come up with a consensus and policies to be put in place to streamline the whole process right from diagnosis through drug administration. The public should be made aware of the dangers of over-counter drug and medicines and the need to have diagnosis done before prescribed medication. The use of these drugs for empirical therapy in uncomplicated infections should be advised only if the antimicrobial agent of involved has resistance prevalence of <10% to <20% but where the resistance exits such rates, an alternative agent need to be considered, Otherwise, the situations may present a risk for treatment and should be closely monitored and treated.

Recommendation

Increased coverage of surveillance of health care associated infections and mass screening to generate more data on infections.

Total adherence to prescribed treatment to help fight the impending antimicrobial resistance pandemic.

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Ethics and Consent to Participate

Ethical approval and permission was obtained from the Scientific and Ethics Review Unit (SERU) of the Kenya Medical Research Institute, (KEMRI/SERU/CMR/P00134/3989), Nairobi Metropolitan (REF:EOP/NMS/HS/7/VOL.1/RS/08) and National Commission for Science, Technology and Innovation (REF: 568570). Written informed consent was obtained from all participants.

Conflict of Interest

Authors declare no conflict of interest.

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