Plasmid-mediated quinolone resistance genes detected in Ciprofloxacin non-susceptible *Escherichia coli* and *Klebsiella* isolated from children under five years at hospital discharge, Kenya

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Abstract

Background The increasing spread of fluoroquinolone resistant enteric bacteria is a global public health concern. Children recently discharged from the hospital are at high risk of carriage of antimicrobial resistance (AMR) due to frequent exposure to antimicrobials during inpatient stays. This study aimed to determine the prevalence, correlates of ciprofloxacin (CIP) non-susceptibility, and distribution of plasmid-mediated quinolone resistance (PMQR) genes in *Escherichia coli (E. coli)* and *Klebsiella* spp isolated from children under five years being discharged from two Kenyan Hospitals.

Methods *E. coli* and *Klebsiella* spp were isolated from fecal samples from children discharged from hospital and subjected to antimicrobial susceptibility testing (AST) by disc diffusion and E-test. CIP non-susceptible isolates were screened for seven PMQR genes using multiplex polymerase chain reaction (PCR). Poisson regression was used to determine the association between the carriage of CIP non-susceptible isolates and patient characteristics.

Results Of the 280 CIP non-susceptible isolates: 188 *E. coli* and 92 *Klebsiella* spp isolates identified among 266 discharged children, 195 (68%) were CIP-non-susceptible with minimum inhibitory concentrations (MICs) of ≥ 1 μg/mL. Among these 195 isolates, 130 (67%) had high-level CIP MIC = ≥ 32 μg/mL. Over 80% of the isolates had at least one PMQR gene identified: *aac(6')lb-cr (60%)*, *qnrB (24%)*, *oqxAB (22%)*, *qnrS (16%)*, and *qepA (6%)*, however, *qnrA* was not identified in any isolates tested. Co-carriage of *qnrB* with *acc(6')-lb-cr* was the most predominant accounting for 20% of all the isolates. Ceftriaxone use during hospital admission and the presence of extended spectrum beta-lactamase (ESBL) production were significantly associated with the carriage of CIP non-susceptible *E. coli* and *Klebsiella* spp.

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Conclusion CIP non-susceptibility is common among *E. coli* and *Klebsiella* spp isolated from hospital discharged children in Kenya. Carriage and co-carriage of PMQR, including the newly identified *qepA* gene, were frequently observed. These findings suggest that children leaving the hospital may serve as an important reservoir for transmission of resistant *E. coli* and *Klebsiella* spp to the community. Enhanced surveillance for AMR determinants is critical to inform interventions to control antimicrobial-resistant bacteria.

Keywords Ciprofloxacin, *Escherichia coli*, *Klebsiella* spp, Post-hospital discharge, Fluoroquinolones, Antimicrobial resistance

Background

AMR is a global public health threat associated with morbidity, rehospitalization, longer hospital stays, and mortality [1, 2]. In sub-Saharan Africa (SSA), antibiotic-resistant pathogens are major drivers of morbidity and mortality in children under five years of age, fuelled by inappropriate antibiotic use and poor sanitation [3] leading to the selection and spread of antibiotic-resistant bacteria [4]. Estimates by the 2019 report on the Global Burden of Bacterial AMR identified SSA as the highest contributor to the global AMR burden [5].

Commensal resident gut bacteria such as *E. coli* and *Klebsiella pneumoniae* (*K. pneumoniae*) play a critical role in AMR as they act as reservoirs for the carriage of AMR determinants [6, 7]. These enteric bacteria can become pathogenic or may transfer AMR genes to other pathogenic Enterobacterales, such as *Salmonella* and *Shigella* [8]. The acquisition of AMR and virulence factors by commensal *E. coli* and *Klebsiella* spp is mediated by mobile genetic elements, such as plasmids and transposons, via horizontal gene transfer [9]. Antibiotic resistance in commensal enteric bacteria, such as *K. pneumoniae* and *E. coli*, has been reported in 85–90% of World Health Organization (WHO) member state regions [10].

Fluoroquinolones such as ciprofloxacin (CIP) are effective broad-spectrum antibiotics used for the treatment of bacterial infections making them a recommended choice of therapy for enteric infections such as salmonellosis and shigellosis [11]. The emergence of fluoroquinolone resistance has reduced therapeutic options, especially for Enterobacterales infections [12]. Fluoroquinolone resistance is mediated by two mechanisms: chromosomal mutations in DNA gyrase and topoisomerase IV enzymes and plasmid-mediated quinolone resistance (PMQR). Mutations in fluoroquinolone binding sites during DNA replication mediate high-level fluoroquinolone resistance[13]. Mechanisms of PMQR genes include protection of DNA gyrase and topoisomerase IV from quinolone activity mediated by *qnr* genes: *qnrA, qnrB, qnrC, qnrD,* and *qnrVC* [14]*.* The aminoglycoside-modifying enzyme encoded by *aac(6')-Ib-cr* is involved in the acetylation of fluoroquinolones leading to reduced susceptibility to CIP and norfloxacin [14]. The final mechanism is enhanced efflux pump activity mediated by quinolone efflux pump *(qepA)* and *oqxAB* associated with reduced susceptibility to fluoroquinolone and increased ESBLs [15].

There is a paucity of data available on fluoroquinolone resistance and PMQR determinants in commensal bacteria, especially in children under five years in Kenya. In this study, we sought to determine the prevalence and the distribution of PMQR determinants mediating CIP non-susceptibility in *E. coli* and *Klebsiella* spp isolates from children being discharged from the hospital. In addition, we identified correlates of carriage of CIP non-susceptible isolates. This in-depth analysis will help inform the burden of CIP resistance carriage in children under five years being discharged from hospitals in SSA.

Results

Participant population and baseline characteristics

Six hundred and fifty-one isolates (406 *E. coli* and 245 *Klebsiella* spp) were isolated from 568 children and subjected to AST (Fig. 1). Among the 568 children, 343 (60.4%) were discharged from Kisii Teaching and Referral Hospital (KTRH) and 225 (39.6%) were discharged from Homabay County Referral Hospital (HCRH), 348 (61.3%) were less than two years of age and 230 (40.5%) were female (Table 1). Prior to discharge, the median duration of hospitalization was 3 days [interquartile range (IQR) 2,6 days] with 203 (35.7%) children being hospitalized for ≥ 4 days. The majority of the children (97.7%) had their human immunodeficiency virus (HIV) status determined with 12 (2.1%) being HIV-infected while 70 (12.3%) were HIV-exposed. There were more HIV-exposed children in HCRH 51 (22.7%) compared to KTRH 19, (5.5%). Common diagnoses at discharge were pneumonia 164 (28.9%), malaria 155 (27.3%), and diarrhoea 108 (19%) (Table 1). The majority of the children 502 (88.4%) had an antibiotic prescribed during their hospitalization with penicillins 359 (63.2%) being the most prescribed antibiotic followed by gentamicin 316 (55.6%) and ceftriaxone 187 (32.9%). Fluoroquinolones were rarely administered during hospitalization with CIP being prescribed to only 2 (0.9%) children in HCRH and none among children from KTRH. Children at KTRH 328 (95.6%) compared to
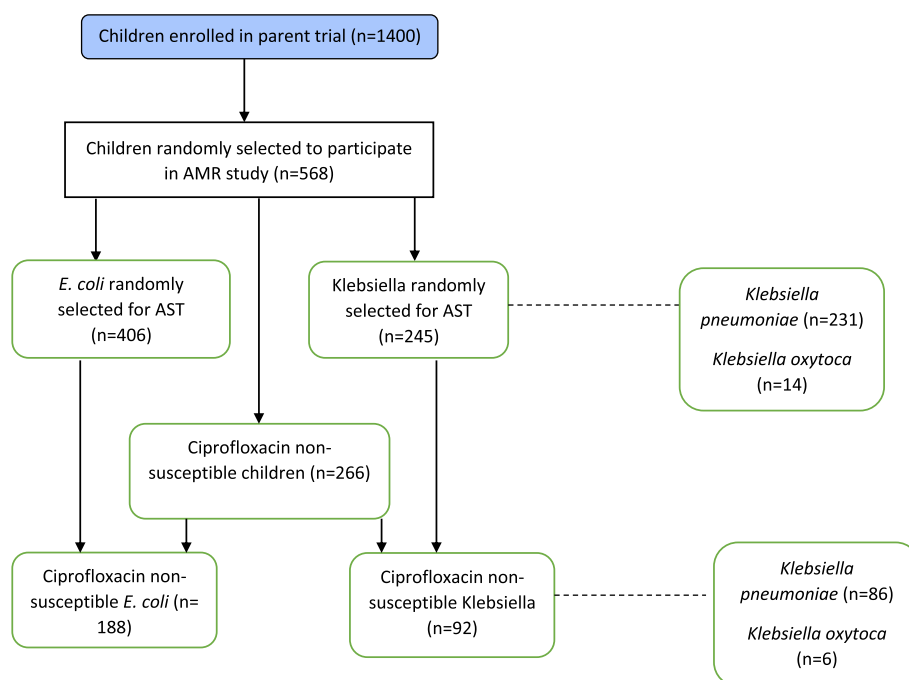
Fig. 1 Flowchart of participants isolates. AMR = Antimicrobial resitanace; AST = Antimicrobial susceptiability testing; CIP = Ciprofloxacin; *E. coli* = Escherichia coli; n = Number

HCRH 174, (77.3%) were more likely to be prescribed an antibiotic. Nearly half of the children, 266 (46.8%) had CIP non-susceptible isolates (46.3% *E. coli* and 37.6% *Klebsiella* spp). Of the 83 children who had both *E. coli* and *Klebsiella* spp isolated, 14 (2.46%) had CIP non-susceptibility in both isolates.

Correlates for carriage of fluoroquinolone non-susceptible *E. coli* or *Klebsiella* at hospital discharge

Children who received an antibiotic during hospitalization were 69% more likely to have a CIP non-susceptible *E. coli* isolate (PR 1.69, [95%CI = 1.09, 2.63], $p = 0.01$) and over two times more likely to have a CIP non-susceptible *Klebsiella* spp isolate (PR 2.61, [95%CI = 1.05, 6.53], $p = 0.01$). The presence of ESBL carriage was also associated with the presence of either a CIP non-susceptible *E. coli* or *Klebsiella* spp isolate. Length of hospital stay was associated with CIP non-susceptible *E. coli* and children with hospitalizations extending more than 4 days were nearly 40% more likely to have CIP non-susceptible *E. coli* (PR 1.38 [95%CI 1.07, 1.78] $p = 0.01$). The use of either CIP or ceftriaxone were equally associated with CIP non-susceptible *Klebsiella* or *E. coli*. Children hospitalized for diarrhoea were 27% less likely to have a CIP non-susceptible *E. coli* compared to those who did not present with diarrhoea (PR 0.73 [95%CI 0.53, 1.0] $p = 0.03$). Similar magnitudes of

association for hospital length and diarrhoea diagnosis were observed in *Klebsiella* isolates but were not statistically significant (Table S1).

Distribution of CIP non-susceptible isolates

Among the 266 children with CIP non-susceptible isolates, we isolated 280 CIP non-susceptible isolates: 188 and 92 *E. coli* and *Klebsiella* spp, respectively. Of the 266 children, 14 had both a CIP non-susceptible *E. coli* and *Klebsiella* spp isolated. Among the 92 *Klebsiella* spp, 86 were *K. pneumoniae* and 6 were *Klebsiella oxytoca (K. oxytoca)* (Fig. 1).

CIP MICs values for the 280 CIP non-susceptible isolates ranged between 0.25 – 32 µg/mL with CIP MIC_{50} (µg/mL) of 32 µg/mL. Of the 280 isolates, 214 (76.4%) were resistant while 61 (21.8%) were intermediate. Among the resistant isolates, high-level CIP resistance ($MIC \geq 32$ µg/mL) was common in almost half of the isolates (46.4%) most commonly among *E. coli* 102 (54.3%) and slightly less in *Klebsiella* spp (28/92, 30.4%). The CIP MIC distribution, MIC_{50} and MIC_{90} among the isolates are shown in Table 2.

Distribution of PMQR determinants

Six different PMQR determinants: *qnr* (*qnrB,* and *qnrS*), enzyme modifying *aac(6')-Ib-cr,* and efflux pumps *(qepA, oqxA,* and *oqxB*) were detected (Table 3). At least one

Table 1 Participant baseline characteristics

	Kisii		Homa Bay		Total	
n	N:343		N:225		N:568	
Sociodemographic						
Age(months)						
1 to 5	53	15.5%	20	8.9%	73	12.9%
6 to 11	73	21.3%	44	19.6%	117	20.6%
12 to 23	87	25.4%	71	31.6%	158	27.8%
24 to 59	130	37.9%	90	40%	220	38.7%
Sex						
Male	205	59.8%	133	59.1%	338	59.5%
Female	138	40.2%	92	40.9%	230	40.5%
Duration of Hospitalization(days)						
0 to 2	100	29.2%	66	29.3%	166	29.2%
3 to 4	121	35.3%	73	32.4%	194	34.2%
>4	117	34.1%	86	38.2%	203	35.7%
Unknown[a]	5	1.5%	0	0	5	0.9%
Median (25%, 75%)	3	(2, 6)	4	(2, 6)	3	(2, 6)
HIV Status						
HIV unexposed	309	90.1%	164	72.9%	473	83.3%
HIV exposed, uninfected	19	5.5%	51	22.7%	70	12.3%
HIV infected	6	1.7%	6	2.7%	12	2.1%
HIV-uninfected/exposure status unknown	9	2.6%	4	1.8%	13	2.3%
Diagnosis at Discharge[b,c]						
Diarrhoea	65	19%	43	19.1%	108	19%
Lower respiratory tract infection	117	34.1%	47	20.9%	164	28.9%
Malaria	68	19.8%	87	38.7%	155	27.3%
Malnutrition	24	7%	18	8%	42	7.4%
Pneumonia	117	34.1%	47	20.9%	164	28.9%
Upper respiratory tract infection	36	10.5%	10	4.4%	46	8.1%
Any antibiotic used during admission (enrollment visit)						
Any antibiotics used	328	95.6%	174	77.3%	502	88.4%
Azithromycin	2	0.6%	2	0.9%	4	0.7%
Ceftriaxone	102	29.7%	85	37.8%	187	32.9%
Penicillin	268	78.1%	91	40.4%	359	63.2%
Gentamicin	241	70.3%	75	33.3%	316	55.6%
Ciprofloxacin	0	0	2	0.9%	2	0.4%

[a] Missing either admission or discharge dates (*n* = 5)

[b] Diagnosis are not mutually exclusive

[c] No documented diagnosis at discharge (*n* = 23)

of the PMQR genes was detected in nearly all (224/280, 80%) of the screened isolates. Most *E. coli* and *Klebsiella* spp (40%) isolates had at least one *qnr* determinant detected. Of the *qnr* genes, *qnrB* was the most commonly detected *qnr* gene (20/188, 11%) and 47/92(51%) in *E. coli* and *Klebsiella* spp, respectively. In *E. coli*, *qnrS* was the most detected *qnr* gene (27/188, 14%). The *qnrA* gene was not detected in any of the *E. coli* or *Klebsiella* spp isolates.

The most predominant PMQR gene was *aac(6')-lb* (167/280, 59%) identified in more than half of all CIP non-susceptible isolates. *Klebsiella* spp had more *aac(6')-lb* positive isolates with (78/89, 85%) *Klebsiella* compared to (89/188, 47%) of *E. coli* isolates. All isolates carrying the *aac(6')-lb* gene were positive for the *cr* variant. The *qepA* gene was only detected in (16/188, 9%) *E. coli* CIP non-susceptible isolates. The o*qxAB* complex was the most dominant efflux pump detected

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Table 2 Distribution of MIC (µg/mL) per organism

	Frequency of isolates with indicated MIC value								CIP MIC$_{50}$ (µg/mL)	CIP MIC$_{90}$(µg/mL)
MIC values (µg/mL)	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**		
Bacterial species										
E. coli	2	51	9	7	10	2	5	102	32	32
K. oxytoca	1	0	0	0	0	1	0	4	32	32
K. pneumoniae	1	10	23	23	2	1	2	24	2	32
Number. of occurrences(%)	4 (**1.4**)	61 (**21.8**)	32 (**11.4**)	30 (**10.7**)	12 (**4.3**)	4 (**1.4**)	7 (**2.5**)	130 (**46.4**)		

CIP Ciprofloxacin, *E. coli Escherichia coli, K. oxytoca Klebsiella oxytoca, K. pneumoniae Klebsiella pneumoniae, MIC* Minimum inhibitory concentration, *MIC$_{50}$* MIC value at which > 50% of the isolates are inhibited, *MIC$_{90}$* MIC value at which ≥ 90% of the isolates are inhibited

Table 3 Distribution of PMQR determinants per organism

PMQR genes	*E. coli* N = 188	*K. oxytoca* N = 6	*K. pneumoniae* N = 86	Total N = 280
qnrB	20 (11%)	2 (33%)	45 (52%)	67 (24%)
qnrS	27 (14%)	1 (17%)	18 (21%)	46 (16%)
aac(6')-lb-cr	89 (47%)	5 (83%)	73 (85%)	167 (59%)
qepA	16 (9%)	0 (0%)	0 (0%)	16 (6%)
OqxA	2 (1%)	4 (67%)	83 (97%)	89 (32%)
OqxB	0 (0%)	3 (50%)	59 (69%)	62 (22%)
OqxAB	0 (0%)	3 (50%)	58 (67%)	61 (22%)

E. coli Escherichia coli, K. oxytoca Klebsiella oxytoca, K. pneumoniae Klebsiella pneumoniae, N Number, *PMQR* Plasmid mediated quinolone resistance

(61/92, 66.3%) in *Klebsiella*, however, none were detected in *E. coli*. Only (2/188, 1%) *E. coli* isolates had the *oqxA* gene, however, both of these isolates lacked the *oqxB* gene therefore none of the *E. coli* isolates carried the *oqxAB* complex.

QepA sequence analysis

DNA sequencing of the *qepA* gene from 16 *E. coli* isolates revealed amino acid substitutions at codons 95 and 134. Double amino acid substitutions F95L and V134I were common in 9/16 (56.3%) *E. coli* isolates. Six *qepA* positive *E. coli* isolates carried had no amino acid substitution while one isolate had only V134I amino acid substitution (Table 4). Assigned accession numbers for the *qepA* gene from 8 representative isolates submitted to GenBank are as follows: OP918677, and OQ031499-OQ031505.

Distribution of co-carriage of PMQR determinants per organism

A total of 225/280 (80%) isolates had at least one PMQR gene including; all 92 *Klebsiella* spp and most *E. coli* 133/189 (70.37%). Interestingly, *qnrB* and *qnrS* co-occurred in two *K. pneumoniae* (0.71%) isolates. Co-carriage of *qnrB* with *acc (6') lb-cr* was present in 12/188 (6.4%) *E. coli* and *aac(6')-Ib-cr* with *oqxAB* detected in 47/92 (51%) *Klebsiella* spp were the most prevalent combination of PMQR gene combinations. *E. coli* isolates had three notable combinations of different PMQR

Table 4 MICs and amino acid changes in *qepA E. coli* isolates

Isolate ID	Accession Number	Site	Organism	CIP MIC	INTER	*qepA* Variants	Amino acid variation	
							F95	**V134**
E24	OQ031502	Kisii	*E. coli*	32	R	-	F95	V134I
E28	OQ031503	Kisii	*E. coli*	2	R	*qepA1*	F95	V134
E40	OQ031504	Kisii	*E. coli*	2	R	*qepA1*	F95	V134
E42	OQ031505	Kisii	*E. coli*	32	R	*qepA1*	F95	V134
E49	OQ031499	Homabay	*E. coli*	32	R	*qepA4*	F95L	V134I
E66	OP918677	Kisii	*E. coli*	32	R	*qepA4*	F95L	V134I
E79	OQ031500	Homabay	*E. coli*	32	R	*qepA4*	F95L	V134I
E80	OQ031501	Homabay	*E. coli*	32	R	*qepA4*	F95L	V134I

CIP Ciprofloxacin, *INTER* Interpretation of susceptibility to Ciprofloxacin according to CLSI guidelines 2020 [16], *Isolate ID* Isolate identification number, *MIC* Minimum inhibitory concentration, *qepA* quinolone efflux pump

determinants with *qnr* combination being predominant in the co-existence of genes. On the other hand, *Klebsiella* spp had as many as nine different combinations and similarly, *qnr* gene combinations were predominant in the different combinations of determinants. The most common co-carriage in both bacterial species was *qnrB* with *acc (6') lb-cr* found in 56/280 (20%). Additionally, *acc(6')-lb-cr* co-existed with a majority of PMQR genes in both *E. coli* and *Klebsiella* spp isolates (Table 5).

Discussion

Fluoroquinolone resistance in Enterobacterales in children under five years recently discharged from the hospital is of great public health concern due to the risk of transmission of these bacteria to the community, and treatment failure, which may require re-hospitalization during the post-hospital discharge period. This study sought to determine the prevalence and correlates of CIP non-susceptibility, and distribution of PMQR genes in *E. coli* and *Klebsiella* spp, isolated from children under five years recently discharged from hospitals. We observed a high level of CIP resistance among children being discharged from two hospitals in western Kenya and multiple fluoroquinolone resistance genes in *Klebsiella* spp and *E. coli*. Our findings show high levels of MICs to CIP in a majority of the CIP non-susceptible isolates, which is disturbing due to the relationship between increasing MICs leading to fluoroquinolone non-susceptibility and fluoroquinolone treatment failure [17]. This is particularly important given that fluoroquinolones are recommended therapies for the treatment of enteric infections such as shigellosis and salmonellosis [18, 19].

CIP non-susceptibility was detected in commensal *E. coli* (46%) and *Klebsiella* spp (38%) isolated from children being discharged from hospital and there was concomitant high carriage of PMQR genes (80%) among the isolates. This is despite less than 1% of the hospitalized children receiving fluoroquinolone antibiotics during hospitalization. The high fluoroquinolone non-susceptibility observed could be attributed to co-selection pressure mediated by non-fluoroquinolone antibiotics especially cephalosporins such as ceftriaxone, facilitating the selection and carriage of PMQR genes as previously reported [20]. This situation is further exacerbated by resistance pressure mediated by ESBL production which is evident in this study; the presence of ESBL production is highly associated with CIP non-susceptibility carriage [21]. Almost all children in the study population received an antibiotic during their in-patient stay with penicillin, gentamicin, and ceftriaxone being the most prescribed antibiotics consistent with other findings in Kenya [22]. Antimicrobial usage has been associated with selective pressure for AMR in gut bacteria [23]; our findings show that there is a strong correlation between antibiotic use and the carriage of CIP non-susceptible bacteria. Notably, children presenting with diarrhoea were less likely to carry a CIP non-susceptible *E. coli.* Children with diarrhoea may be less likely to be treated with an antibiotic [24], treated with fewer antibiotics, and/or treated with shorter courses of antibiotics compared to sepsis, malnutrition and pneumonia. Despite being the guideline-indicated antibiotic, CIP is rarely used for diarrhoea in this setting due to lack of availability and cost. Another reason could be during diarrhoeal episodes, there is gut dysbiosis that alters the gut composition [25] including

Table 5 Co-carriage of PMQR determinants per organism

PMQR genes present	Organism		No. of occurrences
	E. coli	*Klebsiella* spp	
At least one PMQR	133/188	92/92	225/280
qnrB + qnrS	0	2	2
qnrB + qnrS + aac(6')-lb-cr	0	1	1
qnrB + qnrS + aac(6')-lb-cr + oqxAB	0	1	1
qnrB + aac(6')-lb-cr	12	44	56
qnrB + acc (6') lb-cr + oqxAB	0	25	25
qnrB + oqxAB	0	13	13
aac(6')-lb-cr + oqxAB	0	47	47
qnrS + aac(6')-lb-cr	6	15	21
qnrS + aac(6')-lb-cr + oqxAB	0	10	10
qepA + qnrS	3	0	3
qepA + aac(6')-lb-cr	3	0	3

E. coli Escherichia coli, *No.* Number, *PMQR* Plasmid mediated quinolone resistance, *spp* species

resistant bacteria colonizing the gut. Thereby children presenting with diarrhoea would have a lower chance of carrying CIP non-susceptible *E. coli* compared to children without diarrhoea who have had the gut intact with colonized resistant bacteria.

PMQR genes facilitate low-level fluoroquinolone resistance, however, they select for higher-level resistance mediated by mutations on genes encoding gyrase and topoisomerase enzymes [13]. In this study, we detected six (*qnrB, qnrS, aac(6')lb-cr, qepA, oqxA,* and *oqxB*) PMQR genes mediating fluoroquinolone resistance in both *E. coli* and *Klebsiella* spp. One of the six PMQR genes (*qepA*) had not previously been detected in clinical isolates in Kenya. The *qepA* gene has been reported in very few studies within the SSA region: Chad, Malawi, Egypt, Sierra Leon, and Nigeria [26–30]. Other PMQR determinants that have been identified in Kenya are *aac(6')-lb-cr*, *qnrB*, *qnrS* in *E. coli* [31], *qnrS,* and *oqxAB* in *K.pneumoniae* [32, 33]. Intestinal carriage of PMQR genes in these bacteria has been reported in several studies in SSA and globally [34–36]. This is particularly worrying due to their potential transfer of these genetic determinants through mobile genetic elements to pathogenic bacterial species, thereby mediating the transmission of resistant bacteria that may result in treatment failure.

The *aac(6')-Ib-cr* gene was the most commonly detected PMQR determinant. Most of the *Klebsiella* spp (84.78%) harboured the gene aminoglycoside modifying enzyme while almost half of the *E. coli* isolates (90/188, 48%) harbored the gene which is consistent with findings from previous studies [37]. The aminoglycoside acetyltransferase enzymes have not only been associated with reduced susceptibility to fluoroquinolones but also to aminoglycosides, thus limiting effective antibiotic treatment [38]. *Qnr* genes were the second most widely detected PMQR determinants associated with resistance to fluoroquinolones with prevalence rates of 40%. The prevalence of *qnr* genes (*40%*) was higher compared to previous studies from Kenya which reported (2%) and (8.4%) [33, 39]. The *qepA* gene, one of the most recently identified PMQR determinants, has been associated with decreased susceptibility to fluoroquinolones and increased MIC levels [13]. This determinant was detected in (16/188, 8.5%) *E. coli* isolates which was slightly lower compared to previous studies in Nigeria (18.5%) and Sierra Leon (23%) [28, 30]. DNA sequencing confirmed the existence of the *qepA* gene among *E. coli* isolates in Kenya with F95L and V134I amino acid substitution consistent with amino acid substitution reported in the *qepA4* allele [40]. To our knowledge, this is the first report of the detection of *qepA* in Kenya; this

is worrisome for public health and calls for more active fluoroquinolone resistance surveillance.

Co-carriage of PMQR plays a critical role in multidrug resistance as it influences increased MICs leading to decreased susceptibility to fluoroquinolone antibiotics that may lead to treatment failure. We observed high multiple co-carriage in both *E. coli* and *Klebsiella* fluoroquinolone non-susceptible isolates with co-existence of *aac(6')lb-cr* and *qnrB*, or *qnrS* genes, being the most predominant co-carriage in both bacteria. The prevalence of co-carriage between *qnrB* and *aac(6') lb-cr* (20%) was found to be higher compared to findings from previous studies in SSA [41]. We observed co-carriage of *qnrB* with *qnrS* in two *K. pneumoniae* isolates, a phenomenon that has previously been reported in *Klebsiella* spp, however, its prevalence in this study was much lower compared than 18.75% reported in Togo [21]. This co-carriage could be attributed to multiple plasmids carrying the different *qnr* genes within the same genetic environment as has been previously demonstrated [42]. Notably, 12 isolates with the rare *qepA* co-existed with other PMQR genes (*qnrS* or *aac(6') lb-cr*) which was consistent with findings from other previous studies [43]. Interestingly, one *K. pneumoniae* isolate co-harboured all determinants detected in this study except *qnrA* and *qepA.* This is concerning as the co-existence of multiple PMQR genes has been linked to resistance to multiple antibiotic classes due to the carriage of multiple plasmids carrying resistance determinants to other classes of antibiotics.

This is one of the few studies that has characterized AMR determinants in children post-hospital discharge in SSA settings, including screening for a wide range of PMQR genetic determinants, highlighting the greater diversity and distribution of fluoroquinolone resistance genes. In addition, the focus on commensal *E. coli* and *Klebsiella* spp, two commonly isolated Enterobacterales associated with the carriage of AMR determinants as indicator organisms for AMR carriage rather than pathogenic bacteria was important due to their ability to transfer AMR genetic elements.

This study had some limitations. CIP non-susceptibility was determined in a subset of isolates from children after discharge, which means there could be more non-susceptible isolates that were not screened. Only two children in this study received CIP or rather a fluoroquinolone during admission, this may not be sufficient to clearly show the role of fluoroquinolone resistance in poor patient outcomes during the post-discharge period. Limiting the analysis to PCR detection only, other mechanisms mediating resistance such as point mutations which could be detected by comprehensive whole-genome sequencing analyses were not captured.

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Being a cross-sectional study at the point of hospital discharge, we were unable to determine whether AMR was acquired at the community or nosocomially. Having isolates at admission, the point at which treatment decisions are also made would be able to answer this question of the timing of AMR acquisition.

Conclusion
This study detected multiple PMQR genes and the first report of the *qepA* gene among CIP non-susceptible clinical *E. coli* and *Klebsiella* spp. The study observed high levels of CIP non-susceptibility and fluoroquinolone resistance carriage which could form a reservoir for the community spread of resistance, thus posing a great challenge in the effective treatment during hospital stays and subsequently during the post-hospital discharge period. We recommend enhanced surveillance for fluoroquinolone resistance carriage which will be vital to inform interventions to control antimicrobial-resistant bacteria and antimicrobial stewardship in rural and peri-urban populations.

Materials and methods
Study design
This was a cross-sectional nested study from the Toto Bora trial [44] that utilized *E. coli* and *Klebsiella* spp isolates recovered from fecal samples of children under five years discharged from two hospitals in western Kenya. Children being discharged from KTRH and HCRH aged between 1 -59 months were recruited in the parent study [44] to assess the effects of Azithromycin on mortality and rehospitalization in children under five years. The nested study used clinical, sociodemographic, and health history information collected during physical examination from children enrolled in the parent trial or interviews with their caregivers at hospital discharge. Faecal or rectal swab samples were collected before Azithromycin administration was done. The swabs were cultured, isolates recovered and biochemically identified as previously described [44].

Parent trial
Bacterial isolation, identification, and AST
After laboratory culture, *E. coli* and *Klebsiella* spp isolates were identified and AST was performed by disc diffusion as previously described [45]. Briefly, a rectal swab or whole stool was collected from the enrolled child and was inoculated on MacConkey Agar and incubated at 37 °C for 24 h within 24 h of specimen collection time. Mucoid, lactose, or non-lactose fermenting colonies suspected to be *E. coli* or *Klebsiella* spp were isolated and the API 20E (bioMérieux, Inc, Durham, NC, United States) system

confirmed the species of bacteria. A total of 568 children were randomly selected in the parent study, from whom 406 *E. coli* and 245 *Klebsiella* spp isolates were recovered and selected to have AST performed. The isolates were subjected to AST by disc diffusion to 5 µg of CIP (Oxoid, Hampshire, England) representing the fluoroquinolone antibiotic class. The isolates were also screened for ESBL production by the combined disc diffusion test and interpreted using the criteria from the Clinical and Laboratory Standards Institute (CLSI) [16]. *E. coli* ATCC 25922 and *E. coli* NCTC 13351 were used as negative and positive controls respectively for ESBL screening. *E. coli* or *Klebsiella* spp isolates with intermediate (22-25 mm) or resistant (≤21 mm) phenotype zone size interpretations for CIP were considered CIP non-susceptible [16].

Nested study
CIP MIC determination by E-test
In the nested study, MICs for CIP were determined by the E-test method on isolates that were CIP non-susceptible by disc diffusion per CLSI guidelines [16]. Bacterial colonies were suspended in 0.85% normal saline to a turbidity equivalent to 0.5 McFarland standard (bioMérieux, Inc, Durham, NC, United States). The bacterial suspension was inoculated on Mueller Hinton agar (Oxoid, Hants, United Kingdom) plates, and the CIP E-strips were placed at the center of the agar followed by incubation at 35 °C for 16 -18 h. Concentration ranges for MICs for E-test strips for CIP (0.002–32 µg/mL) (bioMérieux Marcy l'Etoile, France) and non-susceptibility interpreted according to 2021 CLSI guidelines [16]. MIC results were classified as follows: susceptible (≤ 0.25 µg/mL), intermediate (0.5 µg/mL), or resistant (≥ 1 µg/mL). *E. coli* ATCC 25922 was used as quality control for determining MICs by the E-test method.

DNA extraction and PMQR characterization
Genomic DNA was extracted using the boiling preparation method [46]. Extracted DNA was subjected to a series of single and multiplex PCR reactions to identify PMQR determinants: *qnrA, qnrB, qnrS, aac(6')-Ib, qepA, oqxA,* and *oqxB*. PCR reactions were performed using previously described primers and PCR conditions [47–49] (Table S2). All isolates positive for the *aac (6')-lb* gene were further analyzed to determine carriage of the (-cr) variant associated with CIP resistance [48]. The PCR products and known positive strains were digested with the restriction enzyme *Bst*CI (New England Biolabs, Ipswich, MA) to identify *aac (6')-lb-cr* which lacks the *Bst*CI restriction site present in CIP susceptible isolates as previously described [48]. The positive controls used in screening for PMQR genes were in-house isolates with

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confirmed target genes by whole genome sequencing and sequence analysis [32]. The identity of the amplified *qepA* gene was confirmed by amplicon sequencing of both the forward and reverse strands. PCR products positive for the *qepA* gene were purified using DNA Clean & Concentrator™-25 Kit (Zymo Research, Orange, CA, USA) and sequenced by the Sanger sequencing method using an ABI 3730 DNA analyzer. The consensus nucleotide sequences were analyzed and compared to available sequences deposited in the GenBank at National Center for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST) program (http://blast.ncbi.nlm.nih.gov). The sequences of the *qepA* gene were submitted to GenBank.

Statistical analysis

Fluoroquinolone resistance was defined by combining resistant and intermediate interpretive breakpoints for CIP. Risk factors for fluoroquinolone resistance previously associated with AMR from published data were chosen to test for association [50]. Patient characteristics including age, sex, hospital site, duration of hospitalization, antibiotic use at admission, HIV status, diagnosis at admission, and ESBL carriage were assessed. Poisson regression was used to determine prevalence ratios (PRs) and associated 95% confidence intervals (CIs) while Chi-square test was used to determine *p*-values. Associations were considered statistically significant at an alpha of 0.05. Analysis was performed in R software version 4.1.3.

Abbreviations

AMR	Antimicrobial Resistance
AST	Antimicrobial Susceptibility Testing
BLAST	Basic Local Alignment Search Tool
CIP	Ciprofloxacin
CLSI	Clinical and Laboratory Standard Institute
CMR	Centre for Microbiology Research
DNA	Deoxyribonucleic Acid
ESBL	Extended Spectrum Beta Lactamase
HCRH	Homabay County Referral Hospital
HIV	Human Immunodeficiency Virus
IQR	Interquantile range
KEMRI	Kenya Medical Research Institute
KTRH	Kisii Teaching and Referral Hospital
MGE	Mobile Genetic Elements
MIC	Minimum Inhibitory Concentration
NCBI	National Center for Biotechnology Information
PCR	Polymerase chain reaction
PMQR	Plasmid Mediated Quinolone Resistance
QRDR	Quinolone Resistance Determining Region
SSA	Sub-Saharan Africa
WHO	World Health Organization

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-023-02849-2.

Additional file 1: Table S1. Correlates of non-susceptibility to Ciprofloxacin.

Additional file 2: Table S2. List of the PCR primer pairs and expected PCR product sizes.

Authors' contributions

KK, PBP, and SK study conceptualization and design. KK, DR, TM, EM Laboratory analysis. JLW and PBP funding acquisition. SK, PBP, SM, and BS supervision. MDM, KDT, STB, data analysis, and curation. KK, PBP, and SK data interpretation and drafting of the manuscript. All authors reviewed, edited, and approved the final manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study will be available from the corresponding author ppav@uw.edu. The *qepA* gene sequences from 8 representative isolates have been submitted to GenBank (https://www.ncbi.nlm.nih.gov/genbank/) under assigned accession numbers: OP918677 (E66), OQ031499 (E49), OQ031500 (E79), OQ031501 (E80), OQ031502 (E24), OQ031503 (E28), OQ031504 (E40), and OQ031505 (E42).

Declarations

Ethics approval and consent to participate

This study was approved by the Scientific and Ethics Review Unit (SERU) of Kenya Medical Research Institute (KEMRI) (SSC No. 4127). The parent study was approved by Kenya Medical Research Institute (SERU 3086). All methods were performed in accordance with the relevant guidelines and regulations for clinical and laboratory research ethics. Informed written consent was provided by the caregivers or the child's legal guardian in their preferred language for the child to participate in this study. If a caregiver was not literate, information was read in the language of their choice and consent was obtained using a witnessed thumbprint.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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