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RESEARCH ARTICLE

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Quinolone resistance in *Escherichia coli* from Accra, Ghana

Sreela S Namboodiri^{1,3}, Japheth A Opintan², Rebecca S Lijek^{1,4}, Mercy J Newman², Iruka N Okeke^{1*}

Abstract

Background: Antimicrobial resistance is under-documented and commensal *Escherichia coli* can be used as indicator organisms to study the resistance in the community. We sought to determine the prevalence of resistance to broad-spectrum antimicrobials with particular focus on the quinolones, which have recently been introduced in parts of Africa, including Ghana.

Results: Forty (13.7%) of 293 *E. coli* isolates evaluated were nalidixic acid-resistant. Thirteen (52%) of 2006 and 2007 isolates and 10 (66.7%) of 2008 isolates were also resistant to ciprofloxacin. All but one of the quinolone-resistant isolates were resistant to three or more other antimicrobial classes. Sequencing the quinolone-resistance determining regions of *gyrA* and *parC*, which encode quinolone targets, revealed that 28 quinolone-resistant *E. coli* harboured a substitution at position 83 of the *gyrA* gene product and 20 of these isolates had other *gyrA* and/or *parC* substitutions. Horizontally-acquired quinolone-resistance genes *qnrB1*, *qnrB2*, *qnrS1* or *qepA* were detected in 12 of the isolates. In spite of considerable overall diversity among *E. coli* from Ghana, as evaluated by multilocus sequence typing, 15 quinolone-resistant *E. coli* belonged to sequence type complex 10. Five of these isolates carried *qnrS1* alleles.

Conclusions: Quinolone-resistant *E. coli* are commonly present in the faecal flora of Accra residents. The isolates have evolved resistance through multiple mechanisms and belong to very few lineages, suggesting clonal expansion. Containment strategies to limit the spread of quinolone-resistant *E. coli* need to be deployed to conserve quinolone effectiveness and promote alternatives to their use.

Background

Following emergence of resistance to inexpensive broad-spectrum antimicrobials across much of Africa, quinolone antibacterials have recently been introduced and are widely used. West African studies that sought quinolone resistance in commensal or diarrhoeagenic *Escherichia coli* before 2004 reported no or very low incidences of resistance to nalidixic acid and the fluor-quinolones [1-4]. Thus, available data suggests that resistance to the quinolones was rare in West Africa until the first decade of the 21st century. More recent anecdotal reports and surveillance studies point to emergence of quinolone resistance among enteric pathogens and faecal enteric bacteria in Ghana and elsewhere in West Africa [5-8]. In a study by Nys et al. (2004) faecal isolates of adult volunteers in eight

different countries were assessed for susceptibility to antimicrobials in the same laboratory [8]. Resistance to broad spectrum first-generation antibiotics was common and ciprofloxacin resistance was found to be slowly emerging in Asian, South American and African countries, including Ghana [8]. Newman et al. (2004) collected 5099 clinical bacterial isolates (1105 of which were *E. coli*) from nine of the ten regions in Ghana and tested them for antimicrobial susceptibility. They found that over 70% of the isolates were resistant to tetracycline, trimethoprim-sulphamethoxazole, ampicillin and chloramphenicol and reported that 11% of the isolates were ciprofloxacin-resistant [7].

Quinolones inhibit the activity of bacterial DNA gyrase and DNA topoisomerase enzymes, which are essential for replication. Single nucleotide polymorphisms (SNPs) in the quinolone resistance determining regions

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(QRDR) of *gyrA* and *parC*, the two genes that encode DNA gyrase and topoisomerase IV respectively, can lead to conformational changes in these enzymes that cause them to block quinolones from binding to the DNA- substrate complex, yet still preserve their enzymatic function [9]. In *Escherichia coli* and related Gram-negative bacteria, DNA gyrase is the first target for fluoroquinolones. If *gyrA* has resistance-conferring mutations, the primary target of fluoroquinolone switches from DNA gyrase to topoisomerase IV [10,11]. Studies from other parts of the world have found that resistance-conferring mutations are typically selected in *gyrA* first, and then *parC*.

Although mutations in the QRDR of *gyrA* and *parC* are the most commonly documented resistance mechanisms, resistance has also been known to be conferred by mutations in the second topoisomerase gene, *parE*. Another mechanism of quinolone resistance relies on upregulation of efflux pumps, which export quinolones and other antimicrobials out of the bacterial cell. For example, mutations in the gene encoding a repressor of the *acrAB* pump genes, *acrR*, are associated with quinolone resistance [12]. Quinolone resistance can also be acquired horizontally through transferable quinolone resistance (*qnr*) or other DNA. The *Qnr* gene product inhibits quinolones binding to target proteins [13]. Other horizontally acquired quinolone resistance genes include *aac(6)-Ib*, encoding a fluoroquinolone acetylating enzyme, as well as *qepA* and *oqxAB*, which encode horizontally transmitted efflux pumps [14-16].

Resistance to the quinolones often emerges at low-levels by acquisition of an initial resistance-conferring mutation or gene. Acquisition of subsequent mutations leads to higher levels of resistance to the first-generation quinolone, nalidixic acid and a broadening of the resistance spectrum to include second-generation quinolones (first-generation fluoroquinolones) such as ciprofloxacin, followed by newer second- and third-generation fluoroquinolones [17].

Although multiple mechanisms of quinolone resistance have been reported from other continents, there are few data from sub-Saharan Africa on the molecular basis for quinolone resistance. We performed antimicrobial susceptibility testing on fecal *E. coli* isolates from Accra, Ghana in 2006, 2007 and 2008. We identified isolates that were resistant to nalidixic acid and screened these strains for mutations in the QRDR of *gyrA* and *parC* as well as horizontally-acquired quinolone-resistance genes. In order to gain some insight into resistance dissemination, we also studied inter-strain relatedness among quinolone-resistant *E. coli* isolates by multilocus sequence typing.

Results

Resistance to commonly used antimicrobials is high and resistance to the quinolones was detected

In 2006, 2007 and 2008 respectively, 156, 78 and 101 stool specimens were collected. A total of 293 *Escherichia coli* isolates were recovered from culture of the 335 stool specimens. Consistent with the results of recent studies from West African countries, including Ghana [1,7,8], 50-90% of the *E. coli* isolates were resistant to the broad-spectrum antimicrobials ampicillin, streptomycin, sulphonamides, tetracycline and trimethoprim (Figure 1). Resistance to chloramphenicol was less common but was seen in 30-41% of the isolates. The proportions of isolates resistant to most agents were comparable between 2006 and 2007. However, the proportion of isolates resistant to each antimicrobial in 2008 was significantly greater than those seen in 2006, for all agents ($p < 0.05$) (Figure 1).

As illustrated in Figure 1 in 2006 and 2007, we recorded resistance rates to nalidixic acid of 12.3% but by 2008, 15 (18.2%) of isolates were nalidixic acid resistant. Ciprofloxacin-resistant isolates represented 7 (5.4%) and 6 (7.7%) of the total number of isolates in 2006 and 2007 respectively. In 2008, 10 (9.9%) of the isolates were fluoroquinolone resistant. Thus, in 2006 and 2007, 13 (52%) quinolone-resistant *E. coli* isolates were ciprofloxacin resistant but in 2008, 10 (67%) of the quinolone-resistant *E. coli* were resistant to ciprofloxacin (Figure 1). Although the numbers were too small to attain statistical significance, organisms with higher nalidixic acid MICs were recovered more commonly in 2008 than in 2006 (Table 1).

Quinolone resistance was almost always seen in multiply-resistant *E. coli*. As shown in Table 1, all quinolone-resistant *E. coli* (QREC) were resistant to at least one other antimicrobial and all but three of the QREC

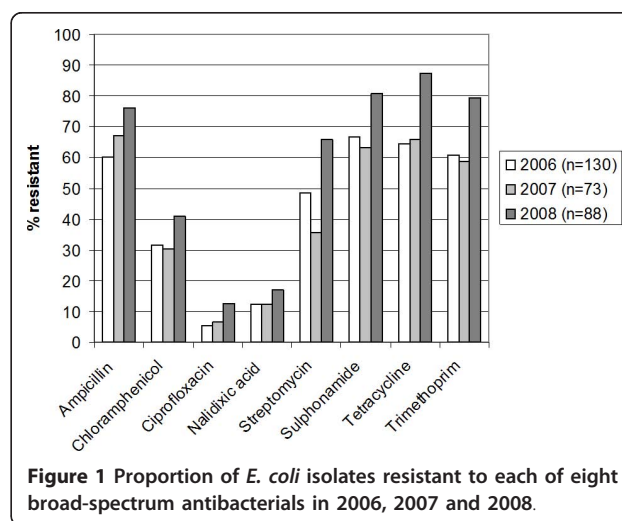


Figure 1 Proportion of *E. coli* isolates resistant to each of eight broad-spectrum antibacterials in 2006, 2007 and 2008.

Table 1 Antimicrobial susceptibility, QRDR mutations and sequence types of quinolone-resistant *E. coli* isolates

Strains	Nali-dixic acid MIC mg/L	Resistance pattern*	QRDR mutations		Horizontally acquired quinolone resistance genes	Allelic profile							Sequence type (ST)	ST complex
			Δ GyrA	Δ ParC		<i>adk</i>	<i>fumC</i>	<i>gyrB</i>	<i>icd</i>	<i>mdh</i>	<i>purA</i>	<i>recA</i>		
2006														
06/073	64	AC NSLTR	none	none	none	6	19	15	16	9	8	7	443	205
06/079	128	AC NSLTR	none	none	none	6	65	2	25	5	5	2	1466	none
06/137	128	AC NSLTR	none	none	none	6	65	2	25	5	5	2	1466	none
06/065	128	A NSLTR	none	none	<i>qnrS1</i>	6	7	5	1	8	18	2	206	206
06/036	256	AC NSLTR	none	none	none	6	8	4	1	9	48	7	210	none
06/027	64	C N	S83L	none	<i>qnrB2</i>	10	11	4	8	8	8	2	10	10
06/045	64	A NSLTR	S83L	none	none	10	174	4	8	8	8	2	1286	10
06/056	128	A NSLTR	S83L	none	none	112	11	5	12	8	8	86	542	none
06/068	128	AC NSLTR	S83L	none	none	76	43	19	37	30	1	2	1465	none
06/151	>1024	ACPN SLTR	S83L, D87N	none	none	43	41	15	18	11	7	6	101	101
06/154	>1024	ACPN TR	S83L, D87N	none	<i>qnrB1</i>	43	41	15	18	11	7	6	101	101
06/030	>1024	ACPN LTR	S83L, D87N	S80I	<i>qnrS1</i>	10	11	4	8	8	13	73	617	10
06/110	>1024	ACPN SLTR	S83L, D87N	S80I	none	92	4	87	96	70	58	2	648	none
06/148	>1024	A PNSLTR	S83L, D87N	S80I	none	92	4	87	96	70	58	2	648	none
06/026	>1024	ACPN SLTR	S83L, D87N	S80I, N105S	none	56	6	22	16	11	1	7	455	None
06/112	>1024	ACPN LTR	S83L, D87N	S80I, E84K	none	6	29	32	16	11	8	44	156	156
2007														
					none									
07/16a	256	A PNSLT	none	none	none	6	95	4	18	11	7	14	1304	none
07/34	128	AC N LTR	none	none	none	10	11	4	12	7	8	2	1467	none
07/12	64	NSLT	S83L	none	none	10	11	4	8	8	8	2	10	10
07/37	128	A N L R	S83L	T66I	none	10	11	4	8	8	8	2	10	10
07/SHA	>1024	A PNSLTR	S83L, D87N	none	none	6	11	95	104	8	7	2	450	none
07/375	>1024	A PNSLTR	S83L, D87N	S80I	<i>qepA</i>	10	11	4	8	8	8	2	10	10
07/337	>1024	A PN LTR	S83L, D87N	S80I	none	10	11	4	8	8	8	2	10	10
07/282	>1024	ACPN SLTR	S83L, D87N	E84K	<i>qepA</i>	6	29	32	16	11	8	44	156	156
07/24A	>1024	ACPN SLTR	S83L, D87N	S80I, E84G	<i>qnrB1</i>	85	88	78	29	59	58	62	354	354
2008														
08/54	64	AC NSLTR	none	none	none	6	99	22	1	8	7	2	1479	206
08/23	64	ACPN SLTR	none	none	none	10	29	4	8	8	8	44	1473	156
08/43	128	A NSLTR	none	none	none	6	7	14	1	8	18	2	1471	1206
08/33	>1024	ACPN SLTR	none	none	<i>qnrS1</i>	6	11	14	8	8	6	6	1469	None
08/91	>1024	ACPN SLTR	none	none	none	10	11	4	1	8	9	2	227	10
08/101	64	A NSLTR	S83A	none	<i>qnrS1</i>	10	11	4	1	8	9	2	227	10
08/78	128	ACPN SLTR	S83L	none	<i>qnrS1</i>	10	11	4	8	8	8	2	10	10

Table 1 Antimicrobial susceptibility, QRDR mutations and sequence types of quinolone-resistant *E. coli* isolates (Continued)

08/90	256	ACPNSLTR	S83L, D87N	S80I	none	6	4	12	1	20	18	7	410	23
08/11	>1024	A PNSLTR	S83L, D87N	S80I	none	6	6	5	26	11	8	6	1468	none
08/26	>1024	A PN LTR	S83L, D87N	S80I	<i>qnrS1</i>	10	11	4	8	8	8	2	10	10
08/87	>1024	A NSLTR	S83L, D87N	S80I	none	10	11	4	8	8	8	2	10	10
08/84	>1024	A NSLTR	S83L, D87N	S80I	<i>qnrS1</i>	10	11	4	8	8	8	2	10	10
08/93	>1024	A PNSLTR	S83L, D87N	S80I	none	10	11	4	8	8	8	2	10	10
08/17	>1024	ACPNSLTR	S83L, D87N	S80I, E84V	none	53	40	47	13	36	28	29	131	none
08/39	>1024	ACPNSLTR	S83L, D87N	S80I, A108V	none	92	40	87	96	70	8	29	1470	none

*A = Ampicillin, C = Chloramphenicol, P = Ciprofloxacin, N = Nalidixic acid, S = Streptomycin, L = Sulphonamide, T = Tetracycline R = Trimethoprim.

isolates were resistant to four or more non-quinolone antibacterials. Most QREC demonstrated high-level resistance to nalidixic acid with 21 of 40 of the QREC isolates showing a nalidixic acid MIC that exceeded 1024 mg/L. Among 2006 isolates, low-level resistance was more common, with the MIC₅₀ in that year being 128 mg/L. In both 2007 and 2008, the MIC₅₀ was >1024 mg/L.

Quinolone resistant *E. coli* predominantly harbour mutations in *gyrA*, *parC* or both

Increasing nalidixic acid MICs, accompanied by resistance to fluoroquinolones is often due to the acquisition of multiple mutations in quinolone targets. We sequenced the quinolone-resistance determining regions (QRDRs) of *gyrA* and *parC* in the 40 QREC isolates. As shown in Table 1, 28 (70%) of the quinolone-resistant isolates had at least one non-synonymous substitution in the QRDR of *gyrA* and 18 of these isolates also had one or more non-synonymous mutations in *parC*. Twenty-seven of the 28 isolates with at least one mutation in *gyrA* had a serine to leucine substitution at position 83, one of the most commonly documented resistance conferring mutations [10]. Twenty of these isolates also harboured the frequently documented aspartic acid to asparagine substitution at position 87 and all of these isolates had a nalidixic acid MIC of at least 256 mg/L. Eighteen of them were resistant to ciprofloxacin as well as nalidixic acid.

Eighteen QREC isolates had non-synonymous mutations in the QRDR of *parC* with a serine to isoleucine substitution at position 80, present in 16 strains, being the most common substitution (Table 1). The 2007 isolate with a Thr66Ile substitution in ParC had a single

GyrA substitution, Ser83Leu. All other isolates with ParC substitutions also had Ser83Leu and Asp87Asn substitutions in GyrA. Five isolates had more than one ParC substitution. Thr66Ile and Asn105Ser substitutions in ParC, seen in two isolates in this study, have not previously been described in *E. coli* but Thr66Ile has been seen in *Salmonella enterica* serovars Heidelberg and Mbandaka [18] (Table 1). Both substitutions occur in strains with other previously described non-synonymous polymorphisms in *parC* and *gyrA*. In each case, the level and spectrum of resistance seen is not significantly greater than that for isolates that lack the novel substitution. Therefore, it is not possible to conclude that either substitution confers additional resistance although confirmation of this assumption, or otherwise, can only be made in isogenic strains.

Multiple horizontally-transmitted quinolone resistance genes were detected among *E. coli* from Accra

We used PCR to screen for *qnrA*, *qnrB*, *qnrS* and *qepA* genes and confirmed all amplicons by sequencing. Of the 40 strains evaluated twelve carried one horizontally acquired quinolone resistance gene. These were *qnrB1* (2 isolates), *qnrB2* (1 isolate), *qnrS1* (7 isolates) and *qepA* (2 isolates). In two isolates, without mutations in *gyrA* and *parC* QRDRs, horizontally-acquired resistance genes could account for the resistance seen. However, in the vast majority of cases, horizontally acquired resistance was seen in combination with QRDR mutations.

Quinolone-resistant *E. coli* from Accra are over-represented among multi-locus sequence type 10

We hypothesized that clonal expansion might account, at least in part, for the rise in resistance seen in the

course of the study. To test this hypothesis, we subjected all the 40 QREC isolates to multi-locus sequence typing by the scheme of Wirth et al [19] and deposited their allelic profiles in the database at <http://www.mlst.net>. We identified 30 Sequence Types (STs) among 40 QREC isolates from Ghana (0.75 STs per strain). As shown in Figure 2, quinolone resistance is seen in diverse lineages that have been detected in Ghana. STs that were recovered more than once among the QREC included ST10 (9 isolates) as well as STs101, 156, 227, 648 and 1466 (2 isolates each) (Table 1). Although there were 10 QREC STs that were identified for the first time in this study (reflecting the low proportion of strains from West Africa in the database), only one of these (1466) was seen more than once among QREC (Figure 2, Table 1). Three others were related to STs that were also seen among QREC - ST1471 was a single-locus variant of ST206, and STs1286 and 1467 were respectively single- and double-locus variants of ST10.

Horizontally-transmitted quinolone resistance determinants were expectedly detected in strains belonging to multiple STs. However *qnrS1* alleles were in all but two cases detected among strains belonging to the ST10 complex.

Nine of the 40 QREC isolates obtained in this study belonged to ST10, in contrast to 10 of 125 other *E. coli* from Ghana in the database ($p = 0.02$, Fisher's exact test). Moreover six other QREC isolates were single- or double- locus variants of ST10. Thus ST10 appeared to be over-represented among QREC isolates from Ghana. Because most of the isolates from Ghana were deposited in the database two to four years in advance of our own study, we sequence typed eight non-QREC isolates selected at random from our 2008 isolates. All eight belonged to different sequence types (10, 349, 541, 1474, 1475, 1476, 1477 and 1478), five of the eight sequence types were novel, and only one 2008 non-QREC strain was an ST10 isolate. Therefore our data suggest that

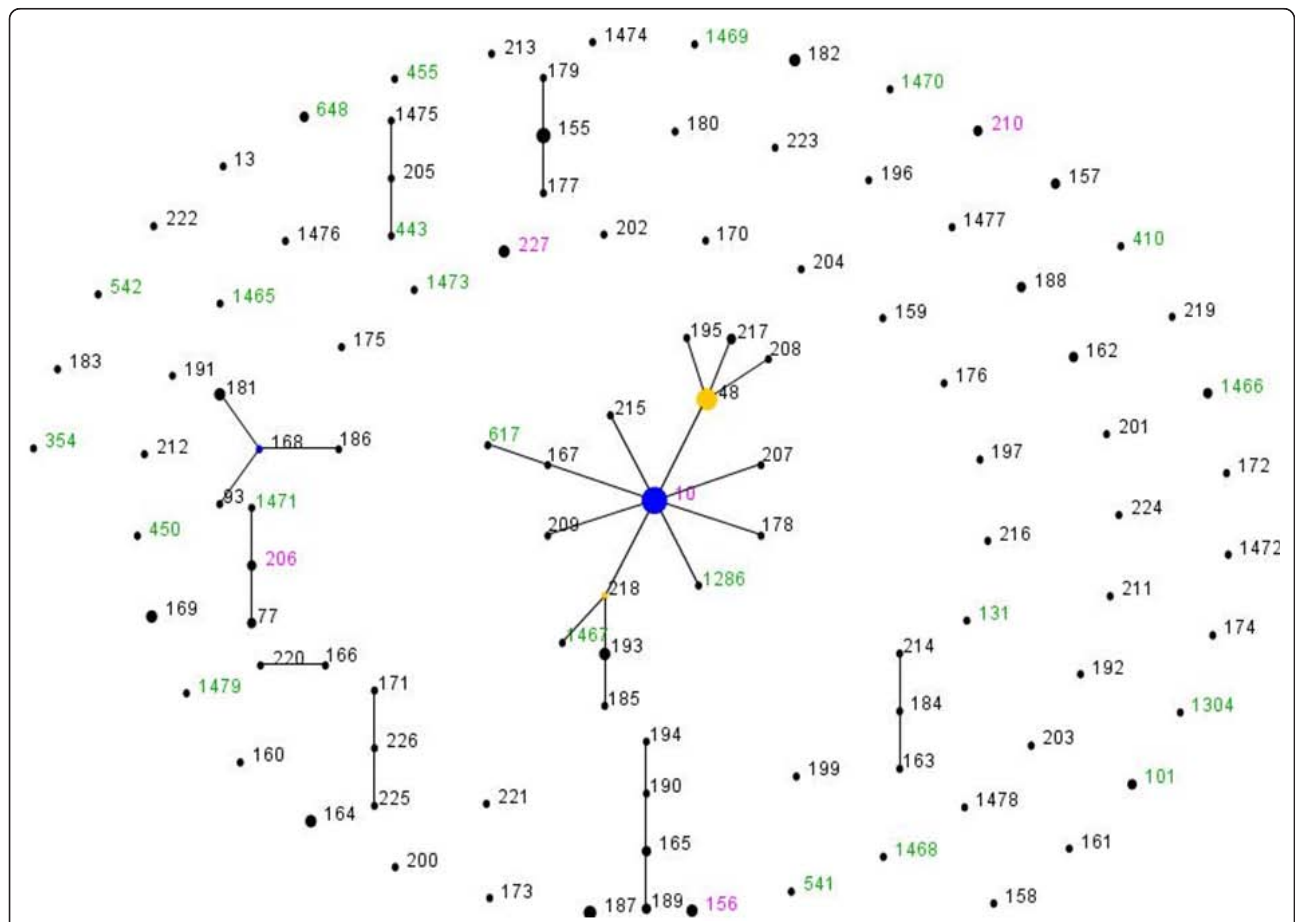


Figure 2 eBURST output for 165 *E. coli* isolates in the <http://www.mlst.net> database that were isolated in Ghana, including 48 isolates sequence-typed in this study. Each ST is marked as a dot or node. The size of the node is proportional to the number of isolates contained in that ST. Blue nodes represent predicted founder STs and sub-founders are indicated in yellow. All other STs marked as black dots. STs annotated in green are comprised of quinolone-resistant strains only and those written in pink contain quinolone-sensitive and quinolone-resistant isolates.

ST10-complex QREC may represent a successful quinolone-resistant lineage.

Discussion

Evolution of reduced susceptibility to the quinolones is causing concern following rapidly rising rates of fluoroquinolone-resistant *E. coli* in many parts of the world [20]. In African countries with a high infectious disease burden, formal and informal health systems depend heavily on broad spectrum orally-administrable antibacterials. In this study, we found that most commensal *E. coli* isolates are resistant to ampicillin, sulphonamides, tetracycline and trimethoprim, as well as streptomycin, which have been used to treat actual and supposed bacterial infections in Ghana for over four decades, and that resistance to these agents is increasing with time. We also found that about a third of isolates were resistant to chloramphenicol. Fluoroquinolone antimicrobials have been recently introduced as an effective alternative to older antibacterials that have been compromised by resistance. However, although resistance rates were markedly lower for this class of drugs, we also found that quinolone resistance was increasingly common among fecal *E. coli* in this study.

We determined that 12-18% of fecal *E. coli* isolated from healthy individuals in Accra in 2006, 2007 and 2008 are quinolone resistant. Twenty-three of the 40 QREC isolated were resistant to the fluoroquinolone ciprofloxacin. Ciprofloxacin-resistant QREC, showing high-level nalidixic acid resistance, were more commonly isolated in 2008 than in 2006 and 2007. Strains with one or no mutations in *gyrA* were typically ciprofloxacin sensitive. However most isolates had accumulated a second *gyrA* mutation and/or mutations in *parC* and were fluoroquinolone resistant. The QRDR polymorphisms most commonly detected in this study are those most frequently reported in the literature [10]. As has been validated experimentally in isogenic strains, high-level nalidixic acid resistance and fluoroquinolone resistance in isolates in this study was associated with *parC* substitutions in strains also harbouring substitutions in *gyrA* [17]. However, *gyrA* and *parC* mutations did not absolutely correlate with nalidixic acid MICs, partly due to horizontally-acquired quinolone-resistance genes. We sought *qnrA*, *qnrB*, *qnrS* and *qepA* genes by PCR and confirmed all amplicons by sequencing. We found that two isolates without mutations in the QRDRs of *gyrA* and *parC*, as well as ten isolates with QRDR mutations carried a *qnrS1*, a *qnrB* or a *qepA* allele. The presence of horizontally-acquired genes accounted in part for elevated nalidixic acid MICs in strains that harboured these genes, but not completely. It is therefore possible that other resistance mechanisms, such as ParE polymorphisms, other horizontally

acquired resistance genes (such as *oqxAB* and *aac(6)-Ib* for example), over-active efflux, or even novel mechanisms are present in some of the isolates.

Resistance patterns in pathogens often mirror those in commensals. This is borne out by our recent documentation of quinolone resistance in *Vibrio cholerae* isolates recovered in the same time frame as the *E. coli* strains presented in this report [21]. Fifteen of the 40 QREC isolates identified in this study belonged to ST10, or were single- or double-locus variants of this ST, pointing to the possibility of clonal expansion. ST10-complex strains were isolated in all three years and therefore over-representation of these STs in our sample cannot be explained by short-term, localized clustering. There are four major *E. coli* phylogenetic clades: ECOR A, B1, B2 and D. Few studies have looked at the geographical variance in the distribution of these groups but overall, QREC from Ghana were predominantly drawn from ECOR group A. Of the STs identified in this study that are classified into ECOR clades at the *E. coli* MLST database, ST10 complex (14 isolates) belong to ECOR group A, ST131 (1 isolate) to ECOR B2, STs101 and 410 (3 isolates) to ECOR B1 and STs 156, 206 and 210 (4 isolates) are hybrids of ECOR A and B1, that is AxB1. Available data appear to suggest that ECOR A strains are highly prevalent in Africa, compared to some other world regions [22]. However, when we compared the sequence types of quinolone-resistant and -susceptible strains from Ghana only, we still found that resistant strains were over-represented in the ST10 complex. Pandemic clonal expansion of some QREC lineages has been reported in the literature [23-28]. For example, ST131 is a globally disseminated multi-resistant clone and was detected once among the QREC in this study. Recent reports suggest that isolates from Europe and North America that belong to ST10- or ST131- clonal complexes may be less likely to carry virulence factors for invasive disease, but more likely to be fluoroquinolone resistant [24-28]. However it is equally likely that mutations to fluoroquinolone resistance are more likely to be stably inherited in a specific genetic background. Our own data also appear to suggest that, although horizontally acquired, *qnrS1* is associated with ST10 complex.

A recent paper by Davidson et al suggests that the anti-malarial chloroquine may select for fluoroquinolone-resistant fecal bacteria in malaria endemic areas and proposes that chloroquine-mediated selection accounts for high levels of QREC in fecal flora in villages in South America [29]. However, data from within Africa (where chloroquine has seen heaviest use) to support this hypothesis are currently lacking and evidence from elsewhere supports a link between quinolone resistance rates in *E. coli* and fluoroquinolone consumption at population levels [30,31]. Our own data suggest that even if

resistance was present at low levels prior to the introduction of the quinolones, an upsurge in resistance may reflect a selective advantage that came with quinolone introduction. This is particularly likely for Ghana where artemisinin combination therapies have recently been introduced to replace chloroquine. Furthermore, because almost all quinolone resistant-*E. coli* were multiply resistant, selective pressure from other, even more commonly applied, antimicrobials will help to maintain the quinolone-resistant clonal groups we identified in this study.

Conclusion

Fluoroquinolones, largely ciprofloxacin, were introduced very recently to Ghana with high expectations. This study demonstrates that resistance to these drugs is already common and occurs through multiple mechanisms, suggesting that heavy use of these valuable drugs may rapidly obliterate their usefulness. In addition to the impact that the emergence and dissemination of quinolone resistant bacteria may have on the use of fluoroquinolone antibacterials, we found that QREC were almost invariably resistant to multiple antimicrobials. This is worrisome because it means that, if the commensal flora is reflective of resistance profiles in pathogens, there may be few low-cost alternatives for managing infections due to Gram-negative enteric organisms. Additionally, horizontally-acquired resistance to the quinolones, and presumably other agents may be present on mobile elements that could be transmitted to pathogens. Recent calls for antimicrobial development have spotlighted hospital pathogens and Gram-positive community-acquired pathogens such as Staphylococci [32]. Our data suggest that there is also a pressing need for orally administrable drugs with activity against Gram-negative organisms, which can be used to manage community enteric infections in Ghana and other parts of Africa. Additionally, known strategies for containing antimicrobial resistance need to be more rigorously applied [33-35].

Methods

Strains

This study was approved by the Institutional Review Board of the University of Ghana Medical School. *E. coli* isolates were recovered from stool specimens collected from consenting, apparently healthy individuals who presented for medical check-ups at the Korle-Bu Teaching Hospital and the Microbiology Department of the University of Ghana Medical School. Colonies with a typical *E. coli* morphology on MacConkey agar were subjected to biochemical testing, and where this was inconclusive, by 16 S amplification and sequencing [36]. Colonies from the same specimen with identical biochemical and susceptibility profiles were treated as identical strains.

Antimicrobial susceptibility testing

Each isolate was tested for susceptibility to eight antimicrobials using the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) disc diffusion method [37]. Antimicrobial discs and control strain *E. coli* ATCC 35218 were obtained from Remel. The antimicrobial discs used contained ampicillin (10 µg), streptomycin (10 µg), trimethoprim (5 µg), tetracycline (30 µg), nalidixic acid (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg) and sulphonamide (300 µg). Inhibition zone diameters were interpreted in accordance with CLSI guidelines with WHONET software version 5.3 [38]. Minimum inhibitory concentrations (MICs) to nalidixic acid were measured using the agar dilution technique on Mueller-Hinton agar as recommended by the CLSI and using *E. coli* ATCC 35218 as control [39].

Mutational analysis of the Quinolone-Resistance Determining Regions of *gyrA* and *parC*

DNA was extracted from each quinolone-resistant isolate, using the Promega Wizard genomic extraction kit. The QRDR of the *gyrA* and *parC* genes were amplified from DNA templates by PCR using Platinum PCR supermix (Invitrogen) and the primer pairs listed in Table 2. PCR reactions began with a two-minute hot start at 94°C followed by 30 cycles of 94°C for 30 s, annealing temperature, 30 s and 72°C for 30 s. *gyrA* amplifications were annealed at 58°C and *parC* reactions were annealed at 52°C. *E. coli* K-12 MG1655 [40] was used as a control. Amplicons were sequenced on both strands and predicted peptide sequences were compared to the corresponding gene from the MG1655 genome [40] by pair-wise FASTA alignments.

Identification of horizontally-acquired quinolone-resistance genes

Horizontally-acquired quinolone-resistance genes were identified by PCR. The primers of Liu et al [41] were used to screen for the *qepA* gene and *qnrA*, *qnrB*, and *qnrS* were identified with PCR using the primer pairs published by Wu et al [42] (Table 2). Amplicons were sequence-verified.

Multi-locus sequence typing

Gene fragments from the *adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA* and *recA* were amplified using primers listed in Table 2, as described by Wirth et al [19]. Amplified DNA products were sequenced from both ends. Allele assignments were made at the publicly accessible *E. coli* MLST database at <http://www.mlst.net>. Phylogenetic inferences about ancestral allelic profiles and strain interrelatedness were made using eBURSTv3 at <http://eburst.mlst.net/> defining clonal complexes based on groups sharing five identical alleles and bootstrapping with 1000 samplings.

Table 2 Oligonucleotide primers used in this study

Target gene	Primer	Primer Sequence	Purpose	Reference
<i>gyrA</i>	gyrA12004	TGC CAG ATG TCC GAG AT	<i>gyrA</i> QRDR amplification	[12]
	gyrA11753	GTA TAA CGC ATT GCC GC		
<i>parC</i>	EC-PAR-A	CTG AAT GCC AGC GCC AAA TT	<i>parC</i> QRDR amplification	[43]
	EC-PAR-B	GCG AAC GAT TTC GGA TCG TC		
<i>qnrA</i>	qnrA-1A	TTC AGC AAG ATT TCT CA	<i>qnrA</i> detection	[42]
	qnrA-1B	GGC AGC ACT ATT ACT CCC AA		
<i>qnrB</i>	qnrB-CS-1A	CCT GAG CGG CAC TGA ATT TAT	<i>qnrB</i> detection	[42]
	qnrB-CS-1B	GTT TGC TGC TCG CCA GTC GA		
<i>qnrS</i>	qnrS-1A	CAA TCA TAC ATA TCG GCA CC	<i>qnrS</i> detection	[42]
	qnr-1B	TCA GGA TAA ACA ACA ATA CCC		
<i>qepA</i>	qepA-F	GCAGGTC CAGCAGCGGGTAG	<i>qepA</i> detection	[41]
	qepA-R	CTTCCTGCCCGAGTATC GTG		
<i>adk</i>	<i>adkF</i>	ATTCTGCTTGGCGCTCCGGG	MLST	[19]
	<i>adkR</i>	CCGTCAACTTTCGCGTATTT		
<i>fumC</i>	<i>fumCF</i>	TCACAGGTGCCAGCGCTTC	MLST	[19]
	<i>fumCR</i>	GTACGCAGCGAAAAAGATTC		
<i>gyrB</i>	<i>gyrBF</i>	TCGGCGACACGGATGACGGC	MLST	[19]
	<i>gyrBR</i>	ATCAGGCCTTCACGCGCATC		
<i>icd</i>	<i>icdF</i>	ATGGAAAGTAAAGTAGTTGTTCCGGCACA	MLST	[19]
	<i>icdR</i>	GGACGCAGCAGGATCTGTT		
<i>mdh</i>	<i>mdhF</i>	ATGAAAGTCGCAGTCCTCGGCGCTGCTGGCGG	MLST	[19]
	<i>mdhR</i>	TTAACGAACTCCTGCCAGAGCGATATCTTTCTT		
<i>purA</i>	<i>purAF</i>	CGCGCTGATGAAAGAGATGA	MLST	[19]
	<i>purAR</i>	CATACGGTAAGCCACGCAGA		
<i>recA</i>	<i>recAF</i>	CGCATTGCTTTACCTGACC	MLST	[19]
	<i>recAR</i>	TCGTCGAAATCTACGGACCGGA		

MLST - multi-locus sequence typing; QRDR - quinolone-resistance determining region.

Statistical analysis

Proportions were compared using the χ^2 or Fisher's exact test with p-values less than 0.05 being considered significant.

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Authors' contributions

SSN performed molecular experiments, analysed and interpreted data, and contributed to writing the paper. JAO collected isolates and performed microbiology experiments. RSL designed and performed molecular experiments. MJN co-conceived the study and collected isolates. INO co-conceived the study, performed microbiology and molecular experiments, analysed and interpreted data and wrote the manuscript. All authors read and approved the final manuscript.

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