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MUTATION OF SERINE-83 TO PHENYLALANINE AND TYROSINE IN THE *GYRA* GENE OF FLUOROQUINOLONE-RESISTANT *SALMONELLA ENTERICA* SEROVAR TYPHI ISOLATES FROM FUNTUA, NIGERIA

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ABSTRACT

Mutation in quinolone resistance determining region of *Salmonella enterica serovar typhi* are mostly associated with fluoroquinolone resistance and typhoid fever treatment failure. This study aims to identify chromosomal mutations in DNA gyrase gene of *Salmonella enterica serovar Typhi* as possible mechanism of fluoroquinolone resistance in patients that are completely resistant to ciprofloxacin, levofloxacin and ofloxacin in Funtua Primary Health, Katsina State.139 stool samples of patients with history of recurring typhoid fever that tested positive to Widal tests were collected and used for this research. *Salmonella typhi* identification was performed using standard microbiological and biochemical procedures while antibiotic susceptibility testing was performed by Kirby-Bauer disc diffusion method. The gene of the isolates with complete resistance to the three drugs were extracted for identification of chromosomal mutations in DNA gyrase gene. The results of the biochemical test confirmed 96(69.06) samples to be positive with the most prevalence seen among 16 – 45years and more positive isolates among the male. Fluoroquinolone susceptibility testing showed that 67 (69.79%) isolates were resistant to a single fluoroquinolone, 27 (28.13%) exhibited resistance to two while 2 (2.08%) were completely resistant to all the three fluoroquinolones tested. Among the three drugs tested, the most prevalent antibiotic resistance was to Ofloxacin. Molecular analysis of gyrase A showed mutation of serine-83 to phenylalanine and tyrosine respective in the two resistance samples. Given that fluoroquinolones remain first-line therapy for typhoid fever caused by S. Typhi in developing countries, decreased susceptibility may pose a threat to the disease management in this area.

Keywords: Fluoroquinolone, DNA Gyrase, Multi-Drug Resistance, Typhoid fever, *Salmonella enterica serovar Typhi*

INTRODUCTION

Quinolones-based drugs have become important antibiotics for the treatment of *Salmonella typhi* infections due to *Salmonella enterica serovars typhi* resistance to first-line antibiotics (Khan *et al*., 2024). Most frequently used quinolones are fluoroquinolones such as Levofloxacin, Ciprofloxacin and Ofloxacin (Hassing *et al*., 2011; Khadka *et al*., 2021). Fluoroquinolones based antibiotics inhibit bacteria DNA gyrase which is important for supercoiling and compacting bacterial DNA molecules inside the bacterial and Topoisomerase IV which is critical for the separation of daughter chromosome during replication (*Shaheen et al.*, 2021). This inhibition results in the release of DNA with single- and double-strand breaks that lead to cell death (*Aldred et al*., 2014).

Fluoroquinolones based antibiotics resistance in Salmonella is mostly linked to point mutations in the *gyr*A gene, which codes for the A subunit of gyrase (Hirose *et al.*, 2002). As demonstrated in multiple studies, the acquisition of resistance *gyr*A and *gyr*B gene in *Salmonella enterica serovar* in Nigeria is on the rise (Abdullahi *et al*., 2015; Yusuf *et al*., 2013). The chromosomal mutations in *gyr*A, results in amino acid changes in the *gyr*A subunit of DNA gyrase (Brown *et al*., 1996). The commonly observed amino acid changes is serine to phenylalanine substitution at position 83 and in less common cases aspartate to tyrosine or glycine at position 87(Adhikari *et al.*, 2022). In *S. Typhi*, one mutation in the *gyr*A gene can cause reduced susceptibility to fluoroquinolone drug while two or more mutations in the DNA quinolone resistance-determining regions (QRDRs) can cause complete resistance to fluoroquinolone drug (Hassing *et al.*, 2011). There is also uncommon resistance in *S. typhi* with a *gyr*B gene mutation (Gupta *et al*., 2015).

Reduced fluoroquinolone susceptibility has been observed in many patients in Nigeria due to misuse and excessive use of antibiotics, poor infection prevention and control and used of antibiotics for treatment of farm animal pathogens (Chukwu *et al*., 2020). In Northern part of Nigeria, antimicrobial resistance among *Salmonella enterica* serovar Typhi isolates has been widely documented. For example, at the University of Abuja Teaching Hospital, exceptionally high resistance rates were reported against nalidixic acid (92.3%), ciprofloxacin (84.6%), and 46.2% to both levofloxacin and ofloxacin (Fasema *et al*., 2024). Similarly, findings from the Abuja Municipal Area Council revealed varying levels of resistance to fluoroquinolones, ranging from 7.3% for ciprofloxacin to 43.5% for ofloxacin (Bur *et al*., 2025). Evidence from General Hospital Minna among patients with typhoid fever further highlights the growing burden of antimicrobial resistance. Most *S. Typhi* isolates (82.4%) demonstrated multidrug resistance, with multiple-antibiotic resistance indices spanning 0.3–0.9. Resistance patterns differed across antimicrobial agents, with the highest resistance observed against ciprofloxacin (76.5%), followed by cefotaxime (64.7%), ceftriaxone (58.8%), azithromycin (58.8%), and levofloxacin (52.9%) (Maryam *et al*., 2026). Comparable trends have been documented in Makurdi, Benue State, where resistance to fluoroquinolones was identified in

28.6% of isolates (Odiniya *et al*., 2024). Furthermore, a multicentre investigation spanning Abuja and Nasarawa States reported that 46.9% of *Salmonella enterica* isolates were multidrug resistant, underscoring the persistence and geographic spread of resistant strains (Uzairue *et al*., 2023).
High resistance of Salmonella to fluoroquinolones such as Ciprofloxacin, Levofloxacin and Ofloxacin has resulted in drastic increase in infection rate, treatment failure and mortality in the Northern part of Nigeria (Abdulkarim et al.,2023;Maryam *et al*., 2026; Obaro *et al*., 2015; Odiniya *et al*., 2024). In view of this, the current study was conducted in Funtua, Katsina State, Nigeria, to discover potential mutations in ser83 of *gryA* of Salmonella *enteria serovar typhi* isolates from patients that are completely resistant to Ciprofloxacin, Levofloxacin and Ofloxacin.

MATERIALS AND METHODS

Research Location

The research was carried out at Funtua Primary health care, Funtua, Katsina state, Nigeria. Funtua Primary health care is the most accessible to the local people and the second largest health care facility in Funtua after the General hospital.

Population and Sampling Technique

The study population included all patients with recurring typhoid fever subjected to Widal tests among the patients visiting the hospital between March and September, 2021.

Ethical Approval

Before the sample collection, the hospital management gave its consent and the ethical approval (FTLG/PHCD/ADM/0368/VOL.II) was obtained. The purpose of the study was discussed with the patients and the risk of losing confidentiality was reduced by the use of identifying numbers and the elimination of personal identifiers other than age and gender. Every ethical procedure involving the use of human beings in a therapeutic setting was followed.

Bacterial Isolation and Identification

For the study, 139 stool samples from patients with recurring typhoid fever who tested positive to Widal test were utilized. The data was sorted into gender and age groups before being analysed.
Standard procedures were used to isolate *Salmonella Enterica serovar Typhi* from the stool samples. Stool samples were directly inoculated into *Salmonella-Shigella* agar plates using sterilized swabs (Himedia, HIMedia laboratories Pvt. Ltd. India). For enrichment, identical sample was plated onto Salenite F broth (BIOTEC, BIOTEC laboratories Ltd UK) and incubated as described below. For 24 hours, agar plates and enrichment broth were incubated aerobically at 37°C. Sterilized wire loop was then used to pick loopful of the selenite F broth before been streaked onto SS Agar plates and incubated in the incubator at 37ºC for a day. Colonies suspected of being salmonella (small colourless colony with black spot) were selected for Gram staining and biochemical characteristics. These include citrate test, urease test, H_2S production, indole test, Triple sugar tests and motility test (Fanissa *et al.*, 2022). Isolates that exhibited motility, citrate positive and H_2S generation but an indole negative reaction were deemed probable Salmonella species and were sub culture and purified for the study.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed on the isolates using the diffusion disc method. As suggested by the Clinical and Laboratory Standards Institute, modified method of disk diffusion test of Kirby-Bauer was employed to determine in vitro sensitivity of the isolates to the fluoroquinolone antibiotics using *Escherichia coli* ATCC 25922 strain as control (Clinical and Laboratory Standards Institute, 2023). Ciprofloxacin (5µg), ofloxacin (5µg) and levofloxacin (5 µg) were employed in commercially manufactured single antibiotic disks. Sensitivity testing was performed using Muller Hinton agar (Oxoid Ltd., UK). To make the inoculum, a colony of the test organism was plucked with a wire loop that has been sterilized before being streaked on Muller Hinton agar plate. A clean cottonwool was put into the inoculum and swirled to drain excess liquid on a wooden applicator. The swab and inoculums were streaked across the entire agar surface of the plate, which was then left to dry for about 15 minutes. The single disk fluoroquinolone antibiotics were carefully placed at least 2mm apart on the agar using sterile forceps. Next, the isolates were incubated 24hours at 37ºC. Growth on the places were then checked for after the overnight incubation period. The diameter of each inhibitory zone was measured in millimetres. Zones of inhibition were recorded and the outcomes were determined using NCCLS's established susceptibility levels (Clinical and Laboratory Standards Institute, 2023). Sensitive, resistant, or intermediate findings were reported.

Primer, DNA Extraction and PCR

The DNA of the isolated *Salmonella typhi* was extracted with DNA extraction kit (Zymo Research) following the manufacturer's guidelines.
Primers for gyrase A were design to amplified the gene and synthesize at Kaduna DNA laboratory, Kaduna State, Nigeria.
gyrA1 5’ GGTACACCGTCGCGTACTTTT3’
gyrA2 5’ ACCGGTACGGTAGGCTTCTT 3’
(Ayana & Surekha, 2008)
Salmonella enterica serovar Typhi: Ty2 strain (GenBank: LR590081.1) was used for reference
The gyrA primers were used to perform PCR in thermal cycler™. The PCR was done on a 25µL of the supernatants, 25 pmol of each primer, 200 µM dNTPs, 1.5mM $MgCl_2$ and 0. 5 U of Taq polymerase. After an initial denaturation step of 180 seconds at 94ºC, amplification was done for more than thirty cycles, each consisting of 60 seconds of denaturation at 94ºC, annealing at 56 °C for 1 minute, extension at 72 °C for 60 s and a final extension step of 10 minutes at 72ºC. The positive control containing the gryase A and negative control without gryase A but nuclease free water was used as control
The PCR product was subjected to Agarose gel electrophoresis. Pure DNA sample was recovered from the gel band corresponding to the required DNA with Zymoclean™ Gel DNA Recovery Kit (ZYMO RESEARCH) following manufacturer’s instruction and stored at – 20ºC for further use. Extracted amplicon from electrophoresed gel as previously described was sent to Kaduna DNA laboratory, Kaduna State for sequencing using DNA sequencer based on the principle of dideoxy method as described by Sanger and Coulson(Sanger & Coulson, 1975). The Basic Local Alignment Search Tool (BLAST) was used to compared the resulting sequences with those of reference *Salmonella* Typhi deposited in the National Centre for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov/BLAST) to confirm *gyrA* gene and check for the presence of mutation in the quinolone determining region of the *GyrA* gene of the isolates.

RESULTS AND DISCUSSION

There were more positive salmonella isolates among the male patients (66.91%) than the female (33.09%) (Table 1), the positive salmonella isolates were most prevalent among the age group 16 – 45years followed by those above 45years with the least being from patients within age range 0 – 15years (Table 2)

Table 1: Positive Salmonella Isolates from Cultured Samples according to Gender

Gender	Sample Number	Percentage (%)
Female	46	33.09
Male	93	66.91
Total	139	100%

Table 2: Positive Salmonella Isolates from Cultured Samples according to Age Group

Age Group	Sample Number	Percentage (%)
0 – 15	15	10.79
16 – 45	91	65.47
> 45	33	23.74
Total	139	100

When subjected to confirmatory biochemical tests, 96 of the probable Salmonella isolates were confirmed positive for Salmonella *enterica serovar typhi* while 43 were negative (Tables 3 & 4)

Table 3: Positive Salmonella Isolates according to Gender after Confirmatory Biochemical Tests

Gender	Sample Number	Percentage
Female	32	33.33%
Male	64	66.67%
Total	96	100%

Table 4: Positive Salmonella Isolates according Age Group after Confirmatory Biochemical Tests

Age Group	Sample Number	Percentage (%)
0 – 15	10	10.42
16 – 45	63	65.63
> 45	23	23.95
Total	96	100

There were more isolates with reduced susceptibility to Ofloxacin only (38.54%) compare to Ciprofloxacin only (15.63%) and Levofloxacin only (15.63%) (Table 5). For resistance to two drugs, the isolates that were resistant to both Ciprofloxacin/Ofloxacin (16.66%) has the highest percentage, followed by Levofloxacin/Ofloxacin while the least were those with resistance to both Ciprofloxacin/ Levofloxacin (3.13%). Only two of the isolates were resistance to all the 3 drugs (ciprofloxacin, levofloxacin and ofloxacin). This represents 2.08% of the samples (Table 5).

Table 5: Susceptibility Tests for Ciprofloxacin, Levofloxacin and Ofloxacin

Drugs	Number of Resistant Isolates
CPX Only	15 (15.63%)
LEV Only	15 (15.63%)
OFL Only	37 (38.54%)
CPX/OFL	16 (16.66%)
LEV/OFL	8 (8.33%)
CPX/LEV	3 (3.13%)
CPX/LEV/OFL	2 (2.08%)
TOTAL	96

CPX = Ciprofloxacin; LEV= Levofloxacin; OFL= Ofloxacin

Figure 1 showed the presence of *gyr*A in two isolates with complete resistance to all the 3 drugs and some isolates with highest resistance to 2 drugs. The gel showed that the gyrase A of Salmonella *enterica* serovar Typhi Isolates sample is approximately 500bp (Figure 1). When the PCR products (Amplicons) of the two isolates that were completely resistant to the three drugs were sequenced, it gave rise to a 487bp for sample1 and 480bp for sample (Appendix).

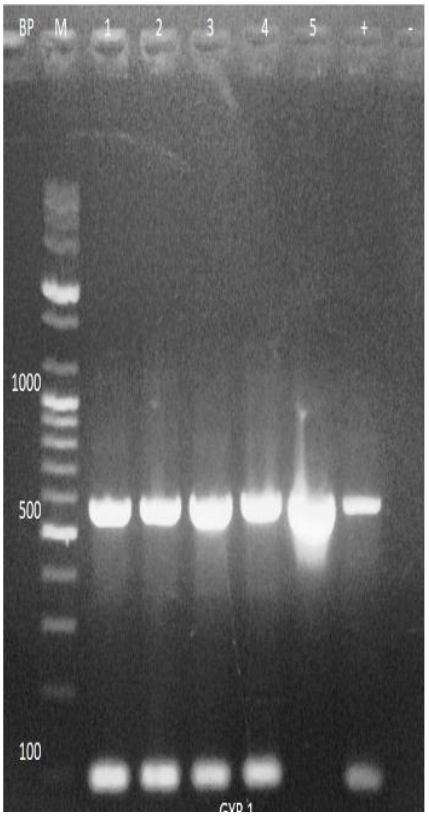

Figure 1: Gel electrophoresis of Salmonella enterica serovar Typhi DNA *gyr*A
M = Molecular weight marker; Well 1= resistance to CPX/OFL; Well 2: resistance to CPX/LEV; Well 3: resistance to LEV/OFL; Well 4= sample1 that is resistance to CPX/LEV/ OFL; Well 5= sample2 that is resistance to CPX/LEV/ OFL; +=Positive control; - = Negative control

The two sequence of the two *gyrA* gene sample that were completely resistant to the three drugs was then subjected to NCBI BLASTX to obtain the amino acids sequence of the nucleotides. The polypeptide obtained contains 162 translated amino acids for sample 1 and 160 translated amino acids for sample 2acids (Figure 2). Further analysis of the nucleotide sequences obtained after the sequencing of the quinolone resistance-determining region (QRDR) of the two *gyrA* gene that are completely resistant to the three drugs using Basic Local Alignment Search Tool (BLAST) with that of *S. enterica serovar Typhi* Ty2 as reference, revealed mutation of Serine 83 codon in *gyrA* gene to Tyrosine(Y) and Phenylalanine(F) in the two samples (shown in bold with asterisk under) (Figure 2)

SAMPLE1
RCTLQCCAMNVLGNDWNKAYKKSARVVGDVIGKYH
PHGDIAVYDTIVVWAQPFSLRYMLV 60
SAMPLE 2
VHRRVLFAMNVLGNDWNKAYKKSARVVGDVIGKY
HPHGDIAVYDTIVVWAQPFSLRYMLV 60

SAMPLE1
DGQGNFGSIDGDSAAAMRYTAL**Y**LAKAMADLEKET
VDFVDNYDGTEKIPDVMPTKIPNLL 120
SAMPLE2 DGQGNFGSIDGDSGAS P
RAVQP**F**MAKAMADLEKETVDFVDNYDGTEKIPDVM
PTKIPNLL 120

SAMPLE1
VNGSSGIAVGMATNIPPHNLTEVINGCLAYIDNVAKST
RRCT 162
SAMPLE
VNGSSGIAVGMATNIPPHNLTEVINGCLAYIDNEDISIE
G-- 160

Figure 2: Comparison of quinolone resistance-determining region (QRDR) of the two completely resistance *gyrA* samples using *S. enterica* serovar Typhi Ty2 genome (GenBank: LR590081.1) as reference

Discussions

This study discovered higher incidence of typhoid infection among male patients compare to the female as reported in previous studies. The high incidence of typhoid fever among the male patients may be due to more involvement in outdoor activities compare to women which resulted in exposure to the sources of infection (Maharjan *et al*., 2021; Ojo & Ogunfowokan, 2025). The prevalence of the disease among the patients within the age bracket 16 – 45years (65.63%) may be ascribe to their active social life in which they are more likely to consume foods and drinks from street vendors. Poor hygiene practice is prevalent among street food vendors according to some studies (Khadka *et al*., 2021; Maharjan *et al*., 2021), consuming such foods can contribute significantly to higher incidence of typhoid fever. Similarly, lack of potable and safe drinking water in most rural communities and public places is believed to be the root cause of high prevalence of typhoid in many communities (Karkey *et al*., 2013; Khadka *et al*., 2021). Similar result in other studies showing high

prevalence among the young people have been observed (Bhetwal *et al*., 2017; Khadka *et al*., 2021).

Fluoroquinolones become the antibiotics of choice following the emergence of multidrug resistance *S.* Typhi (Adhikari *et al*., 2022). Fluoroquinolones are usually under strict prescription of clinicians in advanced countries but freely available in developing countries like Nigeria (Sale *et al*., 2021), indiscriminate use of these antibiotics have resulted in resistance to these antibiotics. The easy access of the public to these drugs must be controlled as this will help to prevent widespread drug-resistant *Salmonella.* Also, extensive use of Fluoroquinolones in treatment of both humans and animals has created a selective pressure that promotes the horizontal transfer of resistance genes and the development of antimicrobial resistance (Piekarska *et al*., 2023). The prevalent antibiotic resistance observed was to Ofloxacin, with more than half of the patients' samples being resistant to it and the least is Levofloxacin. In Northern part of Nigeria, antimicrobial resistance among *Salmonella enterica* serovar Typhi isolates has been widely documented. In a study, conducted at the University of Abuja Teaching Hospital, exceptionally high resistance rates were also reported against ciprofloxacin (84.6%), and levofloxacin and ofloxacin (46.2% each) (Fasema *et al*., 2024) far above the present study. Similarly, findings from the Abuja Municipal Area Council revealed varying levels of resistance to fluoroquinolones, ranging from 7.3% for ciprofloxacin to 43.5% for ofloxacin (Bur *et al*., 2025). Evidence from General Hospital Minna among patients with typhoid fever further demonstrated multidrug resistance with the highest resistance observed against ciprofloxacin (76.5%), and levofloxacin (52.9%). (Maryam *et al*., 2026). Comparable trends have been documented in Makurdi, Benue State**,** where resistance to fluoroquinolones was identified in 28.6% of isolates (Odiniya *et al*., 2024). Levofloxacin was comparatively effective than ciprofloxacin and ofloxacin, this is consistence with the result of Khadka *et al*., (2021) and Adhikari *et al*., (2022). The effectiveness and enhanced activity of Levofloxacin compared to ciprofloxacin and ofloxacin has been linked to the differences in the carbon chain arrangement (Khadka *et al*., 2021).

DNA GyrA subunit of DNA gyrase is the primary target of fluoroquinolones in *Salmonella enterica serovar Typhi,* gyr A breaks and reseal the DNA strand (Hooper, 1998; Nordmann & Poirel, 2005). Apart from antibiotics abuse, mutations in the quinolone resistance-determining region (QRDR) of DNA gyrase and topoisomerase IV have been shown to be responsible for quinolones resistance (Nordmann & Poirel, 2005). Fluoroquinolone antibiotics inhibit Gyr A after they bind to DNA and form a transient complex, in which the enzyme's active-site tyrosine (Tyr) residue forms a covalent bond with a DNA phosphate ester, thus preventing the resealing process leading to accumulation of double-stranded DNA fragments that lead to cell death (Aldred *et al*., 2014). Mutations in *gyrA* genes of quinolone resistance determining region of *Salmonella enterica serovar typhi gyrA* gene is usually associated with fluoroquinolone resistance (Jacoby, 2005; Onken *et al*., 2024). Mutation in the *gyrA* genes result in new codon that form different amino causing structural modifications in DNA gyrase which alter the binding capacity of fluoroquinolones to the DNA- gyrase complex leading to fluoroquinolone resistance (Shaheen *et al*., 2021). The most common mutation in *Salmonella enterica serovar typhi gyrA* gene is serine to phenylalanine substitution at position 83, however, mutation to tyrosine have also been observed, less common cases of aspartate to tyrosine or glycine at position 87 has been recorded (Adhikari *et al*., 2022; Khadka *et al*., 2021). Similar results have been observed in Pakistan, India, Nepal and Bangladesh where Ser83Phe substitution was the most prevalent in resistant patients followed the Ser83Tyr substitution, with MIC increasing to 10- to 60- depending on the number and position of the mutations (Afzal *et al*., 2013; Gaind *et al*., 2006; Nair *et al*., 2006).

Mutation of serine-83 to phenylalanine and tyrosine respective were observed in this study. This is similar to Khadka et al., (2021) that confirmed that 95.65% of fluoroquinolone resistant isolates in Referral Hospital of Kathmandu, Nepal exhibits gyrA ser83 mutation. Results from the study of Sale *et al*., (2021) in Adamawa State showed that 56% of the isolates had point mutations at serine 83 while 24% had mutation at point 87 (Sale *et al*., 2021). Other author has reported similar mutation among *Salmonella* typhi isolates in different places. Gopal *et al*., (2016) have reported that 94% of *Salmonella* typhi isolates from their study had point mutations in *gyrA* position 83 while in Onyenwe *et al.*, (2012) study in in South East Nigeria reported that 64% of the *S* Typhi Isolates possessed mutations in the *gyrA* gene. In *S. Typhi*, one mutation in the *gyr*A gene can cause partial resistance or reduced susceptibility to fluoroquinolone drug while two or more mutations in the DNA quinolone resistance-determining regions (QRDRs) can cause complete resistance to fluoroquinolone drug. Complete resistance of S. Typhi in the absence of a double mutation in the gyrA suggests that these isolates also acquired an active efflux transporter or have impaired outer membrane porins (Hassing *et al*., 2011). From previous studies, a single mutation in *gyrA* alone may not be sufficient for complete resistance to fluoroquinolones, however, mutation in gyrA is a good indicator that fluoroquinolones should not be chosen for treating typhoid in such patient (Piekarska et al., 2023).

CONCLUSION

Among the three drugs tested, the most prevalent antibiotic resistance was to Ofloxacin. Molecular analysis of gyrase A showed mutation of serine-83 to phenylalanine and tyrosine respective in the two resistance samples. Given that fluoroquinolones remain first-line therapy for typhoid fever caused by *Salmonella enterica serovar Typhi* in developing countries, decreased susceptibility may pose a threat to the disease management in this area.

Limitations of the Study

The use of Stool culture for recurrent typhoid infection identification and the use of Disk diffusion method to determine in vitro sensitivity of the isolates to the fluoroquinolone antibiotics in this primary health care without minimum inhibition concentration determination is acknowledged as limitation of the study. Although there are many other mechanisms by which *Salmonella* may develop resistance to fluoroquinolones, this study was limited to investigation of *gyrA* mutation mediated resistance. Further studies can be conducted on other mechanisms.

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APPENDIX

The nucleotide sequence of recovered amplicon from Sample **1 *gyr*A** has a total of 487 base pairs

CGGTGTACCC TTCAATGCTG TGCGATGAAC
GTGCTGGGCA ACGATTGGAA CAAAGCGTAT
AAAAAAAGCG CGCGCGTGGT GGGCGATGTG
ATTGGCAAAT ATCATCCGCA TGGCGATATT
GCGGTGTATG ATACCATTGT GGTGTGGGCG
CAGCCGTTTA GCCTGCGCTA TATGCTGGTG
GATGGCCAGG GCAACTTTGG CAGCATTGAT
GGCGATAGCG CGGCGGCGAT GCGCTATACC
GCGCTATACC TGGCGAAAGC GATGGCGGAT
CTGGAAAAAG AAACCGTGGA TTTTGTGGAT
AACTATGATG GCACCGAAAA AATTCCGGAT
GTGATGCCGA CCAAAATTCC GAACCTGCTG
GTGAACGGCA GCAGCGGCAT TGCGGTGGGC
ATGGCGACCA ACATTCCGCC GCATAACCTG
ACCGAAGTGA TTAACGGCTG CCTGGCGTAT
ATTGATAACG TGGCGAAAAG TACGCGACGG
TGTACCA

The nucleotide sequence of recovered amplicon from Sample **1 *gyr*A**

RCTLQCCAMN VLGNDWNKAY KKSARVVGDV
IGKYHPHGDI AVYDTIVVWA QPFSLRYMLV
DGQGNFGSID GDSAAAMRYT ALYLAKAMAD
LEKETVDFVD NYDGTEKIPD VMPTKIPNLL
VNGSSGIAVG MATNIPPHNL TEVINGCLAY
IDNVAKSTRR CT

5'3' Frame amino acid translation of Sample 1 *gyr*A

The nucleotide sequence of recovered amplicon from Sample **2 *gyr*A** has a total of 480 base pairs

GTGCATCGCC GCGTGCTGTT TGCGATGAAC
GTGCTGGGCA ACGATTGGAA CAAAGCGTAT
AAAAAAAGCG CGCGCGTGGT GGGCGATGTG
ATTGGCAAAT ATCATCCGCA TGGCGATATT
GCGGTGTATG ATACCATTGT GGTGTGGGCG

CAGCCGTTTA GCCTGCGCTA TATGCTGGTG
GATGGCCAGG GCAACTTTGG CAGCATTGAT
GGCGATAGCG GTGCATCGCC GCGTGCTGTT
CAGCCGTTTA TGGCGAAAGC GATGGCGGAT
CTGGAAAAAG AAACCGTGGA TTTTGTGGAT
AACTATGATG GCACCGAAAA AATTCCGGAT
GTGATGCCGA CCAAAATTCC GAACCTGCTG
GTGAACGGCA GCAGCGGCAT TGCGGTGGGC
ATGGCGACCA ACATTCCGCC GCATAACCTG
ACCGAAGTGA TTAACGGCTG CCTGGCGTAT
ATTGATAACG AAGATATTAG CATTGAAGGC

The nucleotide sequence of recovered amplicon from Sample **2 *gyr*A**

VHRRVLFAMN VLGNDWNKAY KKSARVVGDV
IGKYHPHGDI AVYDTIVVWA QPFSLRYMLV
DGQGNFGSID GDSGASPRAV QPFMAKAMAD
LEKETVDFVD NYDGTEKIPD VMPTKIPNLL
VNGSSGIAVG MATNIPPHNL TEVINGCLAY
IDNEDISIEG

5’3’ Frame amino acid translation of Sample 2 *gyr*A