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

Chris Rowe Taitt1,*, Tomasz A. Leski1, Daniel P. Erwin2, Elizabeth A. Odundo3, Nancy C. Kipkemoi3, Janet N. Ndonye3, Ronald K. Kirera3, Abigael N. Ombogo3, Judd L. Watson4,5, Patricia B. Pavlinac5, Christine Hulseberg2, Gary J. Vora1

1 Center for Bio/Molecular Science & Engineering, Naval Research Laboratory, Washington, DC United States of America, 2 US Army Medical Research Directorate-Kenya, Walter Reed Army Institute of Research, Kericho, Kenya, 3 KEMRI/US Army Medical Research Directorate-Kenya, Walter Reed Army Institute of Research, Kericho, Kenya, 4 Department of Global Health, University of Washington, Seattle, WA, United States of America, 5 Departments of Pediatrics, Medicine, and Epidemiology, University of Washington, Seattle, WA, United States of America

* chris.taitt@nrl.navy.mil

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*OXA, *bla*CMY/LAT, *bla*LEN-1, *bla*OKP-1, *bla*OKP-B1, *bla*OKP-1ike family, *bla*OXY-1, *bla*SHV, *bla*TEM, *bla*CTX-M-1 and *bla*CTX-M-2 families), aminoglycosides (*aac(3)-III, aac(6)-lb, aad(A1/A2), aad(A4), aph(AI), aph3/str(A), aph6/str(B), and rmtB*), 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 (*qacE/D*), 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
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 -20°C 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-ampicillin-ampicillin 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-β-D-galactopyranoside production) before analysis on Microflex MALDI Biotyper (Bruker Daltonics, Millerica, 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. Kombeva 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, platensimycin, 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 × n contingency tables). Linear regression was used to compare the number of genes/isolate with age (H₀: 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 compound⁹ | 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%)          |
|                         | S         | 39 (87%)     | 42 (93%)        | 81 (90%)        |
| CTX                     | R         | 1 (2%)       | 1 (2%)          | 2 (2%)          |
|                         | R (ESBL) | 4 (9%)       | 2 (4%)          | 6 (7%)          |
|                         | I         | 2 (4%)       | -               | 2 (2%)          |
|                         | S         | 38           | 42 (93%)        | 80 (89%)        |
| IPM                     | R         | 1 (2%)       | -               | 1 (1%)          |
|                         | I         | -            | 3 (7%)          | 3 (3%)          |
|                         | S         | 44 (98%)     | 42 (93%)        | 86 (96%)        |
| MEM                     | R         | -            | 1 (2%)          | 1 (1%)          |
|                         | I         | 1 (2%)       | -               | 1 (1%)          |
|                         | S         | 44 (98%)     | 44 (98%)        | 88 (98%)        |
| AMK                     | R         | 1 (2%)       | 1 (2%)          | 2 (2%)          |
|                         | I         | -            | -               | -               |
|                         | S         | 44 (98%)     | 44 (98%)        | 88 (98%)        |
| GEN                     | R         | 3 (7%)       | 4 (9%)          | 7 (8%)          |
|                         | I         | 1 (2%)       | 1 (2%)          | 2 (2%)          |
|                         | S         | 41 (91%)     | 40 (89%)        | 81 (90%)        |
| TOB                     | R         | 3 (7%)       | 2 (4%)          | 5 (6%)          |
|                         | I         | -            | 2 (4%)          | 2 (2%)          |
|                         | S         | 42 (93%)     | 41 (91%)        | 83 (92%)        |
| TET                     | R         | 18 (40%)     | 15 (33%)        | 33 (37%)        |
|                         | I         | 5 (11%)      | 3 (7%)          | 8 (9%)          |
|                         | S         | 22 (44%)     | 27 (60%)        | 49 (54%)        |
| CIP                     | R         | 1 (2%)       | -               | 1 (1%)          |
|                         | I         | 1 (2%)       | -               | 1 (1%)          |
|                         | S         | 43 (96%)     | 45 (100%)       | 88 (98%)        |
| LVX                     | R         | 2 (4%)       | -               | 2 (2%)          |
|                         | I         | -            | 1 (2%)          | 1 (1%)          |
|                         | S         | 43 (96%)     | 44 (98%)        | 87 (97%)        |
| SXT                     | R         | 25 (56%)     | 30 (67%)        | 55 (61%)        |
|                         | I         | -            | -               | -               |

(Continued)
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 \(\beta\)-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 \(\beta\)-lactams

The ARDM v.2 content comprises probes for 52 \(\beta\)-lactamase genes, including 12 families of extended-spectrum \(\beta\)-lactamas (ESBLs) and 15 carbapenemases. The ARDM detected \(\text{bla}\)\text{SHV}, a chromosomal gene presumptively carried in all \(K.\ pneumoniae\) [23], in 63 isolates (70%), while PCR detected \(\text{bla}\)\text{SHV} in an additional fourteen (S2 Table); 13 of the 90 isolates were negative for \(\text{bla}\)\text{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. \(\beta\)-lactamase inhibitors such as clavulanate and sulbactam are typically active against \(Klebsiella\) \text{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 \(\beta\)-SHV lactamases [24], this resistance was highly correlated to the presence of \(\text{bla}\)\text{TEM} \((P < 0.0001)\), suggesting either TEM hyperproduction [25,26] or the possible presence of inhibitor-resistant TEM enzymes. The presence of \(\text{bla}\)\text{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 \(\text{bla}\)\text{TEM} genes are also present in both. However, strain MHK01305—positive for \(\text{bla}\)\text{OXA-1-like}, \(\text{bla}\)\text{TEM}, and \(\text{bla}\)\text{CTX-M-1} family genes—is broadly susceptible to almost all tested \(\beta\)-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 |
|------------|-----|--------|---------------|------|
| MHK00504  | 11m | F      | 7/10/2010     | Ku   |
| MHK01305  | 18yr | M      | 5/24/2011     | Ki   |
| MHK01419  | 3yr  | M      | 6/21/2011     | S    |
| MHK01814  | 9m   | M      | 9/28/2011     | Mb   |
| MHK02123  | 21yr | F      | 1/11/2012     | S    |
| MHK02126  | 1yr  | F      | 1/11/2012     | S    |
| MHK02178  | 21yr | M      | 1/21/2012     | S    |
| MHK02303  | 2yr  | M      | 2/11/2012     | S    |
| MHK02499  | 1yr  | F      | 3/29/2012     | S    |
| MHK02590  | 6m   | M      | 4/14/2012     | S    |
| MHK02631  | 54yr | F      | 4/20/2012     | S    |
| MHK02678  | 1yr  | F      | 5/1/2012      | S    |
| MHK02690  | 9m   | M      | 5/4/2012      | S    |
| MHK02780  | 4m   | M      | 5/29/2012     | S    |
| MHK03026  | 8yr  | F      | 7/13/2012     | I    |
| MHK04212  | 11m  | M      | 11/15/2013    | M    |
| MHK04617  | 5m   | M      | 11/16/2013    | M    |
| MHK04622  | 8m   | M      | 11/20/2013    | M    |
| MHK04775  | 2yr  | F      | 2/1/2014      | S    |
| MHK04777  | 51yr | M      | 2/1/2014      | S    |
| MHK04779  | 2yr  | M      | 2/1/2014      | S    |
| MHK04786  | 4yr  | M      | 2/5/2014      | S    |
| MHK04792  | 43yr | M      | 2/5/2014      | S    |
| MHK04804  | 3yr  | F      | 2/7/2014      | S    |
| MHK04812  | 3yr  | M      | 3/27/2014     | S    |
| MHK04813  | 1yr  | M      | 3/28/2014     | S    |
| MHK04819  | 1yr  | M      | 4/1/2014      | S    |
| MHK04821  | 2yr  | F      | 4/2/2014      | S    |
| MHK04822  | 2yr  | M      | 4/3/2014      | S    |
| MHK04834  | 19yr | M      | 4/9/2014      | S    |
| MHK04838  | 4yr  | F      | 4/10/2014     | S    |
| MHK04847  | 28yr | F      | 4/11/2014     | S    |
| MHK04864  | 32yr | F      | 4/17/2014     | S    |
| MHK04872  | 22yr | F      | 4/18/2014     | S    |
| MHK04885  | 2yr  | M      | 4/24/2014     | S    |
| MHK04900  | 3yr  | F      | 4/28/2014     | S    |
| MHK04904  | 22yr | F      | 4/29/2014     | S    |
| MHK04908  | 1yr  | M      | 4/30/2014     | S    |
| MHK04919  | 3yr  | F      | 5/6/2014      | S    |
| MHK04922  | 3yr  | F      | 5/7/2014      | S    |
| MHK04923  | 24yr | F      | 5/7/2014      | S    |
| MHK04926  | 31yr | F      | 5/7/2014      | S    |
| MHK04928  | 5yr  | F      | 5/9/2014      | S    |
| MHK04930  | 28yr | M      | 5/9/2014      | M    |
| MHK04941  | 1yr  | M      | 5/14/2014     | M    |
| MHK04943  | 28yr | F      | 5/15/2014     | M    |
| MHK04946  | 17yr | F      | 5/16/2014     | M    |
| MHK04947  | 36yr | F      | 5/16/2014     | M    |
| MHK04948  | 38yr | M      | 5/16/2014     | M    |

| Antimicrobial compound | Ctrl/Cs |
|------------------------|---------|
| AMC                    | S       |
| SAM                    | S       |
| ATM                    | S       |
| FEP                    | S       |
| CAZ                    | S       |
| CTX                    | S       |
| IPM                    | S       |
| MEM                    | S       |
| AMK                    | S       |
| GEN                    | S       |
| TOB                    | S       |
| TET                    | S       |
| CIP                    | S       |
| LVX                    | S       |
| SXT                    | S       |

(Continued)
| Strain no. | age | gender | date isolated | site | Antimicrobial compound[^a] | Ctrl/Cs[^c] |
|-----------|-----|--------|---------------|------|-----------------------------|------------|
| MHK04957  | 37yr| F      | 5/17/2014     | M1   | S S S S S S I S S S S I S 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 S ctrl |
| MHK04967  | 15yr| M      | 5/22/2014     | M1   | I I I S S S S S S S R S R R | S R R ctrl |
| MHK04980  | 30yr| M      | 5/23/2014     | M1   | I I I S S S S S S S R S R R | S R R cs   |
| MHK04983  | 1yr 1m| M      | 5/24/2014    | Ku   | I R I S S S S S S S R R R | S R R ctrl |
| MHK04984  | 6m  | M      | 5/24/2014     | Ku   | S S S S S S S S S S S S I R | S R R ctrl |
| MHK05010  | 8yr | F      | 5/31/2014     | Ki   | I R R R S S S S S S S S S | S R R ctrl |
| MHK05013a | 35yr| F      | 5/31/2014     | M1   | S S S S S S S S S S S S S S | S S S ctrl |
| MHK05013b | 35yr| F      | 5/31/2014     | M1   | S S S S S S S S S S S S S S | S S S ctrl |
| MHK05014a | 32yr| M      | 5/31/2014     | M1   | I I I S S S S S S S R S R R | S R R ctrl |
| MHK05014b | 32yr| M      | 5/31/2014     | M1   | S 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 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 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 S ctrl |
| MHK05021  | 7yr | M      | 6/5/2014      | Ki   | S R R S S S S S S S S S S S | S R R ctrl |
| MHK05027  | 7yr | M      | 6/6/2014      | Ki   | R R R S S S S S S S S S S S | R R R cs   |
| MHK05028  | 5yr | F      | 6/6/2014      | Ki   | S S S S S S S S S S S S S S | S S S ctrl |
| MHK05042  | 2yr 9m| M      | 6/11/2014   | Ki   | R R R S S S S S S S S S S | S R R ctrl |
| MHK05046  | 4yr 10m| M      | 6/12/2014  | Ki   | S S S S S S S S S S S S S S | S S S ctrl |
| MHK05048  | 6yr | M      | 6/20/2014     | Ki   | R R R R R ESBL ESBL S S S R R | R S R cs   |
| MHK05070  | 1yr | F      | 6/21/2014     | Ku   | S S S S S S S S S S S S S S | S S S 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 S ctrl |
| MHK05080  | 9yr | M      | 6/27/2015     | Ki   | S S S S S S S S S S S S S S | S S S ctrl |
| MHK05084  | 5yr | F      | 6/28/2014     | Ku   | R R R S S S S S S S S S S | R R R 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 S ctrl |
| MHK05091  | 5yr 10m| M      | 7/2/2014    | Ku   | R R R R R ESBL ESBL S S S R R | S R 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 S ctrl |
| NTS01697  | 4yr 1m| M      | 6/12/2014   | Ki   | R R R R R ESBL ESBL S I S R R | R R R cs   |
| NTS01699  | 5yr 5m| M      | 6/12/2014    | Mg   | S S S S S S S S S S S S S S | S S S ctrl |
| NTS01703  | 2yr 8m| F      | 6/13/2014    | Hy   | S S S S S S S S S S S S S S | S S S ctrl |
| NTS01707  | 7m  | M      | 6/13/2014     | Hy   | S R R R R S S S S S S S S | S S S ctrl |
| NTS01707  | 4yr 9m| F      | 6/13/2014    | Mg   | S S S S S S S S S S S S S S | 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 S S ctrl |
| NTS01732  | 2yr 10m| F      | 6/25/2014   | Hy   | S S S S S S S S S S S S S S | S S S ctrl |
| NTS01745  | 11m | F      | 7/2/2014      | Ki   | S S S S S S S S S S S S S S | S S S ctrl |
| NTS01747  | 5yr | M      | 7/3/2014      | Hy   | S S S S S S S S S S S S S S | S S S ctrl |
| NTS01749  | 4yr 3m| M      | 7/3/2014     | Mg   | S S S S S S S S S S S S S S | S S S ctrl |
| NTS01755  | 3yr 1m| F      | 7/5/2014     | Ki   | I R R S S S S S S S S S I S | S S S cs   |
| NTS01793  | 3yr 2m| M      | 8/2/2014     | Hy   | S S I S S S S S S S S S S | S S S ctrl |
| NTS01936  | 5yr 6m| M      | 6/26/2014     | Mg   | S S S S S S S S S S S S S S | S S S ctrl |

[^a]: Antimicrobial 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–ceftazidine; CTX–ceftaxime; 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

[^b]: Collection site: Hy–Homabay; Ke–Kericho; Ki–Kisii; Ko–Kombewa; Ku–Kisumu; Mb–Mbagathi; Mg–Migori; M1 –Moi Barracks at Eldoret

[^c]: ctrl–healthy control; cs–case of acute diarrheal illness

[^d]: K. oxytoca

https://doi.org/10.1371/journal.pone.0178880.002
Table 3. Summary of AMR genes in the tested population.

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

(Continued)
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 \( \text{bla}_{\text{CTX-M-1}} \)-group genes (confirmed by PCR, see S1 and S2 Tables). An additional three isolates also carried \( \text{bla}_{\text{CTX-M-1}} \)-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 \( \text{ampC}/\text{bla}_{\text{DHA}} \) which can mask the ESBL phenotype [27]. One isolate (NTS01708) was positive for the \( \text{bla}_{\text{CTX-M-2}} \)-family, which was also confirmed by PCR. The \( \text{bla}_{\text{CTX-M-2}} \) amplicon sequence (NCBI Accession no. KX377894) identified this gene as encoding a protein most similar to \( \text{CTX-M-2} \) (Toho 1), \( \text{CTX-M-20} \), \( \text{CTX-M-56} \), \( \text{CTX-M-75} \), \( \text{CTX-M-95} \), \( \text{CTX-M-165} \), and \( \text{KLUA-9} \). To our knowledge, this is the first time that a gene from the \( \text{bla}_{\text{CTX-M-2}} \)-family has been identified within Enterobacteriaceae from East Africa. Interestingly, this \( \text{bla}_{\text{CTX-M-2}} \)-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: \( \text{aac(3)-III} \) (five isolates); \( \text{aac(6)-lb} \) family (four isolates); \( \text{aadA1}/\text{A2} \) family (18 isolates); \( \text{aad(A4)} \) (one isolate); \( \text{aphA1} \) (seven isolates); \( \text{aph3/str(A)} \) (44 isolates); \( \text{aph6/str(B)} \) (47 isolates), and \( \text{rmtB} \) (one isolate). As the microarray cannot detect point mutations, we PCR-amplified and sequenced the \( \text{aac(6)-lb} \) genes detected in four isolates to confirm that these alleles were not the \( \text{aac(6)-lb-cr} \) variant conferring resistance to quinolones. The presence of \( \text{aac(3)-III} \) was correlated to phenotypic resistance to gentamicin and tobramycin \( (P < 0.0001) \) and \( \text{aac(6)-lb} \) family genes to amikacin and tobramycin \( (P < 0.0001) \). Not surprisingly, the isolate harboring \( \text{rmtB} \), which confers pan-resistance to aminoglycosides, was resistant to all three aminoglycosides.

| gene        | case \((n = 45)\) | control \((n = 45)\) | overall \((n = 90)\) |
|-------------|-----------------|-----------------|-----------------|
| \( \text{dfrA1} \) | 6 (13%)         | 3 (7%)          | 9 (10%)         |
| \( \text{dfrA12} \) | 1 (2%)           | 2 (4%)          | 3 (3%)          |
| \( \text{dfrA13/21/22/23family} \) | 1 (2%)           | 0 (0%)          | 1 (1%)          |
| \( \text{dfrA14} \) | 8 (18%)         | 10 (22%)        | 18 (20%)        |
| \( \text{dfrA15} \) | 2 (4%)           | 1 (2%)          | 3 (3%)          |
| \( \text{dfrA16} \) | 0 (0%)           | 2 (4%)          | 2 (2%)          |
| \( \text{dfrA17} \) | 1 (2%)           | 0 (0%)          | 1 (1%)          |
| \( \text{dfrA6} \) | 3 (7%)           | 4 (9%)          | 7 (8%)          |
| \( \text{dfrA7} \) | 4 (9%)           | 1 (2%)          | 5 (6%)          |
| \( \text{dfrA8} \) | 3 (7%)           | 2 (4%)          | 5 (6%)          |

https://doi.org/10.1371/journal.pone.0178880.t003
### Table 4. AMR genes present in individual Kenyan *Klebsiella* spp. isolates.

| strain no. | β-Lactams | Aminoglycosides | Macrolides | Tetra-cyclines | Ansa-mycins | Phenicol | Quaternary amines, strepto-thricin | Sulfonamide | Diamino-pyrimidine |
|-----------|-----------|-----------------|------------|----------------|-------------|---------|-----------------------------------|-------------|-------------------|
| MHK00504  | bla<sub>EN</sub>, bla<sub>SHV</sub>-<sub>+</sub>, bla<sub>TEM</sub> | aadA1/A2, aph3′/strA(A), aph6′/strB(B) | macA(A), macB(B) | tetB(B) | | cmr | sat2 | suL2 | dfrA1 |
| MHK01305  | bla<sub>OKP</sub>-<sub>+</sub>, bla<sub>TEM</sub>, bla<sub>TX-K-1</sub> family, (bla<sub>SHV</sub>) | aac(6)-Ib, aph3′/strA(A), aph6′/strB(B) | macA(A), macB(B) | tetB(B) | | arr | catA1/cat4, cmr | qacEΔ1 | suL1, suL2 | dfrA14 |
| MHK01419  | bla<sub>TEM</sub>, (bla<sub>SHV</sub>) | aph3′/strA(A), aph6′/strB(B) | macA(A), macB(B) | tetD(D) | | cmr | qacEΔ1 | suL1, suL2 | dfrA14, dfrA7 |
| MHK01814  | bla<sub>SHV</sub>-<sub>+</sub>, bla<sub>TEM</sub>-<sub>+</sub>, bla<sub>TX-K-1</sub> family, (bla<sub>SHV</sub>) | aph3′/strA(A), aph6′/strB(B) | macA(A), macB(B) | tetD(D) | | cmr | | | |
| MHK02123  | bla<sub>OX-1</sub> | aphA1 | | | | | | | |
| MHK02126  | bla<sub>OX-1</sub> | aphA1 | | | | | | | |
| MHK02178  | bl<sub>TEM</sub>, bla<sub>SHV</sub> | aadA1/A2, aph3′/strA(A), aph6′/strB(B) | macA(A), macB(B) | tetA(A) | | cmr | sat2 | suL2 | dfrA14 |
| MHK02833  | bl<sub>TEM</sub>, bla<sub>SHV</sub> | macA(A), macB(B) | | | | | | | |
| MHK02590  | bl<sub>TEM</sub>, bl<sub>TEM</sub>-<sub>+</sub>, bl<sub>SHV</sub>-<sub>+</sub>, bl<sub>TEM</sub>, bl<sub>TX-K-1</sub> family, (bla<sub>SHV</sub>) | aac(3)-III, aac(6)-Ib, aad (A1/A2), aph3′/strA(A), aph6′/strB(B) | macA(A), macB(B), mphA2, mphK | tetA(A) | | cmr | sat2, cmr | suL1, suL2 | dfrA12, dfrA14 |
| MHK02631  | bl<sub>TEM</sub>, bl<sub>SHV</sub> | aadA1/A2, aph3′/strB(B) | macA(A), macB(B) | cmr | | | | | |
| MHK02778  | bl<sub>TEM</sub>, bl<sub>SHV</sub> | macA(A), macB(B) | | | | | | | |
| MHK03026  | bl<sub>TEM</sub>, bl<sub>SHV</sub> | aadA1/A2, aph3′/strA(A), aph6′/strB(B) | macA(A), macB(B) | tetD(D) | | cmr | | | |
| MHK02122  | bl<sub>TEM</sub>, (bla<sub>SHV</sub>) | aphA1 | | | | | | | |
| MHK04617  | bl<sub>TEM</sub>, (bla<sub>SHV</sub>) | aphA1 | | | | | | | |
| MHK04622  | bl<sub>TEM</sub>, bl<sub>SHV</sub> | aphA1 | | | | | | | |
| MHK04775  | bl<sub>TEM</sub>, bl<sub>SHV</sub> | aphA1/A2, aph6′/strB(B) | macA(A), macB(B) | tetD(D) | | cmr | | | |
| MHK04776  | bl<sub>TEM</sub>, bl<sub>SHV</sub> | aphA1/A2, aph6′/strB(B) | macA(A), macB(B) | tetD(D) | | cmr | | | |
| MHK04777  | bl<sub>SHV</sub>-<sub>+</sub>, bla<sub>TEM</sub> | aphA1/A2, aph6′/strB(B) | macA(A), macB(B) | | | | | | |
| MHK04779  | bl<sub>SHV</sub>-<sub>+</sub>, bla<sub>TEM</sub> | aphA1/A2, aph6′/strB(B) | macA(A), macB(B) | | | | | | |
| MHK04786  | bl<sub>SHV</sub>-<sub>+</sub>, bla<sub>TEM</sub>-<sub>+</sub>, (bla<sub>SHV</sub>) | aphA1/A2, aph6′/strB(B) | macA(A), macB(B) | | | | | | |
| MHK04792  | bl<sub>TEM</sub>, bl<sub>SHV</sub>-<sub>+</sub>, (bla<sub>SHV</sub>) | aphA1/A2, aph6′/strB(B) | macA(A), macB(B) | | | | | | |
| MHK04804  | bl<sub>TEM</sub>, bl<sub>SHV</sub>-<sub>+</sub>, (bla<sub>SHV</sub>) | aphA1/A2, aph6′/strB(B) | macA(A), macB(B) | | | | | | |
| MHK04812  | bl<sub>TX-K-1</sub>, bla<sub>TEM</sub>, bl<sub>TEM</sub>-<sub>+</sub>, (bla<sub>SHV</sub>) | aphA1/A2, aph6′/strB(B) | macA(A), macB(B) | | | | | | |
| MHK04813  | bl<sub>TEM</sub>, (bla<sub>SHV</sub>) | aphA1/A2, aph6′/strB(B) | macA(A), macB(B) | | | | | | |
| MHK04819  | bl<sub>TEM</sub>, bl<sub>SHV</sub>-<sub>+</sub>, (bla<sub>SHV</sub>) | aphA1/A2, aph6′/strB(B) | macA(A), macB(B) | | | | | | |
| MHK04821  | (bla<sub>SHV</sub>) | aphA1/A2, aph6′/strB(B) | macA(A), macB(B) | | | | | | |
| MHK04822  | bl<sub>TEM</sub>, bl<sub>SHV</sub>-<sub>+</sub>, (bla<sub>SHV</sub>) | aphA1/A2, aph6′/strB(B) | macA(A), macB(B) | | | | | | |
| MHK04834  | (bla<sub>SHV</sub>) | aphA1/A2, aph6′/strB(B) | macA(A), macB(B) | | | | | | |

(Continued)
### Table 4. (Continued)

| Strain no. | Resistance determinant(s) |
|------------|---------------------------|
| MHK04847   | bla<sub>TEM</sub>, bla<sub>SHV</sub>, tet<sub>(A)</sub>, qacE<sub>Δ1</sub>, sul<sub>1</sub>, dfr<sub>A1</sub> |
| MHK04864   | bla<sub>TEM</sub>, tet<sub>(D)</sub>, qacE<sub>Δ1</sub>, sul<sub>1</sub>, dfr<sub>A5</sub> |
| MHK04872   | bla<sub>TEM</sub>, tet<sub>(A)</sub>, qacE<sub>Δ1</sub>, sul<sub>1</sub>, dfr<sub>A1</sub> |
| MHK04885   | tet<sub>(D)</sub>, qacE<sub>Δ1</sub>, sul<sub>1</sub>, dfr<sub>A5</sub> |
| MHK04900   | mac(A), mac(B), cmr |
| MHK04904   | tet<sub>(D)</sub>, qacE<sub>Δ1</sub>, sul<sub>1</sub>, dfr<sub>A5</sub> |
| MHK04908   | aph<sub>3</sub>/str(A), aph<sub>6</sub>/str(B), macro<sub>(A), macro<sub>(B)</sub>, cmr |
| MHK04919   | aph<sub>3</sub>/str(A), aph<sub>6</sub>/str(B), macro<sub>(A), macro<sub>(B)</sub>, cmr |
| MHK04922   | aac(3)-III, aac(6)-Ib, aph<sub>(A1), aph<sub>3</sub>/str(A), aph<sub>6</sub>/str(B), mttB, tet<sub>(A), tet<sub>(D), qacE<sub>Δ1</sub>, sul<sub>1</sub>, dfr<sub>A12</sub> |
| MHK04923   | bla<sub>TEM</sub>, bla<sub>SHV</sub>, bla<sub>CTX-M-1 family</sub> |
| MHK04926   | bla<sub>TEM</sub>, bla<sub>SHV</sub> |
| MHK04928   | bla<sub>TEM</sub>, bla<sub>SHV</sub> |
| MHK04930   | aac(3)-III, aad(A1/A2), qacE<sub>Δ1</sub>, sul<sub>1</sub>, dfr<sub>A12</sub> |
| MHK04941   | bla<sub>SHV</sub>, aad(A1/A2) |
| MHK04943   | tet<sub>(A)</sub>, sul<sub>2</sub>, dfr<sub>A14</sub> |
| MHK04946   | mac(A), mpp<sub>(A), mpp(K)</sub> |
| MHK04948   | tet<sub>(D)</sub>, qacE<sub>Δ1</sub>, sul<sub>1</sub>, dfr<sub>A7</sub> |
| MHK04949   | apo<sub>3</sub>/str(A), aph<sub>6</sub>/str(B) |
| MHK04957   | apo<sub>3</sub>/str(A), aph<sub>6</sub>/str(B) |
| MHK04967   | apo<sub>3</sub>/str(A), aph<sub>6</sub>/str(B) |
| MHK04980   | apo<sub>3</sub>/str(A), aph<sub>6</sub>/str(B) |
| MHK04983   | apo<sub>3</sub>/str<sub>(A), aph<sub>6</sub>/str<sub>(B)</sub>) |
| MHK04984   | apo<sub>3</sub>/str<sub>(A), aph<sub>6</sub>/str<sub>(B)</sub>) |
| MHK05010   | apo<sub>3</sub>/str<sub>(A), aph<sub>6</sub>/str<sub>(B)</sub>) |
| MHK05013a  | apo<sub>3</sub>/str<sub>(A), aph<sub>6</sub>/str<sub>(B)</sub>) |
| MHK05013b  | apo<sub>3</sub>/str<sub>(A), aph<sub>6</sub>/str<sub>(B)</sub>) |
| MHK05014a  | apo<sub>3</sub>/str<sub>(A), aph<sub>6</sub>/str<sub>(B)</sub>) |
| MHK05014b  | apo<sub>3</sub>/str<sub>(A), aph<sub>6</sub>/str<sub>(B)</sub>) |
| MHK05017   | apo<sub>3</sub>/str<sub>(A), aph<sub>6</sub>/str<sub>(B)</sub>) |
| MHK05018   | apo<sub>3</sub>/str<sub>(A), aph<sub>6</sub>/str<sub>(B)</sub>) |
| MHK05018-1 | apo<sub>3</sub>/str<sub>(A), aph<sub>6</sub>/str<sub>(B)</sub>) |
| MHK05021   | apo<sub>3</sub>/str<sub>(A), aph<sub>6</sub>/str<sub>(B)</sub>) |

(Continued)
**Table 4. (Continued)**

| strain no. | β-Lactams | Aminoglycosides | Macrolides | Tetra-cyclines | Ansa-mycins | Phenicol | Quaternary amines, streptomycin | Sulfonamide | Diamino-pyrimidine |
|------------|------------|----------------|------------|----------------|-------------|---------|---------------------------------|-------------|-------------------|
| MHK05027   | bla<sub>LEN</sub>, bla<sub>SHV</sub>, bla<sub>TEM</sub> | aph3/str(A), aph6/str(B) | mac(A), mac(B) | tet(A), tet(B) | cmr         |         |                                | su2         | dfrA14            |
| MHK05028   | bla<sub>LEN</sub>, bla<sub>SHV</sub>, bla<sub>TEM</sub> | aad(A1/A2), aph3/str(A), aph6/str(B) | tet(A) | qacEΔ1 | su1, su2 |             |                                |             | dfrA1             |
| MHK05042   | bla<sub>LEN</sub>, bla<sub>SHV</sub>, bla<sub>TEM</sub> | aph3/str(A), aph6/str(B) | tet(A), tet(D) | qacEΔ1 | su1, su2 |         |                                |             | dfrA5             |
| MHK05046   | bla<sub>SHV</sub> | aph3/str(A), aph6/str(B) |         |         |             |         |                                |             |                  |
| MHK05068   | bla<sub>LEN</sub>, bla<sub>SHV</sub>, bla<sub>TEM</sub>, bla<sub>CTX-M-1 family</sub> | aac(3)-III, aac(6)-Ib family, aph(A), aph3/str(A), aph6/str(B) | mph(A)/mph(K) | qnrS | qacEΔ1 | su1, su2 |                                |             | dfrA15, dfrA17    |
| MHK05070   | bla<sub>OKP</sub>, aad(A1/A2) | tet(A) | qacEΔ1 | su1 | dfrA1 |             |                                |             |                  |
| MHK05072   | bla<sub>LEN</sub>, bla<sub>SHV</sub>, bla<sub>TEM</sub> | aad(A1/A2), aph3/str(A), aph6/str(B) | mac(A), mac(B) | cmr | qnrS | qacEΔ1 | su1, su2 | dfrA1, dfrA14 |             |
| MHK05080   | bla<sub>LEN</sub>, bla<sub>SHV</sub> | aph3/str(A), aph6/str(B) | mac(A), mac(B) | tet(B) | cmr |         |                                | su2 | dfrA8             |
| MHK05090   | bla<sub>OKP</sub>, aad(A1/A2) | aph3/str(A), aph6/str(B) |         |             |         |         |                                |             |                  |
| MHK05091   | bla<sub>SHV</sub>, bla<sub>TEM</sub>, bla<sub>CTX-M-1 family</sub> | aad(A1/A2), aph3/str(A), aph6/str(B) | mac(A), mac(B) | tet(A) | catA1/cat4, fl/oR, cmr | qacEΔ1 | su1, su2 | dfrA14, dfrA15 |             |
| MHK05094   | bla<sub>LEN</sub>, (bla<sub>SHV</sub>) |         |         |             |         |         |                                |             |                  |
| NTS01697   | bla<sub>LEN</sub>, bla<sub>SHV</sub>, bla<sub>TEM</sub>, bla<sub>CTX-M-1 family</sub> | aac(3)-III, aph3/str(A), aph6/str(B) |         |             |         |         |                                | su2 | dfrA14 |             |
| NTS01699   | bla<sub>LEN</sub>, bla<sub>SHV</sub> |         |         |             |         |         |                                |             |                  |
| NTS01703   | bla<sub>SHV</sub> | aad(A1/A2) | tet(A) | qacEΔ1 | su1, su2 | dfrA7 |             |             |                  |
| NTS01705   | bla<sub>LEN</sub>, bla<sub>SHV</sub>, bla<sub>TEM</sub> | aph3/str(A), aph6/str(B) | mac(A) | catA1/cat4, cmr | qacEΔ1 | su1, su2 | dfrA14 |             |
| NTS01707   | bla<sub>LEN</sub>, bla<sub>SHV</sub> |         |         |             |         |         |                                |             |                  |
| NTS01708   | bla<sub>LEN</sub>, bla<sub>OKP</sub>, bla<sub>SHV</sub>, bla<sub>TEM</sub>, bla<sub>CTX-M-2 family</sub> |         |         |             |         |         |                                |             |                  |
| NTS01732   | bla<sub>LEN</sub>, bla<sub>SHV</sub> | aph3/str(A), aph6/str(B) | mac(A) | cmr |         |         |                                | su2 |             |
| NTS01745   | bla<sub>LEN</sub>, bla<sub>SHV</sub>, bla<sub>TEM</sub> |         |         |             |         |         |                                |             |                  |
| NTS01747   | bla<sub>LEN</sub>, (bla<sub>SHV</sub>) | aph3/str(A), aph6/str(B) | mac(A), mac(B) | tet(B) | cmr |         |                                | su2 | dfrA14 |             |
| NTS01749   | bla<sub>LEN</sub>, bla<sub>SHV</sub>, bla<sub>TEM</sub> | aad(A1/A2), aph3/str(A), aph6/str(B) | mac(A), mac(B) | tet(A) | cmr | qacEΔ1 | su1, su2 | dfrA1, dfrA8 |             |
| NTS01755   | bla<sub>LEN</sub>, bla<sub>SHV</sub>, bla<sub>TEM</sub> | aad(A1/A2), aph3/str(A), aph6/str(B) | mac(A), mac(B) | tet(D) | cmr | qacEΔ1 | su1, su2 | dfrA7 |             |
| NTS01793   | bla<sub>SHV</sub> | aph3/str(A), aph6/str(B) |         |         |         |         |                                |             |                  |
| NTS01936   | bla<sub>SHV</sub> |         |         |             |         |         |                                |             |                  |

**Bold indicates that microarray-detected bla<sub>CTX-M</sub> or bla<sub>SHV</sub> genes were PCR-confirmed (see S1 and S2 Tables). Results shown in parentheses indicates that bla<sub>SHV</sub> was detected by PCR but not by microarray.**

https://doi.org/10.1371/journal.pone.0178880.t004

**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/catA4 (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 $qacE\Delta1$. Although association of $qac$ genes with phenotypic antiseptic resistance is currently under debate, co-carriage of $qacE\Delta1$ 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 $qacE\Delta1+/sul1+$ 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)

Acknowledgments
Some authors are employed by the US Government and this work was prepared as part of their official duties. Title 17, US code, section 105 provides that ‘Copyright protection under this title is not available for any work of the US Government’ as defined as ‘prepared by a military service member or employee of the US Government as part of that person’s official duties.’

“This work is presented with the permission of the Director, KEMRI. The work presented here represents the opinions of the authors and should not be seen to represent the policy of the Kenya Medical Research Institute, US Army Medical Research Directorate–Kenya, Walter Reed Army Institute of Research, US Department of the Army, US Navy, or the US Department of Defense.”

Author Contributions
Conceptualization: CRT TAL CH GJV.
Data curation: CRT TAL DPE EAO JLW PBP CH GJV.
Formal analysis: CRT TAL PBP CH.
Funding acquisition: DPE JLW PBP GJV.
Investigation: CRT TAL DPE EAO NCK JNN RKK ANO CH.
Methodology: CRT TAL DPE CH GJV.
Project administration: CRT TAL DPE EAO JLW PBP CH GJV.
Resources: CRT TAL DPE EAO NCK JNN RKK ANO JLW PBP CH GJV.
Supervision: CRT DPE JLW PBP CH GJV.
Validation: CRT TAL DPE CH.
Visualization: CRT.
Writing – original draft: CRT TAL PBP CH GJV.
Writing – review & editing: CRT TAL DPE EAO NCK JNN RKK ANO JLW PBP CH GJV.

References
1. World Health Organization, http://www.who.int/drugresistance/documents/surveillancerreport/en/; 27 May 2015.
2. Global Antibiotic Resistance Partnership-Kenya Working Group. Situational analysis and recommendations—Antibiotic use and resistance in Kenya. Washington, DC Center for Disease Dynamics, Economics & Policy. 2011.

3. Thi PLN, Yassibanda S, Aidara A, Le Bouguénec C, Germany I. Enteropathogenic Klebsiella pneumoniae HIV-Infected Adults, Africa. Emerg Infect Dis. 2003; 9: 135–137.

4. Fung C-P, Lin Y-T, Lin J-C, Chen T-L, Yeh K-M, Chang R-Y, et al. Klebsiella pneumoniae in gastrointestinal tract and pyogenic liver abscess Emerg Infect Dis. 2012; 18: 1322–1325. https://doi.org/10.3201/eid1808.111053 PMID: 22840473

5. Selden R, Lee S, Wang W, Bennett JV, Eickhoff TC. Nosocomial klebsiella infections: intestinal colonization as a reservoir. Ann Intern Med. 1971; 74: 657–664. PMID: 5559431

6. Martin RM, Cao J, Brisse S, Passet V, Wu W, Zhao L, et al. Molecular epidemiology of colonizing and infecting isolates of Klebsiella pneumoniae. mSphere. 2016; 1: e00261–00216. https://doi.org/10.1128/mSphere.00261-16 PMID: 27777984

7. Wanyiri JW, Kanyi H, Maina S, Wang DE, Ngugi P, O’Connor R, et al. Infectious diarrhoea in antiretroviral therapy-naive HIV/AIDS patients in Kenya. Trans R Soc Trop Med Hyg. 2013; 107: 631–638. https://doi.org/10.1093/trstmh/trt078 PMID: 24026463

8. Huddleston JR. Horizontal gene transfer in the human gastrointestinal tract: potential spread of antibiotic resistance genes. Infect Drug Res. 2014; 7: 167–176. https://doi.org/10.2147/IDR.S48820 PMID: 25018641

9. Schijerring S, Krogfelt KA. Assessment of bacterial antibiotic resistance transfer in the gut. Int J Microbiol. 2011; 2011: article 312956.

10. Salyers AA, Gupta A, Wang Y. Human intestinal bacteria as reservoirs for antibiotic resistance genes. PLOS ONE. 2013; 8: e69507. https://doi.org/10.1371/journal.pone.0069507 PMID: 23936031

11. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012; 18: 268–281. https://doi.org/10.1111/j.1469-0691.2011.03570.x PMID: 21793988
23. Ford PJ, Avison MB. Evolutionary mapping of the SHV β-lactamase and evidence for two separate IS26-dependent blaSHV mobilization events from the Klebsiella pneumoniae chromosome. J Antimicrob Chemother. 2004; 54: 69–75. https://doi.org/10.1093/jac/dkh251 PMID: 15163647

24. French GL, Shannon KP, Simmons N. Hospital outbreak of Klebsiella pneumoniae resistant to broad-spectrum cephalosporins and b-lactam-b-lactamase inhibitor combinations by hyperproduction of SHV-5 b-lactamase. J Clin Microbiol. 1996; 34: 358–363. PMID: 8789016

25. Shannon K, Williams H, King A, Philippis I. Hyperproduction of TEM-1 β-lactamase in clinical isolates of Escherichia coli serotype O15. FEMS Microbiol Lett. 1990; 67: 319–324.

26. Wu PJ, Shannon K, Phillips I. Mechanisms of hyperproduction of TEM-1 β-lactamase by clinical isolates of Escherichia coli. J Antimicrob Chemother. 1995; 36: 927–939. PMID: 8821592

27. Garrec H, Drieux-Roulet L, Golmard JL, Jarlier V, Robert J. Comparison of nine phenotypic methods for detection of extended-spectrum β-lactamase production by Enterobacteriaceae. J Clin Microbiol. 2011; 49: 1048–1057. https://doi.org/10.1128/JCM.02130-10 PMID: 21248086

28. Albrechtova K, Dolejka M, Cizek A, Tausova D, Klimes J, Bebora L, et al. Dogs of nomadic pastoralists in Northern Kenya are reservoirs of plasmid-mediated cephalosporin- and quinolone-resistant Escherichia coli, including pandemic clone B2-O25-ST131. Antimicrob Agents Chemother. 2012; 56: 4013–4017. https://doi.org/10.1128/AAC.05859-11 PMID: 22508313

29. Kiiru J, Kariuki S, Goddeeris B, Buraye P. Analysis of β-lactamase phenotypes and carriage of selected b-lactamase genes among Escherichia coli strains obtained from Kenyan patients during an 18-year period. BMC Microbiol. 2012; 12: 155. https://doi.org/10.1186/1471-2180-12-155 PMID: 22838634

30. Nguyen MCP, Woerther P-L, Bouvet M, Andremont A, Leclercq R, Canu A. Escherichia coli as reservoir for macrolide resistance genes. Emerg Infect Dis. 2009; 15: 1648–1650. https://doi.org/10.3201/eid1510.090696 PMID: 19861604

31. Kariuki S. Antibiotic resistance in enteric pathogens in developing countries. In: Sosa ADJ, Byarugaba DK, Amabile-Cuevas CF, Hsuheh PR, Kariuki S et al., editors. Antimicrobial Resistance in Developing Countries. New York: Springer; 2010. pp.

32. Nys S, Okeke IN, Kariuki S, Dinant GJ, Diels J, Stobberingh EE. Antibiotic resistance of faecal Escherichia coli from healthy volunteers from eight developing countries. J Antimicrob Chemother. 2004; 54: 952–955. https://doi.org/10.1093/jac/dkh448 PMID: 15471998

33. Okeke IN, Aboderin OA, Byarugaba DK, Ojo KK, Opintan JA. Growing problem of multidrug-resistant enteric pathogens in Africa. Emerg Infect Dis. 2007; 13: 1640–1646. https://doi.org/10.3201/eid1311.070674 PMID: 18217545

34. DuPont HL. For the record: A history of the definition & management of travelers’ diarrhea. In: Brunette GW, Kozarsky PE, Gershman MD, Magill AJ, Ostroff SM et al., editors. CDC Health Information for International Travel. Atlanta, GA: CDC; 2016. pp.

35. Steffen R, Sack DA, Rojel L, Jiang ZD, Sturchler M, Ericsson CD, et al. Therapy of travelers’ diarrhea with rifaximin on various continents. Am J Gastroenterol. 2003; 98: 1073–1078. https://doi.org/10.1111/1572-0241.2003.07283.x PMID: 12809830

36. Vila J, Vargas M, Casals C, Urassa H, Mshinda H, Schellemberg D, et al. Antimicrobial resistance of diarrheagenic Escherichia coli isolated from children under the age of 5 years from Ifakara, Tanzania. Antimicrob Agents Chemother. 1999; 43: 3022–3024. PMID: 10582903

37. Frank T, Gautier V, Talarmin A, Bercion R, Arlet G. Characterization of sulphonamide resistance genes and class 1 integron gene cassette in Enterobacteriaceae, Central African Republic (CAR). J Antimicrob Chemother. 2004; 54: 69–75. https://doi.org/10.1093/jac/dkh251 PMID: 15163647

38. Labar AS, Millman JS, Ruebush E, Opintan JA, Bishar RA, Aboderin AO, et al. Regional dissemination of a trimethoprim-resistance gene cassette in Enterobacteriaceae, Central African Republic (CAR). J Antimicrob Chemother. 2007; 59: 742–745. https://doi.org/10.1093/jac/dkl538 PMID: 17350987

39. Kiiro J, Butaye P, Goddeers B, Kariuki S. Analysis for prevalence and physical linkages amongst integrons, ISEcp1, ISCR1, Tn21 and Tn7 encountered in Escherichia coli strains from hospitalized and non-hospitalized patients in Kenya during a 19-year period (1992–2011). BMC Microbiol. 2013; 13: 109. https://doi.org/10.1186/1471-2180-13-109 PMID: 23632924

40. Jaglic Z, Cervinkova D. Genetic basis of resistance to quaternary ammonium compounds—the qac genes and their role: a review. Vet Med (Praha). 2012; 57: 275–281.

41. Conlan S, Park M, Deming C, Thomas PJ, Young AC, Coleman H, et al. Plasmid dynamics in KPC-positive Klebsiella pneumoniae during long-term patient colonization. mBio. 2016; 7: e00742–00716. https://doi.org/10.1128/mBio.00742-16 PMID: 27393756

42. van Schaik W. The human gut resistome. Philos Trans R Soc London, Ser B. 2015; 370: 20140087.

43. Mitema ES, Kikuvi GM, Wegener HC, Stohr K. An assessment of antimicrobial consumption in food producing animals in Kenya. J Vet Pharmacol Ther. 2001; 24: 385–390. PMID: 11903868
44. Ministry of Medical Services and Ministry of Public Health and Sanitation. Kenya essential medicines list. Nairobi: Government of Kenya with the World Health Organization. 2010.

45. Bwakura-Dangarembizi M, Kendall L, Bakeera-Kitaka S, Nahirya-Ntege P, Keishanyu R, Nathoo K, et al. A randomized trial of prolonged co-trimoxazole in HIV-infected children in Africa. N Engl J Med. 2014; 370: 41–53. https://doi.org/10.1056/NEJMoa1214901 PMID: 24382064

46. Hamel MJ, Greene C, Chiller T, Ouma P, Polyak C, Otieno K, et al. Does cotrimoxazole prophylaxis for the prevention of HIV-associated opportunistic infections select for resistant pathogens in Kenyan adults? Am J Trop Med Hyg. 2008; 79: 320–330. PMID: 18784222

47. Nelson N, Joshi M, Kirika R. Antibacterial resistance: The need for action in the East, Central, and Southern Africa region. Arlington, VA: USAID. 2009.

48. Mwambete KD, Kamuhawba AAR. Resistance of commensal intestinal Escherichia coli and other enterics to co-trimoxazole and commonly used antibiotics in HIV/AIDS patients. Clin Microbiol Rev. 2013; 3: 1000134.

49. Kohli R, Omuse G, Revathi G. Antibacterial susceptibility patterns of blood stream isolates in patients investigated at the Aga Khan University Hospital, Nairobi. East Afr Med J. 2010; 87: 74–80. PMID: 23057259

50. Blomberg S, Jureen R, Manji KP, Tamim BS, Mwakagile DS, Urassa WK, et al. High rate of fatal cases of pediatric septicaemia caused by Gram-negative bacteria with extended-spectrum β-lactamases in Dar es Salaam, Tanzania. J Clin Microbiol. 2005; 43.

51. Maina D, Revathi G, Kariuki S, Ozwara H. Genotypes and cephalosporin susceptibility in extended-spectrum beta-lactamase producing Enterobacteriaceae in the community. J Infect Dev Ctries. 2012; 6: 470–477. PMID: 22706188

52. Muvunya CM, Masaisa F, Bayingana C, Mutesa L, Musemakweri A, Muhirwa G, et al. Decreased susceptibility to commonly used antimicrobial agents in bacterial pathogens isolated from urinary tract infections in Rwanda: Need for new antimicrobial guidelines. Am J Trop Med Hyg. 2011; 84: 923–928. https://doi.org/10.4269/ajtmh.2011.11-0057 PMID: 21633029

53. Rogaski ET, Platts-Mills JA, Seidman JC, John S, Mahfuz M, Ulak M, et al. Use of antibiotics in children younger than two years in eight countries: a prospective cohort study. Bull World Health Organ. 2017; 95: 49–61. https://doi.org/10.2471/BLT.16.176123 PMID: 28053364

54. Cameron A, Even M, Ross-Degnan D, Ball D, Laing R. Medicine prices, availability, and affordability in 36 developing and middle-income countries: a secondary analysis. The Lancet. 2009; 373: 240–249.

55. Ministry of Medical Services and Ministry of Public Health and Sanitation. Kenya: Service provision assessment survey 2010. Nairobi, Kenya: Ministry of Medical Services and Ministry of Public Health and Sanitation. 2010.

56. World Health Organization. Medicine prices in Kenya. Geneva: World Health Organization. 2004.

57. Okoche D, Asiimwe BB, Katabazi FA, Kato L, Najjuka CF. Prevalence and characterization of carbapenem-resistant Enterobacteriaceae isolated from Mulago National Referral Hospital, Uganda. PLOS ONE. 2015; 10: e0135745. https://doi.org/10.1371/journal.pone.0135745 PMID: 26284519

58. Mushiri MF, Mshana SE, Imirzalioglu C, Bwanga F. Carbapenemase genes among multidrug resistant Gram negative clinical isolates from a tertiary hospital in Mwanza, Tanzania. Biomed Res Int. 2014; 2014: 303104. https://doi.org/10.1155/2014/303104 PMID: 24707481

59. Poirel L, Revathi G, Bernabeu S, Nordmann P. Detection of NDM-1-producing Klebsiella pneumoniae in Kenya. Antimicrob Agents Chemother. 2011; 55: 934–936. https://doi.org/10.1128/AAC.01247-10 PMID: 21115785

60. Musembi KP (2016) Drug consumption patterns with clinical and financial implications at Kenyatta National Hospital. Nairobi, Kenya: University of Nairobi.

61. Kariuki S, Okoro C, Kim J, Njoroge S, Omuse G, Langridge G, et al. Ceftriaxone-resistant Salmonella enterica serotype Typhimurium sequence type 313 from Kenyan patients is associated with the blaCTX-M-15 gene on a novel IncHI2 plasmid. Antimicrob Agents Chemother. 2015; 59: 3133–3139. https://doi.org/10.1128/AAC.00078-15 PMID: 25779570

62. Ruiz J. Mechanisms of resistance to quinolones: target alterations, decreased accumulation, and DNA gyrase protection. J Antimicrob Chemother. 2003; 51: 1109–1117. https://doi.org/10.1093/jac/dkg222 PMID: 12697644

63. Weigel LM, Steward CD, Tenover FC. gyrA mutations associated with fluoroquinolone resistance in eight species of Enterobacteriaceae. Antimicrob Agents Chemother. 1998; 42: 2661–2667. PMID: 9756773

64. Sims D, Sudbery I, Illott NE, Heger A, Ponting CP. Sequencing depth and coverage: key considerations in genomic analyses. Nat Rev Genet. 2014; 15: 121–132. https://doi.org/10.1038/nrg3642 PMID: 24434847