# Multiple PMQR genes including the rare qepA 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 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 by disc diffusion and E-test. Ciprofloxacin non-susceptible isolates were screened for seven PMQR genes using multiplex PCR. Poisson regression was used to determine the association between carriage of ciprofloxacin non-susceptible isolates and patient characteristics.

**Results**: Of the 280 ciprofloxacin non-susceptible isolates: 188 *E. coli* and 92 *Klebsiella spp* isolates identified among 266 discharged children, 195 (68%) were ciprofloxacin-resistant (MIC ≥ 1µg/mL). Among these 195 isolates, 130 (67%) had high level ciprofloxacin minimum inhibitory concentrations (MICs) (≥32 µg/mL). Over 80% of the isolates had at least one PMQR gene identified: *aac(6')Ib-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')-Ib-cr* was the most predominant accounting for 20% of all the isolates. Ceftriaxone use during hospital admission and the presence of ESBL production were significantly associated with the carriage of ciprofloxacin non-susceptible *E. coli* and *Klebsiella* spp.

**Conclusion:** Ciprofloxacin 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.


# Background

Antimicrobial resistance (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* play a critical role in antimicrobial resistance 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 (MGEs), such as plasmids and transposons, via horizontal gene transfer [9]. Antibiotic resistance in commensal enteric bacteria, such as *Klebsiella pneumoniae* and *E. coli*, has been reported in 85–90% of 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 ciprofloxacin 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 MICs [15].

There is 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 ciprofloxacin non-susceptibility in *E. coli* and *Klebsiella* spp isolates from children being discharged from the hospital. In addition, we identified correlates of carriage of ciprofloxacin non-susceptible isolates. This in-depth analysis will help inform the burden of ciprofloxacin 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 (**Figure 1)**. Among the 568 children, 343 (60.4%) were discharged from Kisii Teaching and Referral Hospital (KTRH) and 103 (39.6%) were discharged from Homabay County Referral Hospital (HCRH), 348 (60%) were less than two years of age and 348 (61.3%) were female (**Table 1**). Prior to discharge, the median duration of hospitalization was 3 days [IQR 2,6 days] with 203 (35.7%) children being hospitalized for ≥ 5 days, 12 (2.1%) were 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 ciprofloxacin being prescribed to only 2 (0.9%) children in HCRH and none among children from KTRH. Children at KTRH 328 (95.6%) compared to HCRH 174, (88.4%) were more likely to be prescribed an antibiotic. Nearly half of the children, 266 (46.8%) had ciprofloxacin non-susceptible isolates (46.3% *E. coli* and 37.6% *Klebsiella* spp). Of the 83 children who had both *E. coli* and *Klebsiella* isolated, 14 (2.46%) had ciprofloxacin non-susceptibility in both isolates.

**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% |
| 2 to 4 | 121 | 35.3% | 73 | 32.4% | 194 | 34.2% |
| >5 | 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]** | | | | | | |
| Diarrhea | 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% |

| | Kisii | | Homa Bay | | Total | |
|---|---|---|---|---|---|---|
| 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)

**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 ciprofloxacin 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 ciprofloxacin 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 ciprofloxacin non-susceptible *E. coli* or *Klebsiella* spp isolate. Length of hospital stay was associated with ciprofloxacin non-susceptible *E. coli* and children with hospitalizations extending 4 or more days were nearly 40% more likely to have ciprofloxacin non-susceptible *E. coli* (PR 1.38 [95%CI 1.07, 1.78] p= 0.01). The use of either ciprofloxacin or ceftriaxone were equally associated with ciprofloxacin non-susceptible *Klebsiella* or *E. coli*. Children hospitalized for diarrhoea were 27% less likely to have a ciprofloxacin non-susceptible *E. coli* compared to those who did not present with diarrhea (PR 0.73 [95%CI 0.53, 1.0] p=0.03). Similar magnitudes of association for hospital length and diarrhea diagnosis were observed in *Klebsiella* isolates but were not statistically significant. (**Table S1)**

**Distribution of ciprofloxacin non-susceptible isolates.**

Among the 266 children included in this study, we isolated 188 and 92 ciprofloxacin-susceptible *E. coli* and *Klebsiella* spp, respectively, totaling 280 ciprofloxacin non-susceptible isolates. Of the 266 children, 14 had both a ciprofloxacin non-susceptible *E. coli* and *Klebsiella* spp isolated. Among the 92 *Klebsiella* spp, 86 were *Klebsiella pneumoniae* and 6 were *Klebsiella oxytoca* (**Figure 1)**.

**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 | 0 | 0 | 4 | 32 | 32 |
| *K. pneumoniae* | 1 | 10 | 23 | 23 | 2 | 1 | 2 | 24 | 2 | 32 |
| No. 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 MICs values for the 280 ciprofloxacin non-susceptible isolates (188 *E. coli* and 92 *Klebsiella* [86 *Klebsiella pneumoniae* and 6 *Klebsiella oxytoca*) 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 ≥32µg/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 and $MIC_{50}$ 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 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%). *qnrA* was not detected in any of the *E. coli* or *Klebsiella* spp isolates.

**Table 3: Distribution of PMQR determinants per organism**

| | *E. coli* | *K. oxytoca* | *K. pneumoniae* | Total |
|---|---|---|---|---|
| PMQR genes | N=188 | N=6 | N=86 | N=280 |
| *qnrB* | 20 (11%) | 2 (33%) | 45 (52%) | 67 (24%) |
| *qnrS* | 27 (14%) | 1 (17%) | 18 (21%) | 46 (16%) |
| *aac(6')-Ib-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%) |

The most predominant plasmid-mediated quinolone resistance gene was *aac-(6')-Ib* (167/280, 59%) identified in more than half of all fluoroquinolone non-susceptible isolates. *Klebsiella* spp had more *aac(6')-Ib* positive isolates with (78/89, 85%) *Klebsiella* compared to (89/188, 47%) of *E. coli* isolates. All isolates carrying the *aac(6')-Ib* gene were positive for the *cr* variant. *qepA* was only detected in (16/188, 9%) *E. coli* fluoroquinolone non-susceptible isolates (**Figure 2**). The o*qxAB* complex was the most dominant efflux pump detected (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: ACC.No:OP918677, and ACC.No OQ031499-OQ031505 (sequences awaiting processing by NCBI). Additional details (See **Additional file 4**)

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

| Isolate ID | Accession No. | 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 |

### 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 Klebsiella spp (0.71%) isolates. Co-carriage of *qnrB* with *acc (6') Ib-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 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') Ib-cr* found in 56/280 (20%). Additionally, *acc (6') Ib-cr* co-existed with a majority of PMQR genes in both *E. coli* and *Klebsiella* spp isolates (**Table 5**).

**Table 5: Co-carriage of PMQR determinants per organism**

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

# 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, correlates of ciprofloxacin 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 ciprofloxacin 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 ciprofloxacin in a majority of the ciprofloxacin non-susceptible isolates, which is disturbing due to the relationship between increasing MICs leading to fluoroquinolone non-susceptibility and fluoroquinolone treatment failure [16]. This is particularly important given that fluoroquinolones are recommended therapies for the treatment of enteric infections such as shigellosis and salmonellosis [17, 18].

Ciprofloxacin 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 [19]. 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 ciprofloxacin non-susceptibility carriage [20]. 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 [21, 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 ciprofloxacin non-susceptible 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 [24]. In this study, we detected six (*qnrB*, *qnrS, aac(6')Ib-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 [25–29]. Other PMQR determinants that have been identified in Kenya are *aac(6')-Ib-cr*, *qnrB*, *qnrS* in *E. coli* [30, 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 [34–38]. This is particularly worrying due to their potential transfer of these genetic determinants through MGEs 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 [39]. The aminoglycoside acetyltransferase enzymes have not only been associated with reduced susceptibility to fluoroquinolones but also to aminoglycosides, thus limiting effective antibiotic treatment [40]. *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, 41]. The *qepA* gene, one of the most recently identified PMQR determinants, has been associated with decreased susceptibility to fluoroquinolones and increased MIC levels [24]. 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%) [27, 29]. 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 [42]. 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')Ib-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') Ib-cr* (20%) was found to be higher compared to findings from previous studies in SSA [43]. 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 [20]. This co-carriage could be attributed to multiple plasmids carrying the different *qnr* genes within the same genetic environment as has been previously demonstrated [44]. Notably, 12 isolates with the rare *qepA* co-existed with other PMQR genes (*qnrS* or *aac(6')Ib-cr*) which was consistent with findings from other previous studies [45, 46]. 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 FQ 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. Ciprofloxacin 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 ciprofloxacin 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. Being a cross-sectional study, we were unable to determine when AMR was acquired, either at the community level or hospital-acquired. Having analyzed isolates at discharge, this may not reflect the resistance at admission (which is when treatment decisions need to be made) and therefore unable to tease out whether children had resistance at admission or whether it developed during the hospitalization.

# Conclusion

This study detected multiple PMQR genes and the first report of the *qepA* gene among ciprofloxacin non-susceptible clinical *E. coli* and *Klebsiella* spp. The study observed high levels of ciprofloxacin 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 [47] 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 Kisii Teaching and Referral Hospital (KTRH) and Homabay County Referral Hospital (HCRH) aged between 1–59 months were recruited in the parent study [47] 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 [47].

### Parent trial

### Bacterial isolation, identification, and Antimicrobial Susceptibility Testing.

After laboratory culture, *E. coli* and *Klebsiella* spp isolates were identified and AST was performed by disc diffusion as previously described [48]. 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 hours within 24 hours of specimen collection time. Lactose fermenting colonies suspected to be *Escherichia 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 5µg of ciprofloxacin (Oxoid, Hampshire, England) representing the fluoroquinolone antibiotic class. The isolates were also screened for extended-spectrum beta-lactamase (ESBL) production by the combined disc diffusion test [49] and interpreted using the criteria from the Clinical and Laboratory Standards Institute (CLSI) [50]. *E. coli* ATCC 25922 and ECO NCTC 13351 were used as negative and positive controls respectively for ESBL screening. *E. coli* or *Klebsiella* spp isolates with intermediate (22-25mm) or resistant (≤ 21mm) phenotype zone size interpretations for ciprofloxacin were considered ciprofloxacin non-susceptible [50].

### Nested study.

### Ciprofloxacin MIC determination by E-test.

In the nested study, MICs for ciprofloxacin were determined by the E-test method on isolates that were ciprofloxacin non-susceptible by disc diffusion per CLSI guidelines [50]. 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 ciprofloxacin E-strips were placed at the center of the agar followed by incubation at 35°C for 16–18 hours. Concentration ranges for MICs for E-test strips for ciprofloxacin CI (0.002-32 µg/mL) (bioMérieux Marcy l'Etoile, France) and non-susceptibility interpreted according to 2021 CLSI guidelines [51]. 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 [52]. 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 [53–55] (**Table S2**). All isolates positive for the *aac (6')-Ib* gene were further analyzed to determine carriage of the (-cr) variant associated with ciprofloxacin resistance [56]. The PCR products and known positive strains were digested with the restriction enzyme *Bst*CI (New England Biolabs, Ipswich, MA) to identify *aac (6')-Ib-cr* which lacks the *Bst*CI restriction site present in ciprofloxacin susceptible isolates as previously described [56]. The positive controls used in screening for PMQR genes were in-house isolates with confirmed target genes by whole genome sequencing and sequence analysis [32, 57]. 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$^{TM}$-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 interpretative breakpoints for ciprofloxacin. Risk factors for fluoroquinolone resistance previously associated with AMR from published data were chosen to test for association [58]. 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

AME Aminoglycoside Modifying Enzyme

AMR Antimicrobial Resistance

AST Antimicrobial Susceptibility Testing

ESBL Extended Spectrum Beta Lactamase

CLSI Clinical and Laboratory Standard Institute

CMR Centre for Microbiology Research

DNA Deoxyribonucleic Acid

FQ Fluoroquinolone

KEMRI Kenya Medical Research Institute

MDR Multidrug Resistance

MGE Mobile Genetic Elements

MIC Minimum Inhibitory Concentration

PID Patient Identifier

PMQR Plasmid Mediated Quinolone Resistance

QRDR Quinolone Resistance Determining Region

# 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.

### Availability of data and material

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).

### Competing interests

The authors did not provide a conflict of interest statement.


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### Authors' contribution

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.


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## Figures

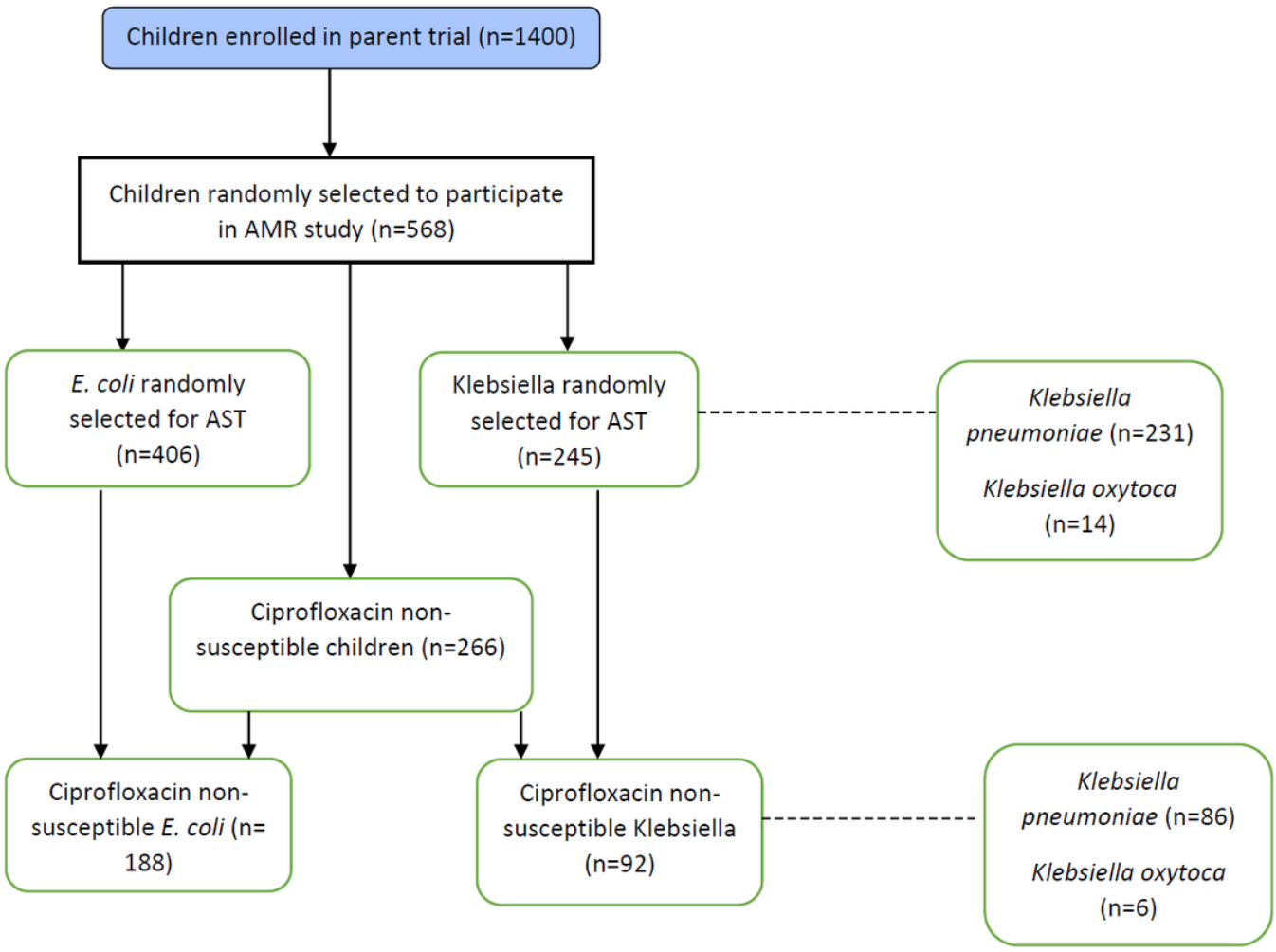


**Figure 1**

Flowchart of participants and isolates

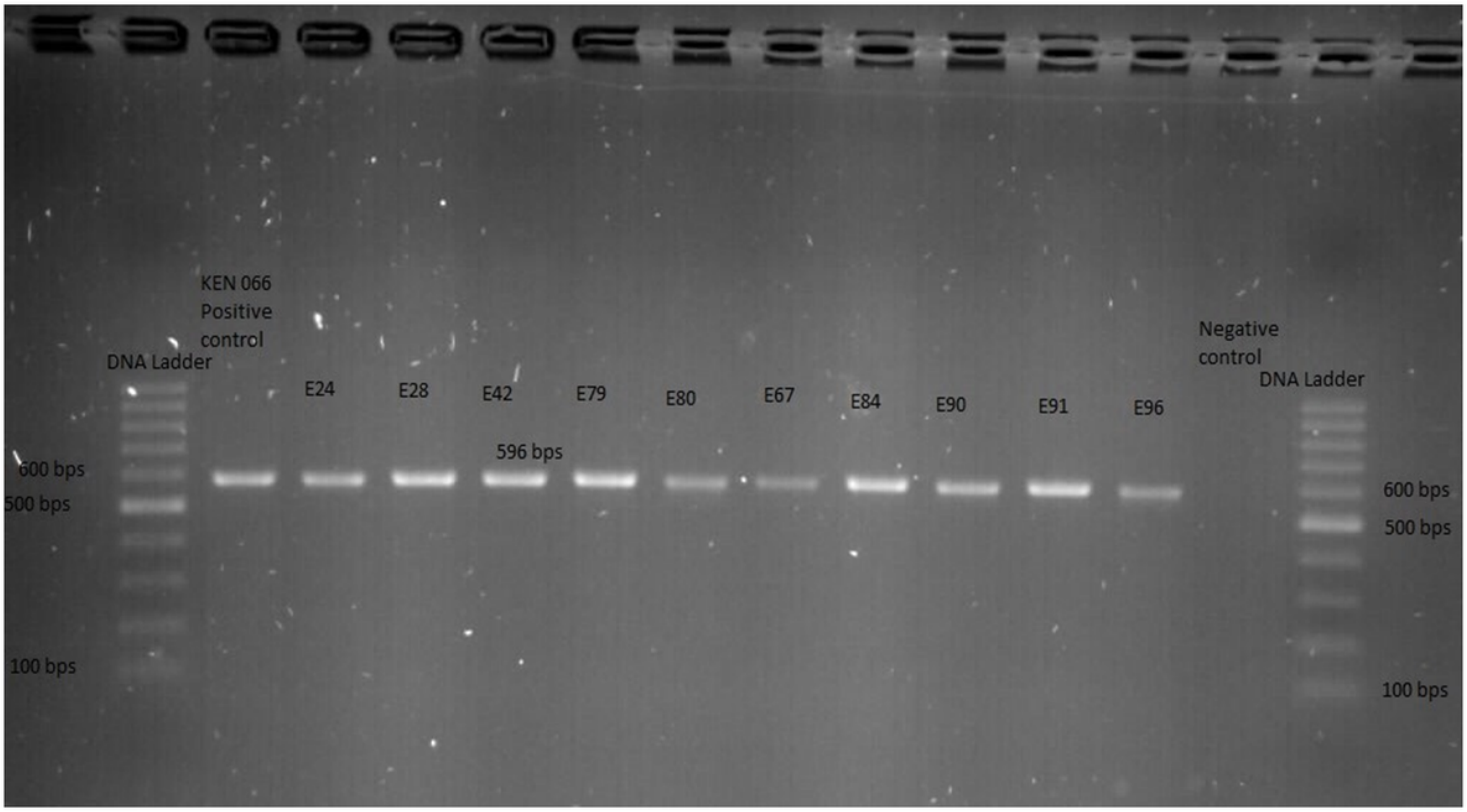


**Figure 2**

*E. coli qepA*gel image

Cropped gel picture showing the 596 bp amplicons for 10 *E.coli* isolates positive for the *qepA* gene. The full-length gel image is presented in Supplementary **Figure S2-1**

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- BMCFQAdditionalfile1.pdf
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