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Research Article

Molecular characterization of fluoroquinolone resistance genes in isolates obtained from patients with diarrhea in Machakos District Hospital, Kenya

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Background: Diarrhea caused by Enterobacteriaceae such as Shigella species and *Escherichia coli* (*E. coli*) is endemic throughout the world, and is one of the most important causes of global childhood mortality and morbidity. There is a range of antibiotics that can be used for treatment among them quinolones. However, there is emerging increase in microbial resistance to quinolones use, with *E. coli* and Shigellae among the species of bacteria commonly associated with quinolone resistance.

Objective: To investigate the prevalence of quinolone resistance genes in *Shigellae* and *E. coli* from patients presenting with diarrhea in Machakos District Hospital.

Methods: Bacteria isolates were identified to species level by biochemical methods and serology and thereafter tested for 12 different antibiotics including quinolones, cephalosporins and aminoglycosides. Those resistant to quinolones with a zone diameter of ≤20 mm were tested for the presence of quinolone resistance genes using PCR. The *gyr*A resistance genes were further analyzed by sequencing to determine mutations within the quinolone resistance regions.

Results: There were different *E. coli* pathotypes and Shigellae spp. They resisted more than four antibiotics: Ciprofloxacin (4%), (Chloramphenical (28%), Cotrimoxazole (78%), Co-amoxilav (70%) Erythromycin (98%) Cefotoxime (18%) and Tetracycline (56%). Mutations responsible for fluoroquinolone resistance in the *gyr*A, *gyr*B, *par*C, and *par*E genes of *E. coli* and *Shigella* spp were: *gyr*A (17/30, 36%) *gyr*B (7/30, 23.3%) topoisomerase (parC 3/30, 10%) *par*E (3/30, 10**%).**

Discussion: There is an increase in fluoroquinolone resistance in *Shigellae* and *E.coli* which points to a major challenge in current treatment strategies. In addition, detection of high resistance found to commonly used antibiotics should serve as a warning call for close surveillance and understanding of the epidemiology of the resistance.

Key words: Quinolone antibiotics, resistance, *Shigella*, *Escherichia coli*

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1. Introduction

Shigellosis caused by Shigella species is endemic throughout the world, and is one of the most important causes of global childhood mortality and morbidity. Globally, every year there are about 165 million cases of Shigella infection and 1.1 million Shigella-related deaths [Kotloff wt al., 1999]. Shigella is transmitted efficiently in low-dose via fecal-oral route in areas, with poor hygienic conditions, and limited access to clean and potable water [Weissman et al, 1975]. Of four Shigella species, shigellosis is predominantly caused by S. flexneri in the developing world especially in Asia, whereas S. sonnei is the predominant causative agent of this disease in developed as well as industrialized countries [Kotloff et al, 1999]. A recent multicenter study of the epidemiology and microbiology of shigellosis in Asia revealed that the incidence of this disease might even exceed previous estimations, as Shigella DNA could also be detected in up to one-third of culture-negative specimens [von Seidlein et al, 2006].

Antimicrobial therapy is effective for the treatment of shigellosis. Increased resistance to commonly used antibiotics including ampicillin, streptomycin, sulfamethoxazole-trimethoprim, nalidixic acid and tetracycline among Shigella poses a major therapeutic challenge to control this disease [Dutta et al, 2002]. One of the reasons for emergence of multi-drug resistant Shigella spp. is the unique capability of the pathogen to acquire resistance factors (transmissible genes) from the environment or from other bacteria [Sack et al, 1997]. Besides, indiscriminate use of antibiotics for the treatment of human infection and in animal husbandries in endemic areas triggers the increase of resistance to newer antibiotics [Barbosa et al, 2000]. Ciprofloxacin, a third generation fluoroquinolone, has been used successfully for the treatment of infectious diseases including shigellosis. Following a successful clinical trial, ciprofloxacin has been recommended for the treatment of both childhood and adult shigellosis caused by multiple antibiotic resistant Shigella spp. in Bangladesh [Salam et al, 1998]. However, this antibiotic is no longer effective for the treatment of shigellosis in south Asian region including Bangladesh because of the emergence of fluoroquinolone resistant S. dysenteriae type 1 and their (same clone) dissemination across the countries [Talukder et al, 2004; Talukder et al, 2006].

Quinolone resistance emerges due to i) point mutations that result in amino acid substitution in chromosomal genes for DNA gyrase and topoisomerase IV, the targets of quinolone action and ii) changes in expression of efflux pumps and outer membrane permeability that control the accumulation of these agents inside the bacterial cell [Drlica et al, 1997; Poole., 2000]. In addition, a novel mechanism of plasmid-mediated quinolone resistance has recently been reported, and this involves DNA gyrase protection by a protein from the pentapeptide repeat family called Qnr [Wang et al, 2004; Tran et al, 2005]. In Gram-negative organisms, the primary target of fluoroquinolones is the enzyme DNA gyrase, which is essential for DNA synthesis [Tran et al, 2005]. DNA gyrase consists of two A and two B subunits, encoded by the *Gyr*A and *Gyr*B genes, respectively. Most mutations have been shown to reside in a small region near the start of the *Gyr*A gene, termed as quinolone resistance-determining region (QRDR), although mutations have also been reported in gyrB [Piddock., 1995]. Genes encoding topoisomerase IV consists of two subunits *par*C and *par*E which have also been shown to be inhibited by fluoroquinolones. In Gram-negative bacteria, topoisomerase IV has been considered as the secondary target to flouroquinolones and alteration in *par*C is involved in the mechanism of resistance [Everett et al, 1996]. The level of MICs of fluoroquinolones has been shown to correlate with the type and number of amino acid substitution of the target genes within the QRDR [Truong et al, 1997; Komp Lindgren et al, 2003; Turner et al, 2006; Morgan-Linnell et al, 2007]. In our previous study, possible mechanisms of fluoroquinolone resistance were analyzed in clinical strains of *S. dysenteriae* type 1 isolated from India, Nepal and Bangladesh [Talukder et al, 2006].

It has also been reported that fluoroquinolone resistance is often the result of a combination of target site mutations and enhanced expression of genes encoding efflux pumps in resistant bacteria [Kim et al, 2008]. In this study, we report the first isolation of fluoroquinolone resistant *S. flexneri, S. boydii and S. sonnei* in Bangladesh. In addition, the mechanism of chromosome mediated fluoroquinolone resistance in *S. flexneri* strains, which results from the combination of target site mutation and energy dependent active pumps involvement has been determined.

2. Methodology

2.1 Study design and Study population

This was a cross sectional study carried out from 2010 to 2013 in Machakos District Hospital.

The study targeted patient from three months and above who presented to the hospital for treatment due to diarrhea infection. Eligibility for inclusion into the study were patients with three or more episodes of loose, watery, mucoid or bloody diarrhea in a day; those who had given consent to participate in the study and those who had not taken any antibiotic before visiting the hospital.

2.2 Patient recruitment

Based on the studies done in Kenya (Karuiki et al, 2002), and using Kaper formula (Kapar et al, 1980), we arrived at a sample size of 300 patients. Before enrollment, the details of study were explained to the patient by the study nurse and allowed to ask questions for clarifications. Every third patient was recruited voluntarily into the study after signing of informed consent.

2.3 Sample collection

After the patient or guardian had been thoroughly briefed about the study and given signed consent to participate, the patient was given a sterile stool cup to collect a single stool sample. In case of a minor, the guardian or parent gave the consent. Care was taken during stool collection to avoid contamination with urine, soil or water. Part of the collected stool was transferred into Carry Blair transport medium and shipped to Kenya Medical Research Institute

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Microbiology laboratory for processing and identification.

2.4 Bacterial identification

Bacteria isolates were identified to species level by biochemical methods and serology (Kariuki et al, 2002) and thereafter tested for susceptibility to 12 different antibiotics including quinolones, cephalosporins and aminoglycosides by the agar diffusion method using Kirby Buer method.

2.5 Antimicrobial susceptibility testing:

Antimicrobial susceptibility testing (disc diffusion and Minimum inhibitory concentration{MIC}) were carried out based on Clinical and Laboratory Standards Institute (CLSI,2012) recommendations using the following discs; (Amp; 10 µg), sulphamethoxazole-trimethoprim (Sxt; 25 µg), mecillinam (Mel; 25 µg), nalidixic acid (Na; 30 µg), ciprofloxacin (Cip; 5 µg), norfloxacin (Nor; 10 µg), ofloxacin (Of; 5 µg), azithromycin (Azm; 15 µg), and ceftriaxone (Cro; 30 µg) [CLSI, 2012]. The minimum inhibitory concentrations (MICs) of nalidixic acid, ciprofloxacin, norfloxacin, ofloxacin, azithromycin, and ceftriaxone were determined by the E-test (AB Biodisk, Solna, Sweden) using a 0.5 McFarland standard. Those that were resistant to quinolones were tested for the presence of the gyrase and topoisomerase resistance genes using PCR for detection of *GyrA, GyrB*, *PerC and per*E. Thereafter conjugation and sequencing was carried out to determine resistance factors mutations in the quinolone resistance regions respectively.

2.6 Amplification of GyrA, GyrB, perE, perC:

Multiplex PCR of some representative strains was performed to detect the GyrA, GyrB, qnrS and aac(6¢)-Ib-cr according to the procedure described earlier [kim et al, 2008]. The primer sequences are:

5′-AGAGGATTTCTCACGCCAGGA-3′ and
5′-GGCTGGCCGATTATGATTGGT-3′ for qnrA;

5′-GGCTGGCCGATTATGATTGGT-3′ and
5′-CGCGTGCGATGAGATAACC-3′ for *Gyr*B;

5′-TGCCACTTTGATGTCGCAGAT-3′ and
5′-CGCACGGAACTCTATACCGTAG-3′ for *per*E; and

5′-ATCTCATATCGTCGAGTGGTGG-3′ and
5′-CGCTTTCTCGTAGCATCGGAT-3′ for perC.

2.7 Plasmid profile analysis

Plasmid DNA was prepared by the alkaline lysis method of Kado and Liu, with some modifications [Talukder et al, 2003]. The molecular weight of the unknown plasmid DNA was assessed by comparing the mobility of the plasmids of known molecular mass as described previously [Talukder et al, 2004].

2.8 Determination of resistance factor

A conjugation experiment between the multidrug-resistant (AmpR SxtR CipR) donors, *S. flexneri* serotype 2a K9482and K9563 strains, and the recipient, *E. coli* K-12 (Lac− F−), was carried out by a procedure described elsewhere [Munshi et al, 1987]. Transconjugant colonies were selected on MacConkey agar plates containing Amp (50 mg/L). Plasmid analysis and antimicrobial susceptibility testing of the transconjugants were carried out to determine the transfer of plasmids with antibiotic resistance.

2.9 Pulsed Field Gel Electrophoresis (PFGE)

Genomic DNA of various strains of E. coli, Shigella spp and Salmonella spp were prepared in agarose plugs as described by Kariuki et al. (2000: 2001) from an overnight culture in Luria batani broth. For complete digestion of DNA, the XbaI (Life Technologies, Paisley, UK) enzymes were added according to the manufacturer's instructions. PFGE of agarose plug inserts was performed in the contour clamped homogenous electric field CHEF-DRIII (Bio-Rad Laboratories, Hercules, CA, USA) on a horizontal agarose 1% gel for 22 h at 120V, pulse time of 1 to 40 sec at 14oC. A DNA size standard (lambda ladder, Bio-Rad) consisting of (c. 22 fragments) of increasing size from 48kb -1000kb were used as the DNA size standard makers. The gels were stained with 0.05% ethidium bromide and observed on a gel Doc 2000 (Bio-Rad). Restriction endonuclease digest pattern of all *Xba*I digest genomic DNA were compared and similarities and differences scored basing on Tenovar's method (Tenovar et al, 1997). Using the Dice coefficient method and clustered by the unweighted pair group arithmetic averaging method (molecular Fingerprinting program version 1.4.1, Bio-Rad) that puts the isolates into PFGE clonal groups (Kariuki et al, 2002; Kariuki et al, 2004). Those isolates showing indistinguishable PFGE banding were taken to be part of the outbreak while isolates showing 2-3 band difference in PFGE patterns were said to be closely related and possibly part of the cause of disease. Those with 4-6 band differences were assumed possibly related, and, those with more than 6 band differences were deemed different (Tenovar et al, 1997).

2.10 DNA sequence analysis:

Chromosomal DNA from selected representative strains was prepared and purified by procedures described previously [Tenover et al, 1995]. Polymerase chain reaction (PCR) for gyrA, gyrB, parC were performed according to the procedures described earlier [Jacoby et al, 2003; Jacoby et al, 2003; Rahman et al, 1994; Wang et al, 2001]. PCR amplicons were purified with the GFX™ PCR DNA and gel band purification kit (Amersham Pharmacia, USA). Sequencing of the amplicons was performed using the dideoxy-nucleotide chain termination method with the ABI PRISM® BigDye Terminator Cycle Sequencing Reaction kit (Perkin-Elmer Applied Biosystems, Foster City, California) on an automated sequencer (ABI PRISM™ 310). The chromatogram sequencing files were inspected using Chromas 2.23 (Technelysium, Queensland, Australia), and contigs were prepared using SeqMan II (DNASTAR, Madison, WI). Nucleotide and protein sequence similarity searches were performed using the National Center for Biotechnology Information (NCBI, National Institutes of Health, Bethesda, MD) BLAST (Basic Local Alignment Search Tool) server on GenBank database, release 138.0 [Altschul et al, 1990]. Multiple sequence alignments were developed using CLUSTALX 1.81 [Thompson et al, 1997]. Sequences were manually edited in the GeneDoc version 2.6.002 alignment editor.

2.11 Ethical considerations

Ethical approval for the study was granted by the Kenya Medical Research Institute - Ethical Review Committee (Ref. No.: **SSC 989**).

3. Results

Drug susceptibility profile of *E. coli* and *Shigellae* isolates

The study recruited and analyzed samples from 300 patients (male and female) who presented with diarrhea for treatment. The patient ages ranged from three months and above. The samples analyzed included *Shigellae* 67/300(22.3%), *E. coli* 500 (52%), *Klebsiella pneumoniae* 37(12.3%), *Salmonella* spp 1/300(0.3%), *Proteus* spp 1/300(0.3%) and *Serratia marcescens* 1/300(0.3%). They were resistant to more than four antibiotics including Ciprofloxacin (4%), Nalidixic acid (12%), Erythromycin (98%), co-amoxilav(70%) , Ceftriaxone(6%), Cefotoxime(17%), Ampicilin (80%), Co-tromoxazole (78%), and Chloramphenicol (28%) (**Figure 1**, **Table 1**). Considering quinolones alone; different bacteria varied slightly in their resistance: E. coli (40/500); Shigellae(6/67); K. pneumonia(3/37); Salmonella(0/1); Proteus(0/1) and S. marcescens(1/1).

Minimum inhibitory concentrations (MIC) for bacteria isolates

The MICs for both *Shigellae* and pathogenic *E. coli* isolates indicated raised MICs especially for Quinolones (0.16µg/ml) compared to the normal range (0.002- 4) implying a possibility for limited antimicrobial therapy (**Table 2**).

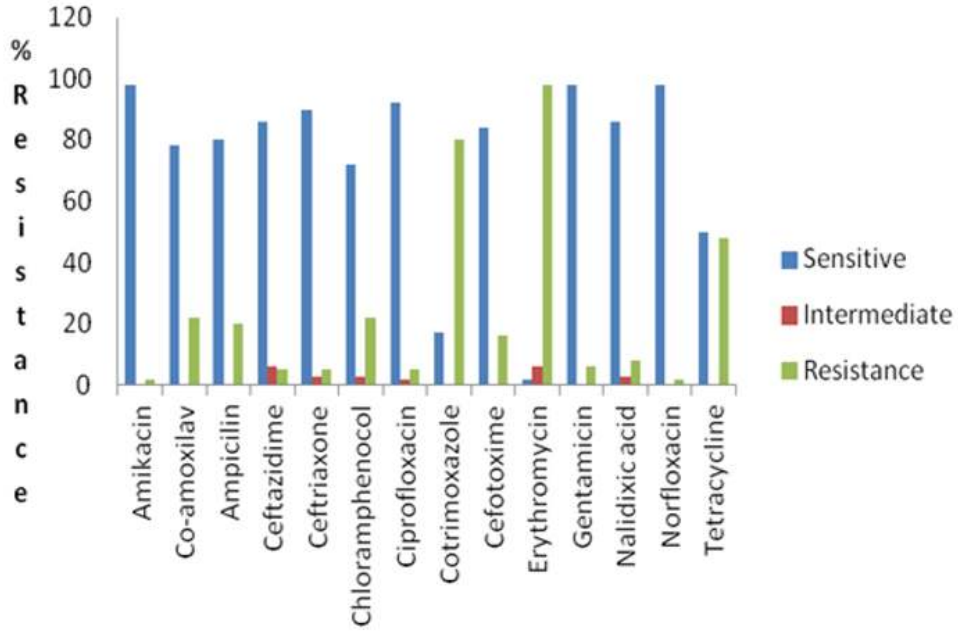

Figure 1. Drug susceptibility profile of *E. coli* and *Shigellae* isolates

Table 1. Antimicrobial resistance phenotypes

Antibiotic Resistance Patterns	Resistance Frequency (%)
AMP	19%
AMP/SXT	28%
AMP/SXT/ TE	42%
AMP/SXT//NA/CIP	14%
AMP/SXT/E	33%
AMP/SXT/CAZ/CN	12%
AMP/SXT/ TET/C/NA	16%
AMP/SXT/TET/NA/CAZ/AMC/CXM	2%

Key: AMC: Augumentin, SXT: Sulfamethoxazole, CN: Canamycin, CXM: Cefuroxime, CIP: Ciprofloxacin, AMP: Ampicilin, C: Chloramphenicol, NA: Nalidixic acid, TE: Tetracyclin, CAZ: Cefetazidine, E:Erythromycin

Table 2: Minimum Inhibitory Concentration for *E. coli* and Shigellae isolates.

Drug	*E. coli*			Shigellae		
	MIC 50 (µg/mL)	MIC 90 (µg/mL)	Ranges (µg/mL)	MIC 50 (µg/mL)	MIC 90 (µg/mL)	Ranges (µg/mL)
Co-trimoxazole	36	38	0.002 - 32	>2/38	>4/38	0.002 - 32
Amikacin	16	16	0.16 - 256	>16	>16	0.16 - 256
Gentamicin	4	6	0.16 - 256	4	8	0.16 - 256
Chloramphenicol	24	64	0.16 - 256	>16	64	0.16 - 256
Ciprofloxacin	0.08	0.16	0.002 - 4	0.16	0.4	0.002 - 4
Cefuroxime	16	16	0.16 - 256	>16	>16	0.16 - 256
Cefotoxime	32	32	0.16 - 32	> 8	32	0.16 - 32
Ceftazidime	32	32	0.16 - 256	8	32	0.16 - 256
Cefpodoxime	16	16	0.16 - 256	8	16	0.16 - 256
Tetracycline	8	24	1.5 - 256	32	64	1.5 - 256

Gyrase A, B and Topoisomerase detection

Detection of resistance genes and determination of mutations that were responsible for fluoroquinolone resistance in the *gyr*A, *gyr*B, *par*C, and *par*E genes of *E. coli* and *Shigellae* spp were investigated by mPCR using primers that were highly specific (**Figure 2**) and sequencing (**Table 3**) respectively. From the isolates tested, 30/300(10%) were positive for the above genes. The genes were distributed as follows: *gyr*A (17/30, 36%) *gyr*B (7/30, 23.3%) topoisomerase (*par*C 3/30, 10%) *par*E 3/30, 10%). Out of the 17/30 *gyr*A genes 9 were extracted from ETEC, 6 from Shigellae, and 2 from atypical *E.coli*. *Gyr*B resistant genes were distributed as follows: 2 from Enterotoxigenic *E. coli* (ETEC), 3 Shigellae, and 2 from atypical Enteropathogenic *E. coli* (EPEC). Topoismerase genes (*par*C and *par*E) were only recovered from Shigellae. The genes were isolated from children below age of five years and those above 40 years.

Test for invasiveness

All 67 Shigella strains harbored the invasive plasmid and had the ability to amplify for ipaH gene.

Plasmid profile analysis

Analysis of plasmid DNA revealed that out of the 33 multidrug resistant strains tested (33/33, 100%) contained multiple plasmids whose sizes ranged from 1.8 to 35 kb, forming a number of unique banding patterns as shown in (**Figure 3**) All strains carried more than two plasmids. They all transferred their resistance phenotypes to four antibiotics (Ampicillin, Nalidixic acid, cotrimoxazole and chloramphenicol) to the recipient *E. coli* K12 100% sensitive for the above antibiotics. After conjugation, the transconjugant *E. coli* K12 was 100% resistant to Ampicillin, Nalidixic acid, cotrimoxazole and chloramphenicol. This confirmed the fact that resistance observed to a range of antibiotics was plasmid mediated.

Figure 2. PCR products of the *Gyr*A, B, *par* C and *par* E quinolone resistance genes in *E. coli* and *Shigella* spp

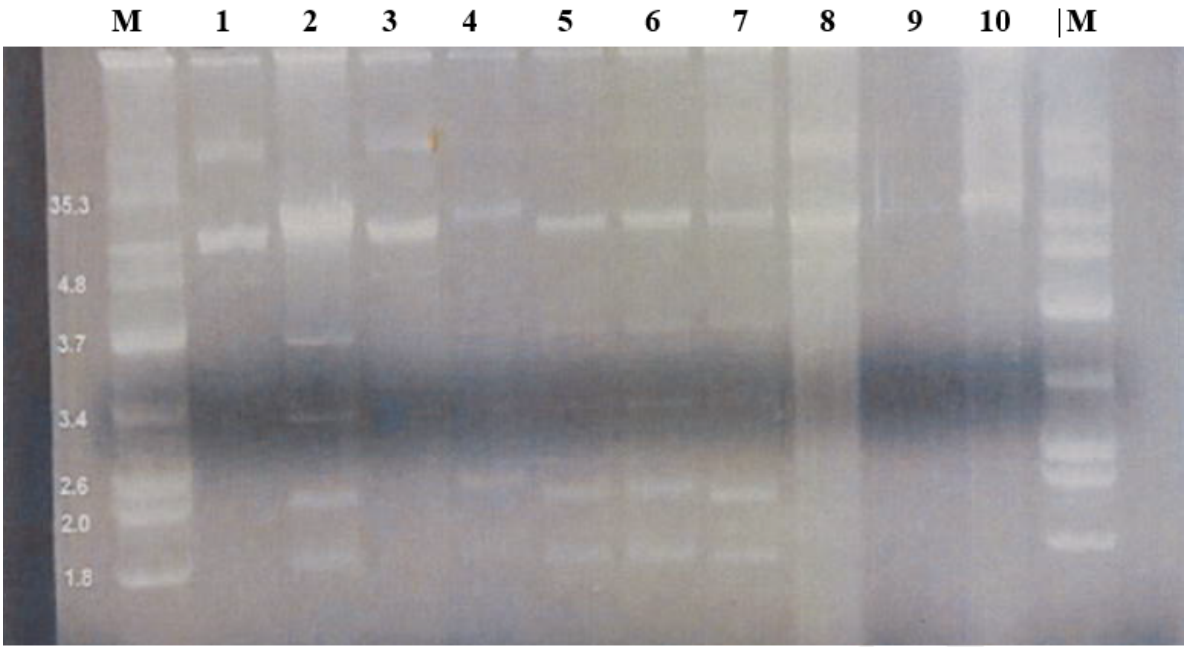

Figure 3: Plasmid profiles of multi drug resistant *E. coli and Shigellae* spp

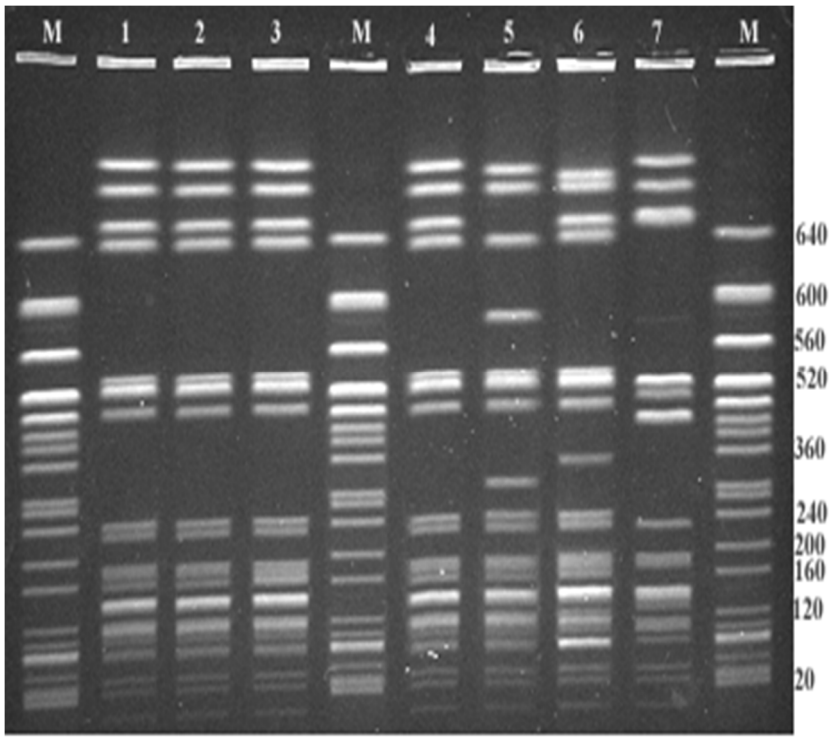

Figure 4. PFGE patterns of *Xba1* digest *Shigella* spp and *E. coli* isolated from patients

Analysis of PFGE profile

PFGE analysis of *Xba1*-digested chromosomal DNAs of the atypical *E. coli, S. flexneri S. dysenteriae*, and *S. boydii* strains yielded 16 to 19 reproducible DNA fragments ranging in size from 20 to more than 640 kb (**Figure 4**).

Shigellae isolates 38/67 (56.7%), 25/67 (37.3%) and 4/67(6%) had 2,3 or 4 band difference respectively, while *E. coli* (350/500(70%) had undistinguishable bands (2-3 bands).

Mutations in quinolone resistance determining regions

Sequencing was carried out on 30 randomly selected isolates that were resistant to quinolones. All sequences of the quinolone resistance-determining region of the *gyr*A gene (17/30, 56.7%) in the isolates which showed decreased susceptibilities or complete resistance had a single or double mutation at either the Ser-83 or the Asp-87 codon. There was no mutations observed in the *gyr*B (7/30, 23.3%), *par* C 3/30, 10%) or *par* E (3/30, 10%) genes (**Figure 5**, **Table 3**).

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Sequence alignment for quinolone resistance regions

The sequences were aligned and analyzed using MRbayes software and phylogenetic tree drawn using the fig tree for determination of relatedness of the isolates in terms of GyrA gene mutations (**Figure 5**). The tree yielded five clusters. Isolates from this study clustered successfully with those from the gene bank bearing the GyrA resistance genes. The conversions and comparisons in base alignment was analysed by ESS using tracer software and black colour outcome indicated a success in the conversions from the 17/30 (56.7%) isolates and a dendrogram drawn by neighbour joining (**Figure 5**). *Gyr*7 was the only different isolate (1/30, 3.3%). It was routed out of the other sequences implying that it was different. The other *gyr*A resistant isolates (16/17, 94.1%) clustered 100% with sequences of quinolone resitance genes obtained from the gene bank. This implied that *Gyr*A reistance genes were resisponsible for observed resistance in ciprofloxacin, Nalidixic acid and Norfloxacin.

The single amino acid switch was thought to be the cause for resistance to the quinolones (**Table 3**). Mutations were detected at amino acid 83 and 87.

Nucleotide sequences accession number

The nucleotide sequences reported in this paper were submitted in GenBank using the National Center for Biotechnology Information (NCBI, Bethesda, MD Sequin, version 5.26) under accession numbers - **JX908762**, **JX908763**, and **JX908764**.

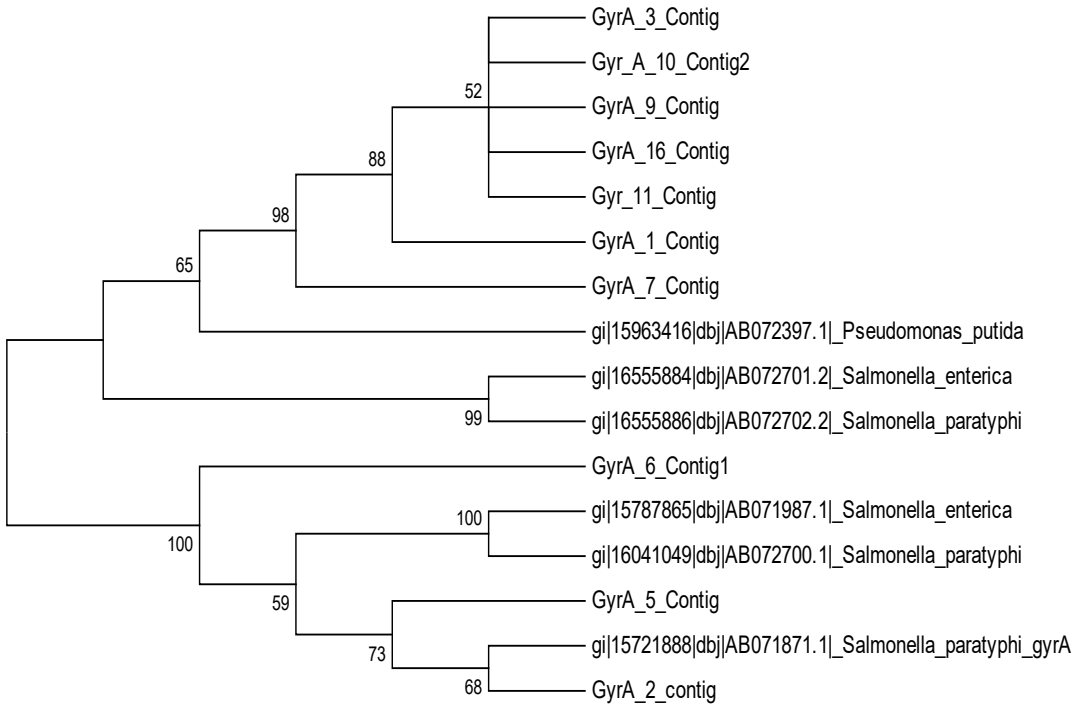

Figure 5. Consensus tree of Gyrase A genes and its nearest neighbours

Table 3. Fluoroquinolone resistance and amino acids substitution in bacteria isolates

Strain	MIC (µ/ml)			Amino acid switch	
	CIP	NOR	NA	*GyrA*	
MKS *S. dysenteriae*	0.5	0.03	1	Ser_{83}	Ser_{87}
MKS *EPEC*	0.09	0.19	>256	Ser_{83}-Leu	Asp_{87}
MKS *ETEC*	6	12	>256	Ser_{83}-Leu	Asp_{87} - Gly
MKS *ETEC*	200	250	>256	Ser_{83}-Leu	Asp_{87} - Gly
MKS *S. flexneri*	0.012	0.023	1.25	Ser_{83}	Ser_{87}
MKS *S. boydii*	64	250	>256	Ser_{83}-Leu	Asp_{87} - Asn
MKS *S. sonnei*	0.16	0.014	>256	Ser_{83}-Leu	Ser_{87}

Key: MKS denotes Machakos

4. Discussion

From the study, different *E. coli* pathotypes and *Shigellae* spp were detected among them Enteropathogenic *E. coli* (EPEC), Enteroinvassive *E. coli* (EIEC) Enteroaggregative *E. coli* (EAEC) and colonizing necrotizing factors (CNF) *S. dysenteriae*, *S. flexineri*, *S. sonnei*, *S. boydii* respectively representing 42.6% and 23% of bacteria isolated respectively. The isolates from the current study exhibited resistance to floroquinolones ranging from 4 to 12% (**Figure 1**) contrally to other studies done in Kenya that indicated no resistance to Ciprofloxacin (Kariuki et al, 2004). Resistance reported in this study was higher (upto 12%) than what has been reported before. Fluoroquinolones have become the first-line drugs for the treatment of bacillary dysentery and typhoid fever (Pazhani et al, 2011; Felmingham et al, 2007). *S. dysenteriae*, *S. flexineri*, *S.boydii*, S. *sonnei*, and *E. coli* strains that exhibit decreased susceptibilities or complete resistance to fluoroquinolones and other antibiotics have been reported elsewhere (Juma, et al, 2016; Mendez et al, 2009; Menendez et al, 2004; Hirose et al, 2001).

Several clinical treatment failures after the administration of ciprofloxacin and other fluoroquinolones to patients with dysentery and typhoid fever due to strains with decreased susceptibilities and /or resistance to fluoroquinolones have also been reported (Martinez-Martinez et al, 2003; Olsen et al, 2001). The emergence and spread of these organisms have been reported in developing countries including Kenya and Nigeria (Kariuki et al, 2004; Aibinu et al, 2007). There is evidence that the incidence of strains that are resistant to Nalidixic acid and that exhibit decreased susceptibilities to the most recent fluoroquinolones used for the treatment of shigellosis is increasing (Strahilevitz et al, 2009; Tran et al, 2002).

In most *E. coli* and *Shigellae* strains in the current study, the detected fluoroquinolone resistance was attributed to mutations due to single amino acid switch (**Table 3**) in the genes encoding DNA gyrase (*Gyr*A) while studies in other regions indicate mutations in *Gyr*A, B and DNA topoisomerase IV (*Par*C and *Par*E) (Kato et al, 1990). In this study, no mutation was detected in *Gyr* B, topoisomerase IV C and E. The resistance was found to be transferable and transconjugant analysis revealed transfer of resistance traits from multidrug resistant *E. coli* and *Shigella* spp to recipient *E. coli* K12. (**Figure 3**).

From the current study, 12% of the isolates were resistant to ciprofloxacin. This was in contrast to the study findings by Sinha et al, (2004) where typical resistant strains for ciprofloxacin were never found among the strains tested in their work. In the current study, the resistant *Shigella dysenteriae, S. flexneri, S. boydii, S. sonnei and E. coli* isolates to fluoroquinolone had single mutation in the *gyr*A gene, at either position 83 or 87 (**Table 3**). This was also contrary to the results of Kariuki et al, 2004 findings where mutations were detected in *Gyr*A, B and topoisomerase IV. This agreed with the PFGE pattern implying no variation in *E. coli* or *Shigelae* (**Figure 4**).

In quinolone resistance, there are three groups distinguished among the strains in which resistance is observed in vitro on the basis of the ciprofloxacin MICs and *gyr*A mutations. The first group consisted of strains which were susceptible to fluoroquinolones and which had no mutations in the QRDR of the *gyr*A gene and this was in agreement with the work done elsewhere (Piddock, 1998 and 2002; Konstantinos et al, 2008). The second group consisted of strains which exhibited slightly reduced susceptibilities to fluoroquinolones by MICs (or intermediate by disc diffusion and which had only a single mutation in the QRDR of the *gyr*A gene. The third group consisted of strains which were resistant to fluoroquinolones with a single mutation in the QRDR of the *gyr*A gene. These findings indicate that *gyr*A mutations are of principal importance for the fluoroquinolone resistance of *Shigella* spp and *E. coli*.

Alterations at position 83 or 87 of the *Gyr*A amino acid sequence have been described for Salmonella strains (Pazhani et al, 2011; Kariuki et al, 2004; Piddock et al, 2002; D'Ignazio et al, 2005). Double mutations at both positions 83 and 87 of the *Gyr*A amino acid sequence were also reported in clinical isolates of serovar Schwarzengrund, which caused nosocomial infections in the United States and which exhibited ciprofloxacin resistance (Rodas et al, 2011), a phenomenon that was not observed in the current study. Although strains with high-level fluoroquinolone resistance due to double mutations at codons 83 and 87 in the *Gyr*A amino acid sequence were not found in clinical isolates of *Shigella* spp, and *E. coli* in the current study. Several cases of the failure of treatment for shigellosis due to strains with decreased susceptibilities or total resistance to fluoroquinolones have been reported elsewhere (Ouyang-Latimer et al, 2011; Smith et al, 2010; Felmingham et al, 2007; Tran et al, 2002). From this study in comparison with the work done in the region (Kariuki et al, 2004), we conclude that fluoroquinolone resistance is increasing. This therefore possess danger in choice of antibiotics in the management of diarrheal illness due to Shigellae and *E. coli*. Active surveillance is therefore recommended in order to minimize the resistance. Observed resistance was attributed to single mutation at either position 83 or 87 of the quinolone resistance determining region.

Conflict of Interest Declaration

The authors declare no conflict of interest.

Disclaimer

The opinions stated in this paper are those of the authors and do not represent the official position of the U.S. Department of Defence.

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