

RESEARCH ARTICLE

Antimicrobial resistance of *Klebsiella pneumoniae* stool isolates circulating in Kenya

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Abstract

We sought to determine the genetic and phenotypic antimicrobial resistance (AMR) profiles of commensal *Klebsiella* spp. circulating in Kenya by testing human stool isolates of 87 *K. pneumoniae* and three *K. oxytoca* collected at eight locations. Over one-third of the isolates were resistant to ≥ 3 categories of antimicrobials and were considered multidrug-resistant (MDR). We then compared the resistance phenotype to the presence/absence of 238 AMR genes determined by a broad-spectrum microarray and PCR. Forty-six genes/gene families were identified conferring resistance to β -lactams (*ampC*/*bla*_{DHA}, *bla*_{CMY/LAT}, *bla*_{LEN-1}, *bla*_{OKP-A/OKP-B1}, *bla*_{OXA-1-like} family, *bla*_{OXY-1}, *bla*_{SHV}, *bla*_{TEM}, *bla*_{CTX-M-1} and *bla*_{CTX-M-2} families), aminoglycosides (*aac(3)-III*, *aac(6)-Ib*, *aad(A1/A2)*, *aad(A4)*, *aph(AI)*, *aph3/str(A)*, *aph6/str(B)*, and *rmt(B)*), macrolides (*mac(A)*, *mac(B)*, *mph(A)/mph(K)*), tetracyclines (*tet(A)*, *tet(B)*, *tet(D)*, *tet(G)*), ansamycins (*arr*), phenicols (*catA1/cat4*, *floR*, *cmlA*, *cmr*), fluoroquinolones (*qnrS*), quaternary amines (*qacEA1*), streptothricin (*sat2*), sulfonamides (*sul1*, *sul2*, *sul3*), and diaminopyrimidines (*dfrA1*, *dfrA5*, *dfrA7*, *dfrA8*, *dfrA12*, *dfrA13/21/22/23* family, *dfrA14*, *dfrA15*, *dfrA16*, *dfrA17*). This is the first profile of genes conferring resistance to multiple categories of antimicrobial agents in western and central Kenya. The large number and wide variety of resistance genes detected suggest the presence of significant selective pressure. The presence of five or more resistance determinants in almost two-thirds of the isolates points to the need for more effective, targeted public health policies and infection control/prevention measures.

Introduction

Antimicrobial resistance (AMR) is of significant concern in developing nations due to over-use of antimicrobial agents, widespread availability of counterfeit or substandard drugs, and poor infection control measures [1,2]. The scarcity of reliable and timely information, particularly in

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sub-Saharan Africa, may further limit epidemiological surveillance and effective stewardship efforts.

While only infrequently associated with diarrheal disease, *Klebsiella pneumoniae* and other klebsiellae are common intestinal commensals with significant potential to cause extraintestinal infections in severely ill patients and diarrhea in HIV/AIDS patients [3,4,5,6,7]. Of additional concern, *Klebsiella* spp. acquire, accumulate, and transfer myriad AMR determinants and therefore may represent a significant reservoir for resistance within the gut [8,9,10] and may increase the risk of resistant infections in hospital environments [5,11]. Indeed, *in vivo* transfer of AMR genes from intestinal klebsiellae to other bacterial species has been well documented [12,13,14,15,16]. Here, we use intestinal *Klebsiella* isolates collected at eight medical treatment facilities in western and central Kenya to interrogate the gut resistome and its potential for rapid evolution and spread.

Materials and methods

Sample collection, processing, antimicrobial susceptibility testing

Stool specimens or rectal swabs were collected into sterile, wide-mouth collection cups and aliquoted into thirds (Cary-Blair transport media, 10% formalin for parasitology, and a vial for freezing at -20C for virology) upon enrollment; previous studies showed no differences in frequency of bacterial isolation between stool samples and rectal swabs [17]. Samples were stripped of all identifiers and were assigned accession numbers before transportation to the WRAIR Microbiology Hub laboratory in Kericho (MHK) within 72 hours of collection. Samples were then plated on primary, selective, and differential media. MacConkey, MacConkey-sorbitol, sheep blood agar, Hektoen enteric agar, thiosulfate-citrate-bile-sucrose agar, cefoperazone-vancomycin-amphotericin agar, and cefsulodin-irgasan-novobiocin agar were the primary media; no specific enrichment step was performed as part of the normal workup. At 24 and 48 hours, colonies were subcultured, Gram stained, and subjected to biochemical testing (indole production, Voges-Proskauer reaction, o-nitrophenyl- Δ -D-galactopyrandoside production) before analysis on Microflex MALDI Biotyper (Bruker Daltonics, Billerica, MA, USA) and MicroScan WalkAway40 (Siemens Healthcare, Sacramento, CA, USA) systems for identification and antibiotic susceptibility testing (AST), respectively. MIC 44 and NC 66 panels were used with LabPro software updated for 2015 CLSI breakpoints [18] and automated interpretation of results. Laboratory personnel performing susceptibility testing were enrolled in External Quality Assurance/Proficiency Testing for both College of American Pathologists (three cycles/year) and United Kingdom National External Quality Assessment Service (monthly). Weekly quality control for AST was performed using recommended ATCC strains [18].

Study sites

Samples were collected from eight Kenyan clinical sites participating in the Walter Reed Army Institute of Research (WRAIR), University of Washington/Kenya Institute of Medical Research Institute (KEMRI) collaborative research group enteric surveillance programs. These surveillance sites serve diverse communities: Mbagathi District Hospital serves a highly urban population near the center of Nairobi. The Eldoret-based clinic at Moi Barracks (MBB1) serves military service members and their families in the Kenyan highlands. Kericho District Hospital, also located in the highlands, serves a relatively rural community of tea pluckers and farmers. Kombewa is similarly considered rural. The remaining sites at the district hospitals of Kisumu, Kisii, Migori, and Homa Bay are located in western Kenya near Lake Victoria and serve both urban and rural populations largely subsistent upon agricultural and fishing economies.

Eligibility criteria

Protocol-trained clinical staff at all sites recruited subjects experiencing acute diarrhea (three or more loose stools within a 24 hour period). The cases were recruited only from outpatient populations, and none were admitted to the hospital. Age-matched asymptomatic controls were recruited from the same sites if the subjects had not experienced acute diarrhea within the previous two week period; when possible, controls were healthy siblings close in age to the index case. Participants experiencing (chronic) diarrhea lasting more than 14 days were excluded. Medical histories were captured for a small subset of samples ($n = 13$). Both cases and controls provided basic clinical, epidemiological (water source and treatment) and demographic (age, gender, residence) information. Enrollment of all subjects required informed consent and custodial assent for subjects under 18 years of age. No diagnostic or therapeutic decisions were based on any phenotypic or genotypic data generated for this study. Work performed on this study was approved by the KEMRI and WRAIR Institutional Review Boards under KEMRI SSC #1549/WRAIR #1549 and KEMRI SSC #2056/WRAIR #1811.

Detection of resistance determinants

The presence/absence of 238 different AMR genes was determined using the Antimicrobial Resistance Determinant Microarray (ARDM) v.2 as previously described [19,20]. Briefly, this microarray was designed for detection of >200 determinants derived from both Gram-positive and -negative bacteria. Chip content covers genes conferring resistance to 15 categories of antimicrobials (β -lactams, aminoglycosides, macrolides, lincosamides, streptogramins, quaternary amines, ansamycins, diaminopyrimidines, antimicrobial peptides, tetracyclines, phenicols, glycopeptides, platensemycin, fluoroquinolones, sulfonamides); several plasmid-borne multidrug efflux pumps are also represented on the chip. Full chip content information is given in [19]. Following sample processing, hybridization, and washing, the signal associated with each probe was determined electrochemically. An AMR gene was identified as detected when > 50% of its representative probes had signals above the mean signal from the lowest 2,128 probes + 3 standard deviations or when >70% of its probes had signals above either of two less stringent thresholds [20,21]. A limited set of detected AMR and integrase genes were confirmed by PCR and DNA amplicon sequencing (S1 Table).

Statistical analysis

Statistical comparisons between populations were performed using two-tailed student's t -tests (assuming unequal variance). Chi-square tests were used to compare binomial proportions in independent samples ($2 \times n$ contingency tables). Linear regression was used to compare the number of genes/isolate with age (H_0 : slope = 0, tested by student's t -test).

Results

Sample set characteristics

A total of 90 *Klebsiella* spp. strains were isolated from participants ranging in age from 4 months to 54 years (median age 57 months). Half of the subjects presented with acute diarrheal illness and half were healthy controls. The majority of isolates came from the Kisii and Kisumu sites (37 [41.1%] and 16 [17.8%] isolates, respectively) (Table 1). Thirty-three of the isolates (36.7%) were non-susceptible to at least three categories of antimicrobials and were considered multidrug resistant (MDR) per Magiorakos [22]. One isolate, MHK02590, was considered extensively drug-resistant (non-susceptible to at least one agent in all but two or fewer antimicrobial categories; Table 2) [22]. As a whole, there were no differences between overall MDR

Table 1. Summary of antimicrobial phenotypic susceptibility for diarrheal and control isolates.

Antimicrobial compound ^a	Phenotype	Case (n = 45)	Control (n = 45)	Overall (n = 90)
AMC	R	11 (24%)	7 (16%)	18 (20%)
	I	8 (18%)	5 (11%)	13 (14%)
	S	26 (58%)	33 (73%)	59 (66%)
SAM	R	18 (40%)	14 (31%)	32 (36%)
	I	4 (9%)	3 (7%)	7 (8%)
	S	23 (51%)	28 (62%)	51 (57%)
ATM	R	4 (9%)	4 (9%)	8 (9%)
	I	1 (2%)	-	1 (1%)
	S	40 (89%)	41 (91%)	81 (91%)
FEP	R	6 (13%)	3 (7%)	9 (10%)
	I	-	-	-
	S	39 (87%)	42 (93%)	81 (90%)
CAZ	R	1 (2%)	1 (2%)	2 (2%)
	R (ESBL)	4 (9%)	2 (4%)	6 (7%)
	I	1 (2%)	-	1 (1%)
CTX	S	39 (87%)	42 (93%)	81 (90%)
	R	1 (2%)	1 (2%)	2 (2%)
	R (ESBL)	4 (9%)	2 (4%)	6 (7%)
IPM	I	2 (4%)	-	2 (2%)
	S	38	42 (93%)	80 (89%)
	R	1 (2%)	-	1 (1%)
MEM	I	-	3 (7%)	3 (3%)
	S	44 (98%)	42 (93%)	86 (96%)
	R	-	1 (2%)	1 (1%)
AMK	I	1 (2%)	-	1 (1%)
	S	44 (98%)	44 (98%)	88 (98%)
	R	1 (2%)	1 (2%)	2 (2%)
GEN	I	-	-	-
	S	44 (98%)	44 (98%)	88 (98%)
	R	3 (7%)	4 (9%)	7 (8%)
TOB	I	1 (2%)	1 (2%)	2 (2%)
	S	41 (91%)	40 (89%)	81 (90%)
	R	3 (7%)	2 (4%)	5 (6%)
TET	I	-	2 (4%)	2 (2%)
	S	42 (93%)	41 (91%)	83 (92%)
	R	18 (40%)	15 (33%)	33 (37%)
CIP	I	5 (11%)	3 (7%)	8 (9%)
	S	22 (49%)	27 (60%)	49 (54%)
	R	1 (2%)	-	1 (1%)
LVX	I	1 (2%)	-	1 (1%)
	S	43 (96%)	45 (100%)	88 (98%)
	R	2 (4%)	-	2 (2%)
SXT	I	-	1 (2%)	1 (1%)
	S	43 (96%)	44 (98%)	87 (97%)
	R	25 (56%)	30 (67%)	55 (61%)
	I	-	-	-

(Continued)

Table 1. (Continued)

Antimicrobial compound ^a	Phenotype	Case (n = 45)	Control (n = 45)	Overall (n = 90)
	S	20 (44%)	15 (33%)	35 (39%)

^aAntimicrobial compounds are grouped together according to categories used to define MDR per Magiorakos [22]. AMC—amoxicillin/clavulanate; SAM—ampicillin/sulbactam; ATM—aztreonam; FEP—cefepime; CAZ—ceftazidime; CTX—cefotaxime; IMP—imipenem; MEM—meropenem; AMK—amikacin; GEN—gentamicin; TOB—tobramycin; TET—tetracycline; CIP—ciprofloxacin; LVX—levofloxacin; SXT—trimethoprim/sulfamethoxazole; S—sensitive; I—intermediate; R—resistant. ESBL—Extended-spectrum β-lactamase

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phenotypes ($P = 0.940$) in the strains isolated from subjects with ADI and asymptomatic controls, nor between genders ($P = 0.463$). Between 80 and 90% of the tested isolates were susceptible to all β-lactams except ampicillin, to one or more aminoglycosides, and to both of the fluoroquinolones tested. Over half were susceptible to tetracycline, but more than 60% were resistant to sulfamethoxazole-trimethoprim (SXT).

A total of 46 AMR genes or gene families covering 11 categories of antimicrobials were identified amongst the 90 isolates using a broad-range microarray (Table 3). PCR was used to verify the presence of a select group of genes detected by microarray, as well as ancillary genes associated with specific combinations of AMR determinants (S1 Table). All but six isolates harbored multiple resistance determinants (Table 4). While there were no differences in MDR phenotype between age quartiles ($P = 0.336$), a small but significant inverse relationship was observed between the total number of genes per isolate and age ($P = 0.029$; *t*-test of linear regression), with isolates from younger subjects harboring a larger number of genes. No significant differences in genes/isolate were observed between diarrheal and control isolates ($P = 0.458$) or between genders ($P = 0.184$). The disparate numbers of isolates collected at the various sites ($n = 4$ to $n = 37$) precluded any statistically valid site-to-site comparisons. However, sites with highest percentages of MDR phenotype, Mbagathi (3 of 4 isolates) and Kisii (17 of 37 isolates), also harbored the widest overall varieties of resistance determinants (28 and 41 determinants, respectively).

Resistance to β-lactams

The ARDM v.2 content comprises probes for 52 β-lactamase genes, including 12 families of extended-spectrum β-lactamases (ESBLs) and 15 carbapenemases. The ARDM detected *bla*_{SHV}, a chromosomal gene presumptively carried in all *K. pneumoniae* [23], in 63 isolates (70%), while PCR detected *bla*_{SHV} in an additional fourteen (S2 Table); 13 of the 90 isolates were negative for *bla*_{SHV} by both methods, but this may be due to point mutations within the primer regions (PCR) or regions used for hybridization on the microarray. β-lactamase inhibitors such as clavulanate and sulbactam are typically active against *Klebsiella* SHV-1 and TEM-1 lactamases, but one-third of the isolates tested here showed resistance to at least one of these inhibitors. While such resistance may arise from hyperproduction of β-SHV lactamases [24], this resistance was highly correlated to the presence of *bla*_{TEM} ($P < 0.0001$), suggesting either TEM hyperproduction [25,26] or the possible presence of inhibitor-resistant TEM enzymes. The presence of *bla*_{OXA-1-like} genes—most often conferring resistance to clavulanate and sulbactam—can also potentially explain phenotypic inhibitor resistance in two strains (MHK01590, MHK05068), although *bla*_{TEM} genes are also present in both. However, strain MHK01305—positive for *bla*_{OXA-1-like}, *bla*_{TEM}, and *bla*_{CTX-M-1} family genes—is broadly susceptible to almost all tested β-lactams and lactam-inhibitor combinations, suggesting that either none of these genes are expressed or that the encoded gene products are non-functional.

Table 2. Metadata and phenotypic antimicrobial susceptibility for individual isolates.

Strain no.	age	gender	date isolated	site ^b	Antimicrobial compound ^a															Ctrl/Cs ^c
					AMC	SAM	ATM	FEP	CAZ	CTX	IPM	MEM	AMK	GEN	TOB	TET	CIP	LVX	SXT	
MHK00504	11m	F	7/10/2010	Ku	R	R	S	S	S	S	I	S	S	S	S	R	S	S	R	ctrl
MHK01305	18yr 6m	M	5/24/2011	Ki	S	R	S	S	S	S	S	S	S	S	S	R	S	S	R	cs
MHK01419	3yr 2m	F	6/21/2011	Ki	S	R	S	S	S	S	S	S	S	R	I	R	S	S	R	ctrl
MHK01814	9m	M	9/28/2011	Mb	R	R	S	S	I	S	R	S	S	S	S	R	S	S	R	cs
MHK02123 ^d	21yr	F	1/11/2012	Ki	S	I	S	S	S	S	S	S	S	S	S	R	S	S	R	ctrl
MHK02126 ^d	1yr	M	1/11/2012	Ki	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	ctrl
MHK02178	21yr	M	1/21/2012	Ki	R	R	S	S	S	S	I	S	S	S	S	R	S	S	R	ctrl
MHK02303	2yr 1m	F	2/11/2012	Ki	R	R	S	S	S	S	S	S	S	R	I	R	S	S	R	ctrl
MHK02499	1yr 3m	M	3/29/2012	Ki	I	R	R	R	ESBL	ESBL	S	S	S	I	S	S	S	S	R	ctrl
MHK02590	6m	M	4/14/2012	Mb	R	R	R	R	R	R	S	S	R	R	R	R	R	R	R	cs
MHK02631	54yr	F	4/20/2012	Ki	S	S	S	S	S	S	S	S	S	S	S	R	S	S	R	ctrl
MHK02678	1yr 10m	F	5/1/2012	Ki	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	ctrl
MHK02690	9m	M	5/4/2012	Mb	R	R	S	S	S	S	S	S	S	S	S	I	S	S	R	cs
MHK02780	4m	F	5/29/2012	Ki	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	cs
MHK03026	8yr	F	7/13/2012	Ku	I	R	S	S	S	S	S	S	S	S	S	R	S	S	R	cs
MHK04212	11m	M	11/15/2013	M1	R	S	S	S	S	S	S	S	S	S	S	S	S	S	R	cs
MHK04617	5m	M	11/16/2013	Mb	I	R	S	S	S	S	S	S	S	S	S	S	S	S	R	cs
MHK04622	8m	M	11/20/2013	Ki	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	cs
MHK04775	2yr 1m	F	2/1/2014	Ku	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	ctrl
MHK04776	2yr 6m	F	2/1/2014	Ku	I	R	S	S	S	S	S	S	S	S	S	S	S	S	R	cs
MHK04777	51yr	M	2/1/2014	Ko	S	R	S	S	S	S	S	S	S	S	S	R	S	S	R	ctrl
MHK04779	2yr 3m	M	2/4/2014	Ke	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	cs
MHK04786	4yr	M	2/5/2014	Ki	I	I	S	S	S	S	S	S	S	S	S	R	S	S	R	cs
MHK04792	43yr	M	2/5/2014	Ke	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	ctrl
MHK04804	3yr 9m	F	2/7/2014	Ki	S	R	S	S	S	S	S	S	S	S	S	R	S	S	R	ctrl
MHK04812	3yr	M	3/27/2014	Ke	R	R	S	R	ESBL	ESBL	S	S	S	S	S	S	S	S	R	cs
MHK04813	1yr 2m	M	3/28/2014	Ku	I	R	S	S	S	S	S	S	S	S	S	S	S	S	R	cs
MHK04819	1yr 4m	M	4/1/2014	Ku	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	ctrl
MHK04821	2yr 3m	F	4/2/2014	Ke	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	cs
MHK04822	2yr 11m	M	4/3/2014	Ki	S	R	S	S	S	S	S	S	S	S	S	R	S	S	R	ctrl
MHK04834	19yr	M	4/9/2014	Ko	S	S	S	S	S	S	S	S	S	S	S	I	S	S	S	ctrl
MHK04838	4yr	F	4/10/2014	Ku	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	cs
MHK04847	28yr	F	4/11/2014	Ko	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	cs
MHK04864	32yr	F	4/17/2014	Ki	S	S	S	S	S	S	S	S	S	S	S	I	S	S	S	ctrl
MHK04872	22yr	F	4/18/2014	Ku	I	R	S	S	S	S	S	S	S	S	S	R	S	S	R	cs
MHK04885	2yr 5m	M	4/24/2014	Ke	S	S	S	S	S	S	S	S	S	S	S	R	S	S	R	cs
MHK04900	3yr 5m	F	4/28/2014	Ke	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	ctrl
MHK04904	22yr	F	4/29/2014	Ki	S	I	S	S	S	S	S	S	S	I	S	S	S	S	R	cs
MHK04908	1yr 3m	M	4/30/2014	Ku	S	R	S	S	S	S	S	S	S	S	S	R	S	S	R	cs
MHK04919	3yr	M	5/6/2014	Ki	I	R	S	S	S	S	S	S	S	S	S	S	S	S	R	ctrl
MHK04922	3yr	F	5/7/2014	Ki	R	R	R	R	R	R	I	S	R	R	R	S	S	I	R	ctrl
MHK04923	24yr	M	5/7/2014	Ki	S	S	S	S	S	S	S	R	S	S	S	S	S	S	R	ctrl
MHK04926	31yr	F	5/7/2014	Ku	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	ctrl
MHK04928	5yr	F	5/9/2014	Ki	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	ctrl
MHK04930	28yr	M	5/9/2014	M1	R	R	R	R	ESBL	ESBL	S	S	S	R	R	S	S	S	R	ctrl
MHK04941	1yr 8m	M	5/14/2014	M1	R	S	S	S	S	S	S	S	S	S	S	R	S	S	R	ctrl
MHK04943	28yr	F	5/15/2014	Ki	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	cs
MHK04946	17yr	F	5/16/2014	Ki	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	cs
MHK04947	36yr	F	5/16/2014	Ko	R	R	S	S	S	S	S	S	S	S	S	I	S	S	R	cs
MHK04948	38yr	M	5/16/2014	Ko	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	ctrl

(Continued)

Table 2. (Continued)

Strain no.	age	gender	date isolated	site ^b	Antimicrobial compound ^a															Ctrl/Cs ^c		
					AMC	SAM	ATM	FEP	CAZ	CTX	IPM	MEM	AMK	GEN	TOB	TET	CIP	LVX	SXT			
MHK04957	37yr	F	5/17/2014	M1	S	S	S	S	S	I	S	S	S	S	S	S	I	S	S	S	S	cs
MHK04960	8m	M	5/20/2014	Ki	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	ctrl
MHK04967	15yr	M	5/22/2014	M1	I	I	S	S	S	S	S	S	S	S	S	S	R	S	S	R	ctrl	
MHK04980	30yr	M	5/23/2014	M1	I	I	S	S	S	S	S	S	S	S	S	S	R	S	S	R	cs	
MHK04983	1yr 1m	M	5/24/2014	Ku	I	R	S	S	S	S	S	S	S	S	S	S	R	S	S	R	ctrl	
MHK04984	6m	M	5/24/2014	Ku	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	ctrl	
MHK05010	8yr	F	5/31/2014	Ki	I	R	R	S	S	S	S	S	S	S	S	S	S	S	S	R	ctrl	
MHK05013a	35yr	F	5/31/2014	M1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	ctrl	
MHK05013b	35yr	F	5/31/2014	M1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	ctrl	
MHK05014a	32yr	M	5/31/2014	M1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	ctrl	
MHK05014b ^d	32yr	M	5/31/2014	M1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	ctrl	
MHK05017	52yr	F	6/5/2014	Ko	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	cs	
MHK05018	32yr	M	6/5/2014	M1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	ctrl	
MHK05018-1b	32yr	M	6/5/2014	M1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	ctrl	
MHK05021	7yr	M	6/5/2014	Ki	S	R	S	S	S	S	S	S	S	S	S	S	S	S	R	ctrl		
MHK05027	7yr	M	6/6/2014	Ki	R	R	S	S	S	S	S	S	S	S	S	S	R	S	S	R	cs	
MHK05028	5yr	F	6/6/2014	Ki	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	R	cs	
MHK05042	2yr 9m	M	6/11/2014	Ki	R	I	S	S	S	S	S	S	S	S	S	S	R	S	S	R	ctrl	
MHK05046	4yr 10m	M	6/12/2014	Ki	S	S	S	S	S	I	S	S	S	S	S	S	S	S	S	S	cs	
MHK05068	6yr	M	6/20/2014	Ki	R	R	R	R	ESBL	ESBL	S	S	S	R	R	S	I	S	R	cs		
MHK05070	1yr	F	6/21/2014	Ku	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	R	ctrl	
MHK05072	4yr 6m	F	6/21/2014	Ki	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	cs	
MHK05080	9yr	M	6/27/2015	Ki	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S	S	cs	
MHK05084	5yr	F	6/28/2014	Ku	R	I	S	S	S	S	S	S	S	S	S	S	R	S	S	S	cs	
MHK05090	4yr 6m	M	7/2/2014	Ku	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	ctrl	
MHK05091	5yr 10m	M	7/2/2014	Ku	R	R	R	R	ESBL	ESBL	S	S	S	S	S	S	R	S	S	R	cs	
MHK05094	23yr	F	7/4/2014	Ki	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	ctrl	
NTS01697	4yr 1m	M	6/12/2014	Ki	R	R	R	R	ESBL	ESBL	S	I	S	R	R	S	S	R	R	cs		
NTS01699	5yr 5m	M	6/12/2014	Mg	S	S	S	S	S	S	S	S	S	S	S	I	S	S	S	cs		
NTS01703	2yr 8m	F	6/13/2014	Hy	S	S	S	S	S	S	S	S	S	S	S	R	S	S	R	cs		
NTS01705	7m	F	6/13/2014	Hy	S	R	S	R	S	S	S	S	S	S	S	S	S	S	R	cs		
NTS01707	4yr 9m	F	6/13/2014	Mg	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	ctrl		
NTS01708	2yr 9m	M	6/14/2014	Ki	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	cs		
NTS01732	2yr 10m	F	6/25/2014	Hy	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	cs		
NTS01745	11m	F	7/2/2014	Ki	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	cs		
NTS01747	5yr	M	7/3/2014	Hy	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	ctrl		
NTS01749	4yr 3m	M	7/3/2014	Mg	S	S	S	S	S	S	S	S	S	S	S	I	S	S	S	cs		
NTS01755	3yr 1m	F	7/5/2014	Ki	I	R	S	S	S	S	S	S	S	S	S	R	S	S	R	cs		
NTS01793	3yr 2m	M	8/2/2014	Hy	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	cs		
NTS01936	5yr 6m	M	6/26/2014	Mg	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	ctrl		

^aAntimicrobial compounds are grouped together according to categories used to define MDR per Magiorakos [22]. AMC—amoxicillin/clavulanate; SAM—ampicillin/sulbactam; ATM—aztreonam; FEP—cefepime; CAZ—ceftazidime; CTX—cefotaxime; IMP—imipenem; MEM—meropenem; AMK—amikacin; GEN—gentamicin; TOB—tobramycin; TET—tetracycline; CIP—ciprofloxacin; LVX—levofloxacin; SXT—trimethoprim/sulfamethoxazole; S—sensitive; I—intermediate; R—resistant. ESBL—Extended-spectrum β-lactamase

^bCollection site: Hy—Homabay; Ke—Kericho; Ki—Kisii; Ko—Kombewa; Ku—Kisumu; Mb—Mbagathi; Mg—Migori; M1—Moi Barracks at Eldoret

^cctrl—healthy control; cs—case of acute diarrheal illness

^d*K. oxytoca*

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Table 3. Summary of AMR genes in the tested population.

gene	case (n = 45)	control (n = 45)	overall (n = 90)
β-lactams			
<i>ampC/bla_{DHA}</i>	0 (0%)	1 (2%)	1 (1%)
<i>bla_{CMY/LAT} family</i>	1 (2%)	0 (0%)	1 (1%)
<i>bla_{LEN-1}</i>	32 (71%)	29 (64%)	61 (68%)
<i>bla_{OKP-A/OKP-B1}</i>	5 (11%)	5 (11%)	10 (11%)
<i>bla_{OXA-1}</i>	3 (7%)	0 (0%)	3 (3%)
<i>bla_{OXY-1}</i>	0 (0%)	4 (9%)	4 (4%)
<i>bla_{SHV} family</i>	43 (95%)	35 (77%)	78 (87%)
<i>bla_{TEM} family</i>	29 (64%)	23 (51%)	52 (58%)
<i>bla_{CTX-M-1} family</i>	5 (11%)	3 (7%)	8 (9%)
<i>bla_{CTX-M-2} family</i>	1 (2%)	0 (0%)	1 (1%)
aminoglycosides			
<i>aac(3)-III</i>	3 (7%)	2 (4%)	5 (6%)
<i>aac(6)-Ib</i>	3 (7%)	1 (2%)	4 (4%)
<i>aad(A1/A2) family</i>	10 (22%)	8 (18%)	18 (20%)
<i>aad(A4)</i>	1 (2%)	0 (0%)	1 (1%)
<i>aph(AI)</i>	3 (7%)	4 (9%)	7 (8%)
<i>aph3/str(A)</i>	23 (51%)	21 (47%)	44 (49%)
<i>aph6/str(B)</i>	25 (56%)	22 (49%)	47 (52%)
<i>rmtB</i>	0 (0%)	1 (2%)	1 (1%)
macrolides			
<i>mac(A)</i>	16 (39%)	13 (29%)	29 (32%)
<i>mac(B)</i>	13 (29%)	12 (27%)	25 (28%)
<i>mph(A)/mph(K) family</i>	4 (9%)	2 (4%)	6 (7%)
tetracyclines			
<i>tet(A)</i>	7 (16%)	9 (20%)	16 (18%)
<i>tet(B)</i>	4 (9%)	5 (11%)	9 (10%)
<i>tet(D)</i>	6 (13%)	5 (11%)	11 (12%)
<i>tet(G)</i>	0 (0%)	1 (2%)	1 (1%)
ansamycins			
<i>arr</i>	1 (2%)	1 (2%)	2 (2%)
phenicols			
<i>catA1/cat4 family</i>	7 (16%)	2 (4%)	7 (8%)
<i>floR</i>	1 (2%)	0 (0%)	1 (1%)
<i>cmlA</i>	1 (2%)	0 (0%)	1 (1%)
<i>cmr</i>	6 (13%)	14 (31%)	32 (36%)
fluoroquinolones			
<i>qnrS</i>	2 (4%)	0 (0%)	2 (2%)
quaternary amines			
<i>qacEΔ1</i>	17 (38%)	11 (24%)	28 (31%)
streptothricin			
<i>sat2</i>	2 (4%)	2 (4%)	4 (4%)
sulfonamides			
<i>sul1</i>	17 (38%)	11 (24%)	28 (31%)
<i>sul2</i>	25 (56%)	22 (49%)	
<i>sul3</i>	1 (2%)	0 (0%)	1 (1%)
diaminopyrimidines			

(Continued)

Table 3. (Continued)

gene	case (n = 45)	control (n = 45)	overall (n = 90)
<i>dfrA1</i>	6 (13%)	3 (7%)	9 (10%)
<i>dfrA12</i>	1 (2%)	2 (4%)	3 (3%)
<i>dfrA13/21/22/23</i> family	1 (2%)	0 (0%)	1 (1%)
<i>dfrA14</i>	8 (18%)	10 (22%)	18 (20%)
<i>dfrA15</i>	2 (4%)	1 (2%)	3 (3%)
<i>dfrA16</i>	0 (0%)	2 (4%)	2 (2%)
<i>dfrA17</i>	1 (2%)	0 (0%)	1 (1%)
<i>dfrA5</i>	3 (7%)	4 (9%)	7 (8%)
<i>dfrA7</i>	4 (9%)	1 (2%)	5 (6%)
<i>dfrA8</i>	3 (7%)	2 (4%)	5 (6%)

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Nine strains were resistant to at least one third or fourth generation cephalosporin (Table 1), with six classified as ESBL producers by the MicroScan. Five of the ESBL-producing isolates were positive for *bla*_{CTX-M-1}-group genes (confirmed by PCR, see S1 and S2 Tables). An additional three isolates also carried *bla*_{CTX-M-1}-family genes, two of which were resistant to the third and fourth generation cephalosporins tested but negative for ESBL production by Microscan; one of these (MHK04922) also carried *ampC/bla*_{DHA}, which can mask the ESBL phenotype [27]. One isolate (NTS01708) was positive for the *bla*_{CTX-M-2}-family, which was also confirmed by PCR. The *bla*_{CTX-M-2} amplicon sequence (NCBI Accession no. KX377894) identified this gene as encoding a protein most similar to CTX-M-2 (Toho 1), CTX-M-20, CTX-M-56, CTX-M-75, CTX-M-95, CTX-M-165, and KLUA-9. To our knowledge, this is the first time that a gene from the *bla*_{CTX-M-2}-family has been identified within *Enterobacteriaceae* from East Africa. Interestingly, this *bla*_{CTX-M-2}-positive isolate were susceptible to both of the lactam/inhibitor combinations tested and all other tested β-lactams except ampicillin, suggesting that this gene was not transcribed or that the encoded proteins was non-functional. None of the 90 isolates were positive for genes encoding the CTX-M-8 and CTX-M-9 families of ESBLs. The preferential carriage of CTX-M-1-type enzymes over other ESBLs agrees with other studies of this region [28,29].

Only three isolates were phenotypically resistant to either imipenem (one isolate) or meropenem (two isolates). However, none of the 15 carbapenemase genes represented on the ARDM v.2 were detected.

Resistance to aminoglycosides

Isolates were tested for the presence of 44 different aminoglycoside resistance determinants. While only nine of the isolates were resistant to the three aminoglycosides tested, a relatively large number harbored genes commonly associated with aminoglycoside resistance: *aac*(3)-III (five isolates); *aac*(6)-Ib family (four isolates); *aadA1/A2* family (18 isolates); *aad*(A4) (one isolate); *aphA1* (seven isolates); *aph3/str*(A) (44 isolates); *aph6/str*(B) (47 isolates), and *rmtB* (one isolate). As the microarray cannot detect point mutations, we PCR-amplified and sequenced the *aac*(6)-Ib genes detected in four isolates to confirm that these alleles were not the *aac*(6)-Ib-cr variant conferring resistance to quinolones. The presence of *aac*(3)-III was correlated to phenotypic resistance to gentamicin and tobramycin ($P < 0.0001$) and *aac*(6)-Ib family genes to amikacin and tobramycin ($P < 0.0001$). Not surprisingly, the isolate harboring *rmtB*, which confers pan-resistance to aminoglycosides, was resistant to all three aminoglycosides.

Table 4. AMR genes present in individual Kenyan *Klebsiella* spp. isolates.

strain no.	Resistance determinant(s)									
	β-Lactams	Aminoglycosides	Macrolides	Tetra-cyclines	Ansa-mysin	Phenicol	Quino-lones	Quaternary amines, strepto-thricin	Sulfon-amide	Diamino-pyrimidine
MHK00504	<i>bla</i> _{LEN} , <i>bla</i> _{SHV} , † <i>bla</i> _{TEM}	<i>aad</i> (A1/A2), <i>aph</i> 3/ <i>str</i> (A), <i>aph</i> 6/ <i>str</i> (B)	<i>mac</i> (A), <i>mac</i> (B)	<i>tet</i> (B)		<i>cmr</i>		<i>sat</i> 2	<i>sul</i> 2	<i>dfr</i> A1
MHK01305	<i>bla</i> _{OXA-1-like} , <i>bla</i> _{TEM} , <i>bla</i> _{CTX-M-1} family, (<i>bla</i> _{SHV})	<i>aac</i> (6)-Ib, <i>aph</i> 3/ <i>str</i> (A), <i>aph</i> 6/ <i>str</i> (B)	<i>mac</i> (A), <i>mac</i> (B)	<i>tet</i> (B)	<i>arr</i>	<i>cat</i> A1/ <i>cat</i> 4, <i>cmr</i>		<i>qac</i> EΔ 1	<i>sul</i> 1, <i>sul</i> 2	<i>dfr</i> A14
MHK01419	<i>bla</i> _{TEM} , (<i>bla</i> _{SHV})	<i>aph</i> 3/ <i>str</i> (A), <i>aph</i> 6/ <i>str</i> (B)	<i>mac</i> (A)	<i>tet</i> (B)					<i>sul</i> 2	<i>dfr</i> A8
MHK01814	<i>bla</i> _{LEN} , <i>bla</i> _{SHV} , <i>bla</i> _{TEM}	<i>aph</i> 3/ <i>str</i> (A), <i>aph</i> 6/ <i>str</i> (B)	<i>mac</i> (A), <i>mac</i> (B)	<i>tet</i> (D)		<i>cmr</i>		<i>qac</i> EΔ 1	<i>sul</i> 1, <i>sul</i> 2	<i>dfr</i> A14, <i>dfr</i> A7
MHK02123	<i>bla</i> _{OXY-1}	<i>aph</i> (AI)				<i>cmr</i>				
MHK02126	<i>bla</i> _{OXY-1}	<i>aph</i> (AI)								
MHK02178		<i>aad</i> (A1/A2), <i>aph</i> 3/ <i>str</i> (A), <i>aph</i> 6/ <i>str</i> (B)	<i>mac</i> (A), <i>mac</i> (B)	<i>tet</i> (A)		<i>cmr</i>		<i>sat</i> 2	<i>sul</i> 2	<i>dfr</i> A14
MHK02303	<i>bla</i> _{LEN} , <i>bla</i> _{SHV}		<i>mac</i> (A), <i>mac</i> (B)			<i>cmr</i>				
MHK02499	<i>bla</i> _{TEM} , <i>bla</i> _{CTX-M-1} family	<i>aph</i> 3/ <i>str</i> (A), <i>aph</i> 6/ <i>str</i> (B)	<i>mac</i> (A), <i>mac</i> (B)	<i>tet</i> (A)		<i>cmr</i>			<i>sul</i> 2	
MHK02590	<i>bla</i> _{LEN} , <i>bla</i> _{OXA-1-like} , <i>bla</i> _{SHV} , <i>bla</i> _{TEM} , <i>bla</i> _{CTX-M-1} family	<i>aac</i> (3)-III, <i>aac</i> (6)-Ib, <i>aad</i> (A1/A2), <i>aph</i> 3/ <i>str</i> (A), <i>aph</i> 6/ <i>str</i> (B)	<i>mac</i> (A), <i>mac</i> (B), <i>mph</i> (A)/ <i>mph</i> (K)	<i>tet</i> (A)		<i>cat</i> A1/ <i>cat</i> 4, <i>cml</i> A, <i>cmr</i>			<i>sul</i> 2, <i>sul</i> 3	<i>dfr</i> A12, <i>dfr</i> A14
MHK02631	<i>bla</i> _{LEN} , <i>bla</i> _{SHV} , <i>bla</i> _{TEM}	<i>aph</i> 3/ <i>str</i> (A), <i>aph</i> 6/ <i>str</i> (B)	<i>mac</i> (A), <i>mac</i> (B)			<i>cmr</i>			<i>sul</i> 2	<i>dfr</i> A14
MHK02678	<i>bla</i> _{TEM} , (<i>bla</i> _{SHV})	<i>aph</i> 3/ <i>str</i> (A), <i>aph</i> 6/ <i>str</i> (B)	<i>mac</i> (A), <i>mac</i> (B)	<i>tet</i> (B)		<i>cmr</i>			<i>sul</i> 2	<i>dfr</i> A8
MHK02690	<i>bla</i> _{TEM} , (<i>bla</i> _{SHV})	<i>aad</i> (A1/A2), <i>aph</i> 6/ <i>str</i> (B)	<i>mac</i> (A), <i>mac</i> (B)	<i>tet</i> (D)		<i>cmr</i>		<i>qac</i> EΔ 1, <i>sat</i> 2	<i>sul</i> 1, <i>sul</i> 2	<i>dfr</i> A1
MHK02780	<i>bla</i> _{SHV}		<i>mac</i> (A), <i>mac</i> (B)			<i>cmr</i>				
MHK03026	<i>bla</i> _{LEN} , <i>bla</i> _{SHV} , <i>bla</i> _{TEM}	<i>aad</i> (A4), <i>aph</i> 3/ <i>str</i> (A), <i>aph</i> 6/ <i>str</i> (B)		<i>tet</i> (D)		<i>cat</i> A1/ <i>cat</i> 4			<i>sul</i> 2	<i>dfr</i> A13/21/22/ 23 family
MHK04212	<i>bla</i> _{LEN} , (<i>bla</i> _{SHV})	<i>aph</i> (AI)				<i>cmr</i>				
MHK04617	<i>bla</i> _{LEN} , <i>bla</i> _{SHV} , <i>bla</i> _{TEM}							<i>qac</i> EΔ 1	<i>sul</i> 1	<i>dfr</i> A5
MHK04622	<i>bla</i> _{LEN} , <i>bla</i> _{SHV}									
MHK04775	<i>bla</i> _{LEN} , <i>bla</i> _{SHV}	<i>aph</i> 3/ <i>str</i> (A), <i>aph</i> 6/ <i>str</i> (B)							<i>sul</i> 2	<i>dfr</i> A14
MHK04776	<i>bla</i> _{LEN} , <i>bla</i> _{SHV}	<i>aph</i> 3/ <i>str</i> (A), <i>aph</i> 6/ <i>str</i> (B)						<i>qac</i> EΔ 1	<i>sul</i> 1, <i>sul</i> 2	<i>dfr</i> A7
MHK04777	<i>bla</i> _{LEN} , <i>bla</i> _{SHV} , <i>bla</i> _{TEM}	<i>aph</i> 3/ <i>str</i> (A), <i>aph</i> 6/ <i>str</i> (B)		<i>tet</i> (D)				<i>qac</i> EΔ 1	<i>sul</i> 1, <i>sul</i> 2	<i>dfr</i> A5
MHK04779	<i>bla</i> _{LEN} , <i>bla</i> _{SHV}									
MHK04786	<i>bla</i> _{LEN} , <i>bla</i> _{OKP-A/-B} , (<i>bla</i> _{SHV}), <i>bla</i> _{TEM}									
MHK04792	<i>bla</i> _{LEN} , <i>bla</i> _{OKP-A/-B} , <i>bla</i> _{SHV} , <i>bla</i> _{TEM}	<i>aph</i> 6/ <i>str</i> (B)								
MHK04804	<i>bla</i> _{LEN} , <i>bla</i> _{SHV} , <i>bla</i> _{TEM}	<i>aph</i> 3/ <i>str</i> (A), <i>aph</i> 6/ <i>str</i> (B)		<i>tet</i> (D)				<i>qac</i> EΔ 1	<i>sul</i> 1, <i>sul</i> 2	<i>dfr</i> A5
MHK04812	<i>bla</i> _{CMY/LAT} , <i>bla</i> _{LEN} , <i>bla</i> _{TEM} , (<i>bla</i> _{SHV})	<i>aad</i> (A1/A2), <i>aph</i> (AI), <i>aph</i> 3/ <i>str</i> (A), <i>aph</i> 6/ <i>str</i> (B)	<i>mac</i> (A), <i>mac</i> (B)			<i>cmr</i>		<i>qac</i> EΔ 1	<i>sul</i> 1, <i>sul</i> 2	
MHK04813	<i>bla</i> _{TEM} , (<i>bla</i> _{SHV})	<i>aph</i> 3/ <i>str</i> (A), <i>aph</i> 6/ <i>str</i> (B)	<i>mac</i> (A), <i>mac</i> (B)			<i>cmr</i>			<i>sul</i> 2	<i>dfr</i> A8
MHK04819	<i>bla</i> _{LEN} , <i>bla</i> _{SHV} , <i>bla</i> _{TEM}	<i>aad</i> (A1/A2)		<i>tet</i> (D)				<i>qac</i> EΔ 1	<i>sul</i> 2	<i>dfr</i> A16, <i>dfr</i> A5
MHK04821	(<i>bla</i> _{SHV})							<i>qac</i> EΔ 1	<i>sul</i> 1	
MHK04822	<i>bla</i> _{LEN} , <i>bla</i> _{SHV} , <i>bla</i> _{TEM}	<i>aph</i> (AI), <i>aph</i> 3/ <i>str</i> (A), <i>aph</i> 6/ <i>str</i> (B)		<i>tet</i> (D)					<i>sul</i> 1, <i>sul</i> 2	<i>dfr</i> A14
MHK04834	<i>bla</i> _{SHV}									
MHK04838	<i>bla</i> _{LEN} , <i>bla</i> _{OKP-A/-B} , <i>bla</i> _{SHV} , <i>bla</i> _{TEM}									

(Continued)

Table 4. (Continued)

strain no.	Resistance determinant(s)									
	β -Lactams	Aminoglycosides	Macrolides	Tetra-cyclines	Ansa-mysin	Phenicol	Quino-lones	Quaternary amines, strepto-thricin	Sulfon-amide	Diamino-pyrimidine
MHK04847	<i>bla</i> _{LEN} , <i>bla</i> _{OKP-A-B} , <i>bla</i> _{SHV} , <i>bla</i> _{TEM}					<i>cmr</i>				
MHK04864	<i>bla</i> _{LEN} , (<i>bla</i> _{SHV})									
MHK04872	<i>bla</i> _{LEN} , <i>bla</i> _{SHV} , <i>bla</i> _{TEM}			<i>tet</i> (D)				<i>qacE</i> Δ 1	<i>sul</i> 1, <i>sul</i> 2	<i>dfr</i> A5
MHK04885	<i>bla</i> _{LEN} , <i>bla</i> _{SHV} , <i>bla</i> _{TEM}	<i>aad</i> (A1/A2)		<i>tet</i> (A)				<i>qacE</i> Δ 1	<i>sul</i> 1	<i>dfr</i> A1
MHK04900	<i>bla</i> _{LEN} , <i>bla</i> _{SHV} , <i>bla</i> _{TEM}		<i>mac</i> (A), <i>mac</i> (B)			<i>cmr</i>				
MHK04904	<i>bla</i> _{LEN} , <i>bla</i> _{SHV}									
MHK04908	<i>bla</i> _{LEN} , <i>bla</i> _{SHV} , <i>bla</i> _{TEM}			<i>tet</i> (D)				<i>qacE</i> Δ 1	<i>sul</i> 1, <i>sul</i> 2	<i>dfr</i> A5
MHK04919	<i>bla</i> _{LEN} , <i>bla</i> _{SHV} , <i>bla</i> _{TEM}	<i>aph</i> 3/ <i>str</i> (A), <i>aph</i> 6/ <i>str</i> (B)	<i>mac</i> (A), <i>mac</i> (B)			<i>cmr</i>			<i>sul</i> 2	<i>dfr</i> A14
MHK04922	<i>ampC/bla</i> _{DHA} , <i>bla</i> _{LEN} , <i>bla</i> _{SHV} , <i>bla</i> _{TEM} , <i>bla</i> _{CTX-M-1 family}	<i>aac</i> (3)-III, <i>aac</i> (6)-Ib, <i>aph</i> (A), <i>aph</i> 3/ <i>str</i> (A), <i>aph</i> 6/ <i>str</i> (B), <i>rmt</i> B,		<i>tet</i> (A), <i>tet</i> (G)	<i>arr</i>			<i>qacE</i> Δ 1	<i>sul</i> 1, <i>sul</i> 2	<i>dfr</i> A12
MHK04923	<i>bla</i> _{LEN} , <i>bla</i> _{SHV} , <i>bla</i> _{TEM}									
MHK04926	<i>bla</i> _{LEN} , <i>bla</i> _{SHV}									
MHK04928	<i>bla</i> _{LEN} , <i>bla</i> _{SHV}									
MHK04930	<i>bla</i> _{SHV} , <i>bla</i> _{TEM} , <i>bla</i> _{CTX-M-1 family}	<i>aac</i> (3)-III, <i>aad</i> (A1/A2)						<i>qacE</i> Δ 1	<i>sul</i> 1	<i>dfr</i> A12
MHK04941	<i>bla</i> _{SHV}	<i>aph</i> 3/ <i>str</i> (A), <i>aph</i> 6/ <i>str</i> (B)		<i>tet</i> (A)					<i>sul</i> 2	<i>dfr</i> A14
MHK04943	<i>bla</i> _{LEN} , <i>bla</i> _{SHV} , <i>bla</i> _{TEM}									
MHK04946	<i>bla</i> _{LEN}									
MHK04947	<i>bla</i> _{TEM} , (<i>bla</i> _{SHV})	<i>aph</i> 3/ <i>str</i> (A), <i>aph</i> 6/ <i>str</i> (B)	<i>mac</i> (A), <i>mph</i> (A)/ <i>mph</i> (K)			<i>cmr</i>			<i>sul</i> 2	
MHK04948	<i>bla</i> _{LEN} , <i>bla</i> _{SHV}									
MHK04957	<i>bla</i> _{SHV}									
MHK04960	<i>bla</i> _{LEN} , <i>bla</i> _{SHV}									
MHK04967	<i>bla</i> _{SHV} , <i>bla</i> _{TEM}	<i>aph</i> 3/ <i>str</i> (A), <i>aph</i> 6/ <i>str</i> (B)		<i>tet</i> (A)		<i>cat</i> A1/ <i>cat</i> 4		<i>qacE</i> Δ 1	<i>sul</i> 1, <i>sul</i> 2	<i>dfr</i> A7
MHK04980	<i>bla</i> _{LEN} , <i>bla</i> _{SHV} , <i>bla</i> _{TEM}	<i>aph</i> 3/ <i>str</i> (A), <i>aph</i> 6/ <i>str</i> (B)	<i>mph</i> (A)/ <i>mph</i> (K)						<i>sul</i> 2	<i>dfr</i> A14
MHK04983	<i>bla</i> _{OKP-A-B} , <i>bla</i> _{TEM}	<i>aad</i> (A1/A2)		<i>tet</i> (A)				<i>qacE</i> Δ 1	<i>sul</i> 1	<i>dfr</i> A16
MHK04984	<i>bla</i> _{SHV}	<i>aph</i> 3/ <i>str</i> (A), <i>aph</i> 6/ <i>str</i> (B)							<i>sul</i> 2	<i>dfr</i> A14
MHK05010	<i>bla</i> _{LEN} , <i>bla</i> _{SHV} , <i>bla</i> _{TEM}	<i>aph</i> 3/ <i>str</i> (A), <i>aph</i> 6/ <i>str</i> (B)	<i>mac</i> (A), <i>mac</i> (B) <i>mph</i> (A)/ <i>mph</i> (K)	<i>tet</i> (B)		<i>cat</i> A1/ <i>cat</i> 4, <i>cmr</i>			<i>sul</i> 2	<i>dfr</i> A14
MHK05013a	<i>bla</i> _{LEN} , <i>bla</i> _{SHV} , <i>bla</i> _{TEM}	<i>aph</i> 3/ <i>str</i> (A), <i>aph</i> 6/ <i>str</i> (B)	<i>mph</i> (A)/ <i>mph</i> (K)						<i>sul</i> 2	<i>dfr</i> A14
MHK05013b	<i>bla</i> _{LEN} , <i>bla</i> _{OKP-A-B} , (<i>bla</i> _{SHV}), <i>bla</i> _{TEM}	<i>aad</i> (A1/A2)						<i>qacE</i> Δ 1	<i>sul</i> 1	<i>dfr</i> A15
MHK05014a	<i>bla</i> _{OXY-1}									
MHK05014b	<i>bla</i> _{OXY-1} , <i>bla</i> _{TEM}									
MHK05017	<i>bla</i> _{LEN} , <i>bla</i> _{SHV}	<i>aph</i> 3/ <i>str</i> (A), <i>aph</i> 6/ <i>str</i> (B)		<i>tet</i> (A)					<i>sul</i> 2	
MHK05018	<i>bla</i> _{LEN}					<i>cmr</i>				
MHK05018-1B	<i>bla</i> _{LEN} , <i>bla</i> _{SHV}		<i>mac</i> (A), <i>mac</i> (B)			<i>cmr</i>			<i>sul</i> 2	
MHK05021	<i>bla</i> _{LEN} , <i>bla</i> _{SHV} , <i>bla</i> _{TEM}	<i>aad</i> (A1/A2), <i>aph</i> 3/ <i>str</i> (A), <i>aph</i> 6/ <i>str</i> (B)	<i>mac</i> (A), <i>mac</i> (B)	<i>tet</i> (A)		<i>cmr</i>		<i>qacE</i> Δ 1	<i>sul</i> 1, <i>sul</i> 2	<i>dfr</i> A1

(Continued)

Table 4. (Continued)

strain no.	Resistance determinant(s)									
	β -Lactams	Aminoglycosides	Macrolides	Tetra-cyclines	Ansa-mysin	Phenicol	Quino-lones	Quaternary amines, strepto-thricin	Sulfon-amide	Diamino-pyrimidine
MHK05027	<i>bla</i> _{LEN} , <i>bla</i> _{SHV} , <i>bla</i> _{TEM}	<i>aph3/str(A)</i> , <i>aph6/str(B)</i>	<i>mac(A)</i> , <i>mac(B)</i>	<i>tet(A)</i> , <i>tet(B)</i>		<i>cmr</i>			<i>sul2</i>	<i>dfrA14</i>
MHK05028	<i>bla</i> _{LEN} , <i>bla</i> _{SHV} , <i>bla</i> _{TEM}	<i>aad(A1/A2)</i> , <i>aph3/str(A)</i> , <i>aph6/str(B)</i>		<i>tet(A)</i>				<i>qacEΔ1</i>	<i>sul1</i> , <i>sul2</i>	<i>dfrA1</i>
MHK05042	<i>bla</i> _{LEN} , <i>bla</i> _{SHV} , <i>bla</i> _{TEM}	<i>aph3/str(A)</i> , <i>aph6/str(B)</i>		<i>tet(A)</i> , <i>tet(D)</i>				<i>qacEΔ1</i>	<i>sul1</i> , <i>sul2</i>	<i>dfrA5</i>
MHK05046	<i>bla</i> _{SHV}	<i>aph3/str(A)</i> , <i>aph6/str(B)</i>								
MHK05068	<i>bla</i> _{LEN} , <i>bla</i> _{OXA-1-like} , (<i>bla</i> _{SHV}), <i>bla</i> _{TEM} , <i>bla</i> _{CTX-M-1 family}	<i>aac(3)-III</i> , <i>aac(6)-Ib</i> family, <i>aph(A)</i> , <i>aph3/str(A)</i> , <i>aph6/str(B)</i>	<i>mph(A)/mph(K)</i>				<i>qnrS</i>	<i>qacEΔ1</i>	<i>sul1</i> , <i>sul2</i>	<i>dfrA15</i> , <i>dfrA17</i>
MHK05070	<i>bla</i> _{OKP-A/B}	<i>aad(A1/A2)</i>		<i>tet(A)</i>				<i>qacEΔ1</i>	<i>sul1</i>	<i>dfrA1</i>
MHK05072	<i>bla</i> _{LEN} , <i>bla</i> _{SHV} , <i>bla</i> _{TEM}	<i>aad(A1/A2)</i> , <i>aph3/str(A)</i> , <i>aph6/str(B)</i>	<i>mac(A)</i> , <i>mac(B)</i>	<i>tet(B)</i>		<i>cmr</i>	<i>qnrS</i>	<i>qacEΔ1</i>	<i>sul1</i> , <i>sul2</i>	<i>dfrA1</i> , <i>dfrA14</i>
MHK05080	<i>bla</i> _{LEN} , <i>bla</i> _{SHV}									
MHK05084	<i>bla</i> _{OKP-A/B} , <i>bla</i> _{TEM}	<i>aph3/str(A)</i> , <i>aph6/str(B)</i>	<i>mac(A)</i> , <i>mac(B)</i>	<i>tet(B)</i>		<i>cmr</i>			<i>sul2</i>	<i>dfrA8</i>
MHK05090	<i>bla</i> _{OKP-A/B}	<i>aph3/str(A)</i> , <i>aph6/str(B)</i>								
MHK05091	<i>bla</i> _{SHV} , <i>bla</i> _{TEM} , <i>bla</i> _{CTX-M-1 family}	<i>aad(A1/A2)</i> , <i>aph3/str(A)</i> , <i>aph6/str(B)</i>	<i>mac(A)</i> , <i>mac(B)</i>	<i>tet(A)</i>		<i>catA1/cat4 floR</i> , <i>cmr</i>		<i>qacEΔ1</i>	<i>sul1</i> , <i>sul2</i>	<i>dfrA14</i> , <i>dfrA15</i>
MHK05094	<i>bla</i> _{LEN} , (<i>bla</i> _{SHV})									
NTS01697	<i>bla</i> _{SHV} , <i>bla</i> _{TEM} , <i>bla</i> _{CTX-M-1 family}	<i>aac(3)-III</i> , <i>aph3/str(A)</i> , <i>aph6/str(B)</i>							<i>sul2</i>	<i>dfrA14</i>
NTS01699	<i>bla</i> _{LEN} , <i>bla</i> _{SHV}									
NTS01703	<i>bla</i> _{SHV}	<i>aad(A1/A2)</i>						<i>sat2</i>		<i>dfrA1</i>
NTS01705	<i>bla</i> _{LEN} , <i>bla</i> _{SHV} , <i>bla</i> _{TEM}	<i>aph3/str(A)</i> , <i>aph6/str(B)</i>	<i>mac(A)</i>			<i>catA1/cat4 cmr</i>		<i>qacEΔ1</i>	<i>sul1</i> , <i>sul2</i>	<i>dfrA7</i>
NTS01707	<i>bla</i> _{LEN} , <i>bla</i> _{SHV}									
NTS01708	<i>bla</i> _{LEN} , <i>bla</i> _{OKP-A/B} , <i>bla</i> _{SHV} , <i>bla</i> _{TEM} , <i>bla</i> _{CTX-M-2 family}									
NTS01732	<i>bla</i> _{LEN} , <i>bla</i> _{SHV}	<i>aph3/str(A)</i> , <i>aph6/str(B)</i>							<i>sul2</i>	
NTS01745	<i>bla</i> _{LEN} , <i>bla</i> _{SHV} , <i>bla</i> _{TEM}		<i>mac(A)</i>			<i>cmr</i>				
NTS01747	<i>bla</i> _{LEN} , (<i>bla</i> _{SHV})	<i>aph3/str(A)</i> , <i>aph6/str(B)</i>	<i>mac(A)</i> , <i>mac(B)</i>	<i>tet(B)</i>		<i>cmr</i>			<i>sul2</i>	<i>dfrA14</i>
NTS01749	<i>bla</i> _{LEN} , <i>bla</i> _{SHV} , <i>bla</i> _{TEM}	<i>aad(A1/A2)</i> , <i>aph3/str(A)</i> , <i>aph6/str(B)</i>	<i>mac(A)</i> , <i>mac(B)</i>	<i>tet(A)</i>		<i>cmr</i>		<i>qacEΔ1</i>	<i>sul1</i> , <i>sul2</i>	<i>dfrA1</i> , <i>dfrA8</i>
NTS01755	<i>bla</i> _{LEN} , <i>bla</i> _{SHV}	<i>aad(A1/A2)</i> , <i>aph3/str(A)</i> , <i>aph6/str(B)</i>	<i>mac(A)</i> , <i>mac(B)</i>	<i>tet(D)</i>		<i>cmr</i>		<i>qacEΔ1</i>	<i>sul1</i> , <i>sul2</i>	<i>dfrA7</i>
NTS01793	<i>bla</i> _{SHV}	<i>aph3/str(A)</i> , <i>aph6/str(B)</i>								
NTS01936	<i>bla</i> _{LEN} , <i>bla</i> _{SHV}									

bold indicates that microarray-detected *bla*_{CTX-M} or *bla*_{SHV} genes were PCR-confirmed (see S1 and S2 Tables). Results shown in parentheses indicates that *bla*_{SHV} was detected by PCR but not by microarray.

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Resistance to tetracyclines, chloramphenicol

Almost half of the isolates were non-susceptible to tetracycline. Phenotypic resistance was positively correlated to the presence of a tetracycline resistance determinant ($P < 0.0005$), although 10 isolates harboring resistance genes were phenotypically sensitive. Of the 38 tetracycline resistance genes on the ARDM v.2, only four were detected: *tet(A)* (18%), *tet(D)* (12%), *tet(B)* (10%), and *tet(G)* (1%).

The ARDM v.2 chip also contains probes directed against 20 chloramphenicol resistance determinants. However, only four were detected in the tested population: *cmr* (32 isolates); two variants of *floR* originating from different species (one isolate); *cmlA* (one isolate); and *catA1/cat4* (seven isolates). Phenotypic resistance to chloramphenicol was not assessed.

Resistance to quinolones

A single isolate (MHK02590) was resistant to both ciprofloxacin and levofloxacin, while the remainder were susceptible to one (three isolates) or both quinolones tested (86 isolates). The plasmid-mediated quinolone resistance gene, *qnrS*, was observed in two isolates, of which one displayed intermediate susceptibility for ciprofloxacin. None of the other plasmid-mediated quinolone resistance genes were detected (*norA*, *qnrA*, *qepA*, *aac(6)-Ib-cr*). The ARDM is unable to identify mutations in gyrase or helicase genes that confer high-level resistance to quinolones.

Genes conferring resistance to macrolides, lincosamides, streptogramins, and ansamycins

Ansamycins and macrolides, lincosamide, and streptogramin (MLS) antibiotics are not typically considered clinically relevant for treatment of Gram-negative infections. However, some researchers have suggested that commensal Gram-negative organisms may serve as a reservoir of AMR genes that can be transferred to other pathogens and organisms responsible for severe intestinal infections [30,31,32,33]. For this reason, the ARDM v.2 chip content includes ten MLS resistance genes derived from Gram-negative species, in addition to 31 MLS resistance genes derived from Gram-positive species. As expected, none of the isolates tested were positive for any of the Gram-positive-derived MLS resistance determinants, but *Escherichia coli*-derived genes, *mph(A)/mph(K)*, *mac(A)*, and *mac(B)*, were detected in six, 29, and 25 isolates, respectively. All isolates positive for *mac(B)* also harbored *mac(A)*. PCR amplification and amplicon sequencing confirmed that the microarray-detected *mac(A)* and *mac(B)* sequences are analogous to those derived from *E. coli* (NCBI accession nos. KX377891 through KX377893), although *Klebsiella*-derived analogs were also detected. Analogous *mac(A)* and *mac(B)* genes derived from *Klebsiella* spp. are only 70% identical to the *E. coli* genes and can be discriminated from the *E. coli*-derived genes by hybridization to the ARDM and amplicon sequencing (S2 Table).

Two isolates were positive for the presence of the rifampicin resistance determinant, *arr*. The presence of *arr* and *mphA/mphK* within stool isolates of *K. pneumoniae*—while not clinically relevant in itself—may portend the spread of azithromycin or rifaximin resistance, respectively, to other intestinal pathogens, potentially limiting the effectiveness of these drugs for treatment of travelers' diarrhea [34,35].

Resistance to sulfonamides, quaternary amines, streptothricin, and trimethoprim

Sixty percent of the tested isolates were resistant to SXT, a first line agent for treatment of enteric infections in many parts of Africa [33,36]. Phenotypic resistance to SXT was highly correlated to the presence of a sulfonamide or trimethoprim resistance determinant ($P < 0.0001$). Approximately half of the tested isolates harbored at least one of the 28 trimethoprim resistance genes present on the ARDM: *dfrA14* (18 isolates), *dfrA1* (nine isolates), *dfrA5* (seven isolates), *dfrA7* or *dfrA8* (5 isolates each), and *dfrA12*, *dfrA13/21/22/23* family, *dfrA15*, *dfrA16*, and *dfrA17* (three or fewer isolates each). The high rate of *dfrA14*-positive samples observed

here contrasts with other studies showing a much higher proportion of *dfrA1* and *dfrA7* amongst African intestinal isolates [37,38]. Seven isolates harbored multiple *dfrA* genes.

Present in 52.2% of the tested isolates, *sul2* was the most frequently encountered sulfonamide resistance determinant. *Sul1* was detected in 28 isolates, 21 of which also harbored *sul2*. In agreement with other studies of the region [37,39], *sul3* was infrequently encountered (1 isolate).

Twenty-seven of the 28 isolates positive for *sul1* also harbored *qacEΔ1*. Although association of *qac* genes with phenotypic antiseptic resistance is currently under debate, co-carriage of *qacEΔ1* with *sul1* within the 3'-conserved sequences of many class 1 integrons is often linked to the presence of other resistance genes, presumptively as gene cassettes within the integrons [40]. The presence of *intI1*—indicative of a class 1 integron—was confirmed in all *qacEΔ1* +/*sul1*+ isolates. *IntI1* was detected in 20 additional strains by PCR, indicating the absence of a full 3'-conserved sequence amongst almost half of the integrons detected here (S2 Table). Carriage of class 1 integrons with alternative structures has previously been documented within Kenya, albeit at lower rates [39]. Similarly, co-carriage of *dfrA1*, *aadA1/A2*, and *sat2* is often associated with the presence of class 2 integrons. PCR amplification of *intI2* confirmed the presence of class 2 integrons in the three isolates harboring all three genes.

Discussion

With improvements in metagenomic sequencing and other methods to characterize intestinal microbiota, a number of recent studies have documented intestinal colonization with klebsiellae as a source of extra-intestinal infections [4] and an initial stage in many nosocomial infections [6,41]. Pertinent to the current study, intestinal klebsiellae and other *Enterobacteriaceae* may serve as reservoirs of AMR determinants, increasing the potential for highly resistant disease [10,12,42]. Here we have assessed a collection of 90 *Klebsiella* spp. intestinal isolates as a model for the accumulation and evolution of resistance assemblages within the gut of Kenyan individuals.

Our data suggest that there is some selective pressure for the establishment and maintenance of bacterial populations resistant to multiple antimicrobial compounds within this region. The high proportion of isolates that were classified as MDR (36.7%), in a sample population *not selected for resistance* underscores this point, although some bias may have resulted from recent antibiotic use by the participants (no participant medical histories were available for most samples). Specific to Kenya, widespread use of tetracycline in livestock production [43], use of SXT and chloramphenicol as first line therapeutics for typhoid [2,44], and prophylactic use of SXT in persons exposed to or infected with HIV [45] may have contributed to the high prevalence of resistance to these compounds. These results are in line with other studies in East Africa showing similar rates of resistance and carriage of AMR genes [46,47,48]. On the other hand, while ciprofloxacin and third generation cephalosporins are widely distributed in Kenya [49,50,51,52], their high costs limit their use [53,54,55,56]. Thus, it was not surprising that only a small percentage of the tested population was resistant to fluoroquinolones or third/fourth generation cephalosporins, with a correspondingly low number of isolates positive for genes conferring resistance to these compounds. Similarly, carbapenem resistance was observed in only three isolates, and none of the 15 carbapenemase genes on the ARDM v.2 were identified here, including those detected in previous studies of the region where higher carbapenem resistance was observed (e.g., *bla*_{OXA-48}, *bla*_{VIM}, *bla*_{NDM}, *bla*_{IMP}, *bla*_{KPC}) [57,58,59]. Differences in the current dataset and those of other studies in East Africa may simply reflect the particular species studied (e.g., *E. coli*, *Klebsiella* spp.), age and medical histories of participants, or the sample sources (e.g., urine, blood, stool). Alternatively, our results may

suggest that availability and use of carbapenems are lower in Kenya than elsewhere in the region [60].

A large number of *K. pneumoniae* strains hybridized to the *mac(A)* and *mac(B)* probes derived from *E. coli* genes, although isolates carrying variants from both species were also identified (S2 Table). Interestingly, the presence of *E. coli*-derived *mac(A)/mac(B)* genes was also correlated with the presence of sequences hybridizing to an *E. coli*-derived *cmr* gene ($P < 0.0001$), which is only ~80% identical to the *Klebsiella* spp. homolog. BLAST searches of the *E. coli*-derived *mac(AB)* sequences indicated that these sequences have not previously been documented in any *klebsiellae*.

The breadth of genes on the microarray allowed us to detect multiple classes of resistance determinants, which may suggest the presence of integrons and/or plasmids associated with AMR. Strain MHK02590, isolated at Mbagathi District Hospital in Nairobi, was resistant to all tested antimicrobials except carbapenems and harbored 21 resistance determinants. Interestingly, Kariuki and colleagues [61] recently isolated an IncHI2 plasmid, pKST313, from a Kenyan *Salmonella typhimurium* carrying 11 of these determinants. While we did not attempt to confirm the presence of pKST313 in strain MHK02590, isolation of this strain within the Nairobi metropolitan area where pKST313 was first identified suggests that this plasmid may be circulating within this urban setting.

This study had several limitations. As with any molecular method, genotype is not always fully predictive of phenotype. Though statistically valid genotypic/phenotypic correlations could be made for many genes in this study, a disconnect was observed between the presence of several β -lactamase and dihydrofolate reductase genes and the predicted resistance profiles. These discrepancies could be due to poor gene expression, non-functionality of the expressed gene products, or the presence of other genes or mechanisms not addressed. On the other hand, we were unable to identify the molecular mechanisms for carbapenem or fluoroquinolone non-susceptibility observed in a number of samples. While carbapenem resistance was likely due to the presence of a carbapenemase gene not currently included in the ARDM chip content, fluoroquinolone resistance is likely due to mutations in DNA gyrase and topoisomerase genes, *gyrA* and *parC* [62,63]. The ARDM cannot detect these mutations. In such an instance, a more comprehensive technique such as whole genome sequencing (WGS) might provide the needed information. An additional advantage of WGS is the ability to discriminate closely related alleles and identification of changes in regulatory sequences affecting gene expression. However, WGS may also miss the presence of important genes or point mutations if coverage is insufficient or error rates are too high [64]. Nonetheless, molecular approaches such as microarray hybridization and WGS can assist in tracking the epidemiological development and spread of AMR, a benefit not realized through phenotypic testing.

Despite these limitations, we identified a high prevalence of MDR amongst a collection of Kenyan *Klebsiella* spp. stool isolates not specifically selected for their resistance characteristics. In most cases, phenotypic resistance was highly correlated to the presence of appropriate AMR determinants. While our results suggest that selective pressure exists for carriage of genes conferring resistance to tetracyclines, phenicols, trimethoprim, and sulfonamides, resistance to fluoroquinolones, third- and fourth-generation cephalosporins, and carbapenems was observed in only a small number of isolates, likely commensurate with regional usage. The wide variety of resistance determinants detected, the large number of isolates harboring five or more of these genes (65.5%) and the high prevalence of MDR phenotype (36.7%) underscore the need for more effective, targeted public health policies and infection control/prevention measures than those likely implemented in the population tested. Timely public health intervention to new and emerging sources of resistance are always important—and unfortunately

often not available—in developing countries where access to second- and third-line antimicrobials may be limited.

Supporting information

S1 Table. PCR primers used for confirmation of specific AMR and integrase genes.

(DOCX)

S2 Table. Comparison of resistance genes detected thru microarray hybridization and by PCR.

(DOCX)

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