gyrA ser83 mutation among fluoroquinolone-resistant *Salmonella enterica* serovars from enteric fever patients in tertiary care hospital, Kathmandu

Prashanna Adhikari1†, Roshani Maharjan1†, Subash Paudel1, Bikram Malla2, Pradeep Kumar Shah1, Anup Bastola3 and Upendra Thapa Shrestha2*

Abstract

**Background:** The management of enteric fever through antibiotics is difficult these days due to the emerging resistance of *Salmonella* to various antimicrobial agents. The development of antimicrobial resistance is associated with multiple factors including mutations in the specific genes. To know the current status of mutation-mediated fluoroquinolone-resistance among *Salmonella enterica* serovars; Typhi, Paratyphi A, B and C, this study was focused on detecting *gyrA* ser83 mutation by restriction digestion analysis of *gyrA* gene using *HinfI* endonuclease.

**Results:** A total of 948 blood samples were processed for isolation of *Salmonella* spp. and 3.4% of them were found to be positive for *Salmonella* growth. Out of the 32 *Salmonella* isolates, 2.2% were *S*. Typhi and 1.2% were *S*. Paratyphi A. More interestingly, we observed less than 5% of isolates were resistant to first-line drugs including chloramphenicol, cotrimoxazole and ampicillin. More than 80% of isolates were resistant to fluoroquinolones accounting for 84.4% to levofloxacin followed by 87.5% to ofloxacin and 100% to ciprofloxacin by disc diffusion methods. However, the minimum inhibitory concentration method using agar dilution showed only 50% of isolates were resistant to ciprofloxacin. A total of 3.1% of isolates were multidrug-resistant. Similarly, 90.6% of the *Salmonella* isolates showed *gyrA* ser83 mutation with resistance to nalidixic acid.

**Conclusions:** The increased resistance to fluoroquinolones and nalidixic acid in *Salmonella* isolates in our study suggests the use of alternative drugs as empirical treatment. Rather, the treatment should focus on prescribing first-line antibiotics since we observed less than 5% of *Salmonella* isolates were resistant to these drugs.

**Keywords:** Enteric fever, *Salmonella enterica*, Fluoroquinolone-resistant, *gyrA*, ser83 mutation

**Background**

Enteric fever is an acute, life-threatening febrile infection caused by *Salmonella enterica* serovars; Typhi, Paratyphi A, B and C. Symptoms may vary from mild to severe and usually begin 6 to 30 days after exposure [1]. These isolates are highly adapted infections to the human population, having no animal and environmental reservoirs. Enteric fever was once considered a major cause of morbidity and mortality throughout the world accounting...
for approximately 15% case fatality rate. World Health Organization (WHO) estimates the annual global incidence of enteric fever is between 11 and 21 million cases and approximately 128,000 to 161,000 deaths [2]. In addition, the infection is more prevalent in developing countries with poor sanitation including Nepal. As per the annual report published by the Department of Health Service (DoHSS), Ministry of Health and Population, Nepal, enteric fever was still one of the top ten causes for inpatient morbidity (8.48%) [3]. Nepal remains endemic to enteric fever for many years. S. Typhi and S. Paratyphi A are the two most predominant bacterial pathogens in the blood cultures of enteric febrile patients in Nepal.

Although enteric fever is endemic in many developing countries including Nepal, effective antimicrobial therapy has significantly reduced morbidity and mortality among the infected cases. S. Typhi and S. Paratyphi A, B and C are susceptible to a variety of antibiotics in vitro testing. However, in vivo responses are not always similar to in vitro susceptibility. It is very difficult to predict accurately because of their predominantly intracellular location within phagocytic cells. These isolates were usually sensitive to the fluoroquinolone antibiotics which were rapidly bactericidal than the third-generation cephalosporins. Unfortunately, the resistance to fluoroquinolones has now developed in S. Typhi and S. Paratyphi isolates from central Asia, southern India and Vietnam [4–7]. The emerging resistance to fluoroquinolones adds another burden to manage enteric febrile cases. This is because of extensive use of fluoroquinolones for the treatment of infections caused by multidrug-resistant Salmonella spp. [8]. S. enterica has developed resistance to fluoroquinolones in many different ways including inactivation of drug, reduced membrane permeability, alteration of the target site and active efflux [9]. Likewise, single point mutations in the “quinolone resistance determining region” of the gyrA gene are usually associated with a mechanism of fluoroquinolone resistance [9]. The mutation may occur in gyrA when there is a change in the amino acid sequence either by addition or deletion leading to the development of resistance. The most commonly identified mutation has been a serine to tyrosine substitution at position 87. Less common mutations have been reported as aspartate to tyrosine or glycine at position 87 [10, 11]. Moreover, the reduced susceptibility to the fluoroquinolones has also been reported in Salmonella isolates without any mutations in gyrA gene suggesting an alternative way of resistance. The mutation in gyrA gene is not only responsible for the reduced susceptibility to the fluoroquinolones but also increases the minimum inhibitory concentration (MIC) breakpoint values to fluoroquinolone antibiotics [12]. Hence, it is necessary to evaluate MIC break points to those antibiotics regularly. The susceptibility testing of Salmonella isolates to a variety of antibiotics are equally important to know the current antimicrobial-resistant status and prescribing potential drugs for disease management. This study was therefore conducted to determine the antibiogram patterns of S. Typhi and S. Paratyphi isolated in blood cultures of enteric febrile patients. Our study also estimated the fluoroquinolones resistance due to mutation of gyrA gene at ser83 position using restriction digestion. Finally, we have also recommended some potential antibiotics to manage fluoroquinolones resistant Salmonella infections.

Methods

Study design, duration, and site
A hospital-based cross-sectional study was conducted from February to July 2019 among the clinically suspected patients with enteric fever visiting Sukraraj Tropical and Infectious Disease Hospital, Kathmandu, Nepal.

Inclusion and exclusion criteria
The patients suspected of enteric fever from all sexes of greater than five years who gave written consent to participate in the study were included for data and sample collection. The collected samples with visible signs of contamination or insufficient volume were excluded.

Consent from the patients
Written consent was taken from each participant. The patients’ information including demographic characteristics, travel history and clinical history were collected using a structured questionnaire.

Sample collection
About 8–10ml venous blood sample was collected from each adult patient and 3–5ml from each child patient [13, 14]. A total of 948 blood samples from the patients were processed during the study period. 5ml of blood from each adult patient and 3ml from each child patient were inoculated in a BACTEC culture bottle and incubated at 37°C for up to five days. The remaining blood samples were used for routine laboratory diagnosis including hematology, serology and biochemical investigation. The culture bottles showing bacterial growth were sub-cultured on Mac-Conkey agar (MA), Xylose lysine deoxycholate agar (XLD) and Blood agar (BA). The isolated colonies were further identified as per standard microbiological techniques including colony morphology, staining reaction, biochemical characteristics and serotyping method [15, 16].
Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of Salmonella isolates was performed by using the modified Kirby-Bauer disk diffusion method as recommended by CLSI guidelines. The antibiotics used in this study were ampicillin (AMP, 10 μg), azithromycin (AZM, 15 μg), cefotaxime (CTX, 30 μg), ceftriaxone (CTR, 30 μg), ciprofloxacin (CIP, 5 μg), chloramphenicol (C, 30 μg), cotrimoxazole (COT, 25 μg), levofloxacin (LEV, 5 μg), nalidixic acid (NA, 30 μg) and ofloxacin (OF, 5 μg) from HiMedia [17]. MDR Salmonella species were identified based on the resistance to the three first-line drugs [18].

Minimum inhibitory concentration (MIC) of ciprofloxacin

Salmonella enterica isolates were further subjected to the minimum inhibitory concentration of ciprofloxacin by the agar dilution method using the antibiotic concentration of 1280 μg/ml. Muller Hinton Agar (MHA) plates were prepared with different concentrations of ciprofloxacin antibiotics (0.002 to 256 μg/ml) as per the recommendations in Andrews and CLSI guidelines [17, 19]. Standardized test inoculum was inoculated on antibiotic-MHA plates using a micropipette capable of delivering 2 μl volume with about 10^6 cells per spot. Each test organism was inoculated in triplicate. QC strain was inoculated for the validity of the test for every batch of agar dilution. The plates incubated aerobically at 37 °C for 18 to 20 h, were observed for visible colonies to confirm the growth of the bacterium. Inoculation of the organism in an antibiotic-free plate was undertaken as a control [19].

DNA extraction and PCR amplification of gyrA gene

Genomic DNA was extracted from Salmonella isolates by phenol:chloroform method and the presence of DNA was confirmed on 0.8% agarose gel electrophoresis at 120 V for one hour with 0.1 μg/ml ethidium bromide (EtBr) concentration [20]. The PCR amplification of gyrA gene was carried out using a set of specific primers; gyrA-F (5'- CGA GAG AAA TTA CAC CGG TCA-3') and gyrA-R (5'- AGC CCT TCA ATG CTG ATG TC-3') from Macrogen Inc., Korea [21]. A total of 25 μl PCR reaction mixture volume containing 21 μl of 1X PCR master mix (Qiagen), 3 μl of template DNA, 0.5 μl of gyrA forward primer and 0.5 μl of gyrA reverse primer was used for PCR amplification. DNA from Salmonella ATCC 35664 was positive control and no template as negative control were run simultaneously in each PCR cycle. The gyrA gene amplification was carried out at an initial denaturation of 95 °C for 15 min followed by 30 cycles of denaturation at 94 °C for 60 s, annealing at 57 °C for 90 s, extension at 72 °C for 60 s and a final extension at 72 °C for 7 min [21]. The amplified gyrA gene was confirmed by its molecular size of 610 bp on 1.5% agarose gel electrophoresis with 0.1 μg/ml EtBr concentration.

Digestion of amplified PCR product of gyrA by HinfI

The amplified PCR product of gyrA gene was further digested by using restriction endonuclease; Fast HinfI from Thermofisher Scientific Inc. A total restriction digestion reaction of 30 μl containing 17 μl of nuclease-free water, 2 μl of fast digest green buffer, 10 μl of amplified gyrA product and 1 μl of fast digest HinfI enzyme was prepared in a tube and mixed properly. The tube was incubated at 37 °C in a water bath for five minutes and then placed into another water bath at 65 °C for 20 min for enzyme inactivation following the manufacturer’s guidelines. The digested product was run through 2% agarose gel electrophoresis at 120 V for 60 min and visualized under a UV transilluminator. The digested products into two fragments of molecular sizes; 343 bp and 149 bp were considered as the mutation in gyrA gene while the digested products from non-mutated isolates were revealed into three fragments of 244 bp, 149 bp and 118 bp sizes. The mutated and non-mutated isolates were further compared with nalidixic acid-resistant (NAS) and nalidixic acid-sensitive (NAS) groups.

Data analysis

The preliminary data was managed by MS Excel and later transcribed to Statistical Package for Social Science (SPSS) software (version25) for statistical analysis. A scatterplot diagram to determine the breakpoint of ciprofloxacin MIC was generated using WHONET 5.0.

Results

Total blood cultures and growth results

Out of 948 blood cultures, 76 (8.0%) samples were culture positive, in which collectively 32 (3.4%) samples were found to be positive for Salmonella spp. including Salmonella Typhi (n = 21) and Salmonella Paratyphi A (n = 11). For the remaining samples, 17 (1.8%) samples were positive for Staphylococcus aureus, 20 (2.1%) for Coagulase Negative Staphylococci (CONS) and 7 (0.7%) were positive for Escherichia coli. Since our study was focused on enteric febrile cases by Salmonella isolates, the other isolates were not further processed.

Distribution of enteric fever cases based on the age of the patients

The highest rate of Salmonella was isolated from blood specimens of the age groups 5–20 years (1.6%; 15/945) and 21–35 years (1.6%; 15/945) followed by 36–50 age group (0.2%; 2/945 (p < 0.0001) (Table 1). No Salmonella infection was observed among patients of higher than...
The highest number of *S. Typhi* was isolated from male patients of age 5–20 years (9/32) followed by 21–35 years (6/32). On the other hand, the highest number of *S. Paratyphi A* was observed among male patients of age 21–35 (5/32) years followed by female patients of 5–20 years (2/32) and 21–35 years (2/32) (Table 2).

### Antimicrobial susceptibility pattern of Salmonella isolates

None of the isolates of *Salmonella* were resistant to cefixime followed by 3.1% of the isolates were resistant to the antibiotics; ampicillin, chloramphenicol, cotrimoxazole and ceftriaxone. In the case of fluoroquinolones, 100% of the isolates were resistant to ciprofloxacin while 84.4% of *Salmonella* isolates were resistant to levofloxacin followed by 90.6% resistant to nalidixic acid and 87.5% non-susceptible to ofloxacin (Table 3). Out of 32 *Salmonella* isolates, only one *S. Typhi* was found to be multidrug-resistant.

### Minimum inhibitory concentration value of ciprofloxacin

The scatter plot correlating the MIC values and disk diffusion method of ciprofloxacin suggests the reduced susceptibility of *Salmonella* isolates. Out of nine resistant isolates by disk diffusion (zone of inhibition < 21 mm), eight were resistant to ciprofloxacin by MIC (MIC value ≥ 1 μg/ml) method and one showed reduced susceptibility (0.12–0.5 μg/ml). Similarly, out of 23 isolates that were intermediately resistant to ciprofloxacin by disk diffusion method, only eight isolates were found to be resistant by MIC, 12 isolates with reduced susceptibility and three were sensitive. A large number of isolates were found in the area of ciprofloxacin intermediate with slightly increased MIC values (Fig. 1).

### PCR amplification of gyrA and HinfI restriction digestion analysis of gyrA

GyrA gene of molecular weight of 610bp was amplified in all 32 *Salmonella* isolates (Fig. 2A). Out of 32 isolates, gyrA ser83 mutation was observed in 90.6% [n=22] isolates showing two fragments of size 343bp and 149bp by HinfI digestion. 28.1% of mutated isolates were resistant and 62.5% showed reduced susceptibility to ciprofloxacin. All those isolates were resistant to nalidixic acid. In contrast, gyrA ser83 mutation was not detected in 9.4% [n=3] of *Salmonella* isolates. All three were sensitive to nalidixic acid antibiotic (Table 4, Fig. 2B).

### Discussion

Our study revealed 3.4% culture-positive blood samples to *Salmonella enterica*. This study agreed with a descriptive study done from 2011 to 2013 which reported 3.05%

---

**Table 1** Distribution of blood cultures and number of *S. enterica* isolates based on the age and sex group of patients

| Age group (years) | Number of cases (%) | Number of *S. enterica* isolates (%) | p-value |
|-------------------|---------------------|-------------------------------------|---------|
|                   | Male | Female | Total | Male | Female | Total |         |
| 5–20              | 86 (9.1) | 59 (6.2) | 145 (15.3) | 10 (1.0) | 5 (0.5) | 15 (1.6) | < 0.0001 |
| 21–35             | 236 (24.9) | 136 (14.3) | 372 (39.2) | 11 (1.2) | 4 (0.4) | 15 (1.6) |         |
| 36–50             | 130 (13.7) | 120 (12.7) | 250 (26.4) | 1 (0.1) | 1 (0.1) | 2 (0.2) |         |
| 51–65             | 79 (8.3) | 50 (5.3) | 129 (13.6) | 0 | 0 | 0 |         |
| 66–80             | 25 (2.7) | 21 (2.2) | 46 (4.9) | 0 | 0 | 0 |         |
| 81–95             | 5 (0.5) | 1 (0.1) | 6 (0.6) | 0 | 0 | 0 |         |
| Total             | 561 (59.2) | 387 (40.8) | 948 (100) | 22 (2.3) | 10 (1.1) | 32 (3.4) |         |

**Table 2** Distribution of *S. Typhi* and *S. Paratyphi A* isolates among different ages and sex groups of patients

| Age group (years) | Number of *S. Typhi* | Number of *S. Paratyphi A* | Total *Salmonella* isolates |
|-------------------|----------------------|---------------------------|----------------------------|
|                   | Male | Female | Total | Male | Female | Total |              |
| 5–20              | 9 | 3 | 12 | 1 | 2 | 3 | 15 |
| 21–35             | 6 | 2 | 8 | 5 | 2 | 7 | 15 |
| 36–50             | 0 | 1 | 1 | 1 | 0 | 1 | 2 |
| 51–65             | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 66–80             | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 81–95             | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total             | 15 | 6 | 21 | 7 | 4 | 11 | 32 |
### Table 3  Antimicrobial susceptibility pattern of *Salmonella* isolates

| Generation of antibiotics | Category of antibiotics | Antibiotics | Antibiotic Susceptibility Pattern (n = 32) |
|---------------------------|-------------------------|-------------|------------------------------------------|
|                           |                         |             | Sensitive n (%) | Intermediate n (%) | Resistant n (%) |
| First                     | Fluoroquinolone         | Nalidixic acid (30 μg) | 3 (9.4) | 0 | 29 (90.6) |
| Second                    | Macrolides              | Azithromycin (15 μg) | 30 (93.8) | 2 (6.2) | 0 |
|                           | Fluoroquinolone         | Ciprofloxacin (5 μg) | 0 (0) | 23 (71.9) | 9 (28.1) |
|                           | Chloramphenicol (bacteriostatic) | Chloramphenicol (30 μg) | 31 (96.9) | 0 | 1 (3.1) |
|                           | Trimethoprim            | Cotrimoxazole (25 μg) | 31 (96.9) | 0 | 1 (3.1) |
|                           | Fluoroquinolone         | Ofloxacin (5 μg) | 4 (12.5) | 26 (81.2) | 2 (6.3) |
| Third                     | Aminopenicillins        | Ampicillin (10 μg) | 31 (96.9) | 0 | 1 (3.1) |
|                           | Cephalosporin           | Cefixime (5 μg) | 32 (100) | 0 | 0 |
|                           | Cephalosporin           | Cefotaxime (30 μg) | 23 (71.9) | 4 (12.5) | 5 (15.6) |
|                           | Cephalosporin           | Ceftriaxone (30 μg) | 31 (96.9) | 1 (3.1) | 0 |
|                           | Fluoroquinolone         | Levofloxacin (5 μg) | 5 (15.6) | 25 (78.1) | 2 (6.3) |

**Fig. 1** Scatterplot analysis of MIC values (μg/ml) vs disk diffusion values (Zone of Inhibition in mm) of ciprofloxacin (output from WHONET after analysis). Disk diffusion interpretative range; susceptible: ≥31 mm, intermediate: 21–30 mm, resistant: ≤20 mm. MIC interpretative range; susceptible: ≤0.06 μg/ml, intermediate: 0.12–0.5 μg/ml, resistant: ≥1 μg/ml. The numeric values; 1, 2 and 3 represent the number of *Salmonella* isolates resistant to the specific concentration of ciprofloxacin.
of culture-positive enteric febrile cases from different tertiary care hospitals in Kathmandu, Nepal [23]. However, another study by Adhikari et al. in 2012 reported a higher prevalence (7.6%) of enteric fever with *Salmonella* infections; *S. Typhi* and *S. Paratyphi* A from a tertiary care hospital in Kathmandu, Nepal [24]. The prevalence of enteric fever in Nepal seems to have fluctuated between 3 to 10% for the last decade without a significant increase or decrease in cases. Among age-wise distribution of enteric fever, the greatest number of cases were observed from the age group of 5–20 years and 21–35 years. Pokharel et al. had reported the highest number of enteric fever from the age group of 21–35 years in a hospital at Kathmandu [25]. Likewise, Bhetwal et al. also reported the highest number of *Salmonella* infections (*S. Typhi* and *S. Paratyphi* A) among the children of age 5–15 years in a community-based teaching hospital in Nepal in 2017 [13]. The higher rate of culture positivity among the age group of 5–35 years might be due to poor food hygiene and their dependency on out-sourcing foods [26]. Similarly, personal hygiene might be the reason for the higher infections among children.

We observed none of *Salmonella* isolates were resistant to cefixime and less than 5% resistance to the four antibiotics namely ampicillin, ceftriaxone, chloramphenicol and cotrimoxazole. The results also agree with the antibiogram report presented by Shrestha and Basnet in 2019 at Patan Hospital, Lalitpur, Nepal [27]. Although cefixime is a preferred drug of choice in developing countries due to the availability of an oral form for uncomplicated enteric fever [28], a study by Pandit et al. reported resistance to cefixime in vivo even if the isolates were susceptible in vitro study [29]. Although third-generation cephalosporins were found to be effective drugs for *Salmonella* isolates in our study, the increasing rate of extended-spectrum β-lactamase (ESBL) producing strains among other members of the *Enterobacteriaceae* family have added a huge challenge in empirical therapy [22, 30–32]. Those isolates could be the sources of ESBL genes to *Salmonella* isolates via horizontal transfer. Compared to a study by Maharjan et al. at the same hospital [33], we observed reduced susceptibility to fluoroquinolone antibiotics. The results were also supported by the data of Shrestha and Basnet [27]. The reason might be due to the extensive use of these drugs as therapeutic management of enteric fever. The rate of multidrug-resistant *Salmonella* was consistently low in the last two decades [22, 24, 34]. We also found only one MDR isolate

![Fig. 2 A Visualization of gyrA gene (610 bp) under UV transilluminator after electrophoresis (L: DNA ladder (GeneRuler 100 bp DNA Ladder, Thermo Fisher Scientific), PC: Positive control, NTC: No template control, S1-S13: Different *Salmonella* isolates); B Restriction pattern of gyrA after digestion by Hindl enzyme on UV transilluminator after electrophoresis. (Three fragments = non-mutated, two fragments = mutated) (The Hindl of gyrA gene in the original photo was not much clear mainly for smaller bands of 118 bp, 149 bp and 244 bp, hence, slight modifications including cropping of the image, labeling and increasing the contrast of bands have been done. The original photo can be submitted as supplementary on request)](image)

**Table 4** Distribution of gyrA restriction pattern based on the fluoroquinolone resistance pattern (n = 32)

| Fluoroquinolone resistance phenotypes | NAR/NAS | No. of *Salmonella* spp (%) | MIC value for Ciprofloxacin (μg/ml) | gyrA ser83 mutation |
|--------------------------------------|---------|-----------------------------|-------------------------------------|-------------------|
| CIP<sub>r</sub>                      | NAR     | 9 (28.1)                    | 0.5–16                              | Mutated (+)       |
| CIP<sub>i</sub>                      | NAR     | 20 (62.5)                   | 0.12–2                              | Mutated (+)       |
| CIP<sub>s</sub>                      | NAS     | 3 (9.4)                     | 0.06                                | Non-mutated (−)   |

**Note** CIP<sub>r</sub> = Ciprofloxacin resistant, CIP<sub>i</sub> = Ciprofloxacin intermediate, NAR = Nalidixic Acid Resistant, NAS = Nalidixic Acid Sensitive.
while Maharajan et al. reported no MDR strain out of 40 *Salmonella* isolates in their study [33]. A low rate of MDR was supported by the findings of Britto et al. 2018 [35]. The abstain use of first-line antibiotics as empirical treatment for a longer period might have caused a loss of *Inch* plasmids leading to emerging back to sensitivity towards these drugs [36–38]. In addition, immunization could theoretically reduce the number of circulating MDR [35].

In our study, the scatterplot correlating the MIC and disk diffusion values of ciprofloxacin illustrates the reduced ciprofloxacin susceptibility among *Salmonella* isolates as compared to the studies at the same hospital [33, 39]. Compared to fluoroquinolone sensitive isolates, MIC values were commonly two or more dilutions higher (ciprofloxacin MIC 0.12 to 2 μg/ml for resistant strains compared with 0.5 to 16 μg/ml for intermediate resistant strains and 0.06 μg/ml for sensitive strains). When compared with nalidixic acid disc, the resistant strains showed ciprofloxacin MIC value of greater than 0.12 μg/ml. Nalidixic acid resistance hence serves as a surrogate marker for *gyrA* mutation associated with diminished fluoroquinolone susceptibility [40]. Out of 90.6% of NAR isolates, 40.6% of the *Salmonella* isolates had decreased susceptibility to ciprofloxacin with MIC value of 0.12 to 2 μg/ml and 50% isolates were resistant to ciprofloxacin with MIC value of up to 16 μg/ml. A similar study undertaken in India reported 47.5% resistance and 36.2% decreased susceptibility to ciprofloxacin among 97.5% of NAR *Salmonella* isolates, [41]. Nalidixic acid resistance showed a 100% predictive value for ciprofloxacin resistance as reported by Agrawal et al. [42]. Besides the *gyrA* gene mutation that occurred usually in the bacterial chromosome, the plasmid-mediated quinolone resistance mechanisms cannot be ignored [43].

Ser-83 mutation is the most commonly occurring point mutations among fluoroquinolone resistance strains. Over 90% of *gyrA* ser83 mutation was observed in *Salmonella* strains in our study which were similar to the studies by Khadka et al., 2021, Gopal et al. 2016 and Renuka et al. 2004 showing mutation rates of 95.7, 94 and 92.1% respectively [39, 44, 45]. Complete resistance or reduced susceptibility to fluoroquinolones is due to the mutation at ser-83 or asp-87 position of *gyrA* [35]. The mutations in the quinolone resistance determining regions of chromosomal genes such as *gyrA*, *gyrB*, *parC* and *parE* and plasmid-mediated *qnr*, *qepA* and *aacs* genes cause fluoroquinolones not to act and induce resistance in *Salmonella* spp. [43]. In our study, we observed that the strains with reduced susceptibility or complete resistance to fluoroquinolones retained ser-83 mutation. However, the isolates resistant to nalidixic acid and ciprofloxacin may have two or more mutations in the *gyrA*, *gyrB*, *parC*, or *parE* gene [46] which were not investigated in this study. In the case of patients with reduced susceptibility to fluoroquinolones for enteric fever, the infections can be managed with a higher dose of fluoroquinolones for a longer duration of time [47].

As a major study limitation, we only focused on *Salmonella* isolates and didn’t process for other bacterial pathogens isolated on the blood culture. Although there are many other mechanisms by which *Salmonella* may develop resistance to fluoroquinolones, due to limited time and budget, we couldn’t analyze all those mechanisms. We could only investigate *gyrA* ser83 mediated mutation among the isolates using restriction endonuclease *HinfI*. In addition, our study has shown the recent trend of the antimicrobial-resistant pattern of *Salmonella* isolates and suggested potential drugs for antimicrobial therapy.

Conclusions

Since fluoroquinolones are the drugs of choice for the management of enteric fever, especially caused by *S. Typhi* and *S. Paratyphi* A in most developing countries, the decreased susceptibility to fluoroquinolones along with the accumulation of mutation in the ser83 position of *gyrA* gene may pose a threat to the disease management. In conclusion, our study suggests reintroducing ampicillin, chloramphenicol and cotrimoxazole drugs as empirical treatment for enteric fever as well as the use of third generation cephalosporins.

**Abbreviations**

AST: Antibiotic Susceptibility testing; ATCC: American Type Culture Collection; BA: Blood Agar; CLSI: Clinical Laboratory Standard Institute; DOHS: Department Of Health Service; ESBL: Extended-Spectrum ß-Lactamase; MA: Mac-Conkey Agar; MDR: Multidrug Resistance; MHA: Mueller Hinton Agar; MIC: Minimum Inhibitory Concentration; min: minute/s; NAR: Nalidixic acid-resistant; NAS: Nalidixic acid-sensitive; PCR: Polymerase chain reaction; QRDR: Quinolone Resistant Determining Region; RFLP: Restriction Fragment Length Polymorphism; SPSS: Statistical Package for Social Science; STIDHH: Sukraraj Tropical and Infectious Disease Hospital; WHO: World Health Organization; XLD: Xylose Lysine Deoxycholate Agar.

**Acknowledgments**

We would like to express our sincere gratitude to all laboratory and technical staff at Microbiology Laboratory, Sukraraj Tropical and Infectious Disease Hospital and Tri-Chandra Multiple Campus for their generous support throughout the research work. We would also like to thank Dr. Gaye Procter; Training Consultant, Mahidol Oxford Tropical Medicine Research Unit, Mahidol University for English language correction.

**Authors’ contributions**

All authors have made substantial contributions to the conception of the study, methodology and interpretation of data. All authors have drafted the work and substantively revised it. All authors have approved the submitted version of the manuscript and agreed to publish it.

**Funding**

The funding supports for this research work and publishing it is not available.
Availibility of data and materials
The datasets used and/or analyzed during the current study will be available from the corresponding author on reasonable request at upendras@gmail.com.

Declarations

Ethics approval and consent to participate
The study was reviewed and approved by the Institutional Review Committee (IRC), Nepal Health Research Council (NHRC) on the registration number 666/2019. A copy of the information sheet and consent form was given to participants to obtain written consent before enrollment in the research and collecting samples. A local language-translated information sheet was read for illiterate participants. Informed consent was obtained from all the participants and in the case of children under 16 years, both written informed consent with assent was obtained from a parent or guardian attending the hospital along with the participant. All the methods were carried out in accordance with the principles stated in the Declaration of Helsinki.

Consent for publication
Not applicable.

Competing interests
We declare no competing interests.

Author details
1 Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal. 2 Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal. 3 Sukraraj Tropical and Infectious Disease Hospital, Teku, Kathmandu, Nepal.

Received: 18 October 2021   Accepted: 25 January 2022
Published online: 10 February 2022

References
1. Wain J, Hendriksen RS, Mikoleit ML, Keddy KH, Ochial RL. Typhoid fever. Lancet. 2015;385(9973):1136–45.
2. WHO. Typhoid and other invasive salmonellosis. WHO Vaccine–Preventable Diseases Surveillance Standards. 2018.
3. DoHs. Annual-Report-FY-2074-75. Department of Health Services. 2018; Ministry of Health and Population, Government of Nepal.
4. Daga MK, Sarin K, Sarkar R. A study of culture positive multidrug resistant enteric fever changing pattern and emerging resistance to ciprofloxacin. J Assoc Physicians India. 1994;42:599–600.
5. Parry C, Wain J, Chinh NT, Vinh H, Farrar JJ. Quinolone-resistant Salmonella typhi in Vietnam. Lancet. 1998;351:1289.
6. Threlfall EJ, Ward LR. Decreased susceptibility to ciprofloxacin in Salmonella enterica serotype typhi, United Kingdom. Emerg Infect Dis. 2001;7:448–50.
7. Parry C, Wain J, Chinh NT, Vinh H, Farrar JJ. Quinolone-resistant Salmonella typhi in Vietnam. Lancet. 1998;351:1289.
8. Klemm EJ, Shakoor S, Page AJ, Qamar FN, Judge K, Saeed DK, et al. Characterization of the quinolone resistance mechanism in foodborne Salmonella isolates with high nalidixic acid resistance. Int J Food Microbiol. 2011;146(1):52–6.
9. Parry CM, Threlfall EJ. Antimicrobial resistance in typhoidal and nontyphoidal salmonellae. Curr Opin Infect Dis. 2008;21(5):531–8.
10. Andrews JM. Determination of minimum inhibitory concentrations. J Antimicrob Chemother. 2001;48(Suppl 1):5–16.
11. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: a laboratory manual, 1989.
12. Kim KY, Park JH, Kwak HS, Woo GJ. Characterization of the quinolone resistance mechanism in foodborne Salmonella enterica serotypes Typhi and Paratyphi a from blood isolates in Nepal: surveillance of resistance and a search for newer alternatives. Int J Infect Dis. 2006;10(6):434–8.
13. Bhetwal A, Maharjan A, Khanal PR, Parajuli NP. Enteric fever caused by Salmonella enterica Serovars with reduced susceptibility of Fluoroquinolones at a community based teaching Hospital of Nepal. Int J Microbiol. 2017;2017:2869458.
14. Wain J, Deep TS, Bay PV, Walsh AL, Vinh H, Duong NM, et al. Specimens and culture media for the laboratory diagnosis of typhoid fever. J Infect Dev Ctries. 2008;2(6):469–74.
15. Cheesebrough M. District laboratory practice in tropical countries; 2006.
16. WHO. Diagnosis treatment prevention of typhoid fever: World Health Organization; 2003.
17. Clinical Laboratory Standards Institute (CLSI): Performance standards for antimicrobial susceptibility testing. 30th ed. CLSI supplement M100 (ISBN 978-1-68440-066-9 [Print]; ISBN 978-1-68440-067-6 [Electronic]. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA. 2020.
18. Parry CM, Threlfall EJ. Antimicrobial resistance in typhoidal and nontyphoidal salmonellae. Curr Opin Infect Dis. 2008;21(5):531–8.
19. Andrews JM. Determination of minimum inhibitory concentrations. J Antimicrob Chemother. 2001;48(Suppl 1):5–16.
20. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: a laboratory manual, 1989.
21. Klemm EJ, Shakoor S, Page AJ, Qamar FN, Judge K, Saeed DK, et al. Characterization of the quinolone resistance mechanism in foodborne Salmonella isolates with high nalidixic acid resistance. Int J Food Microbiol. 2011;146(1):52–6.
22. Parry C, Wain J, Chinh NT, Vinh H, Farrar JJ. Quinolone-resistant Salmonella typhi in Vietnam. Lancet. 1998;351:1289.
23. Adhikari A, Sapkota S, Bhattachar J, Raghubanshi B. Antimicrobial resistance trend of Salmonella typhi and paratyphi from 2011–2013: a descriptive study from tertiary care hospital of Nepal. J Kathmandu Med Coll. 2017;6(1):5.
24. Adhikari D, Acharya D, Shrestha P, Amaty R. Ciprofloxacin susceptibility of Salmonella enteric serovar Typhi and Paratyphi a from blood samples of suspected enteric fever patients. Int J Infect Microbiol. 2012;1(1):5.
25. Parikh P, Rai S, Karki G, Khatiwada V, Vithal K, Shrestha B, et al. Biofilm formation and phenotypic detection of ESBL, MBL, KPC producing Salmonella enterica serovar Typhi Clone Harboring a Promiscuous Plasmid Encoding Resistance to Fluoroquinolones and Third-Generation Cephalosporins. mBio. 2018;9(1):e00105–18.
26. Klemm EJ, Shakoor S, Page AJ, Qamar FN, Judge K, Saeed DK, et al. Emergence of an Extensively Drug-Resistant Salmonella enterica Serovar Typhi Clone Harboring a Promiscuous Plasmid Encoding Resistance to Fluoroquinolones and Third-Generation Cephalosporins. mBio. 2018;9(1):e00105–18.
27. Jacoby GA. Mechanisms of resistance to quinolones. Clin Infect Dis. 2005;41:7.
28. Brown JC, Shanahan PM, Jesudason MV, Thomson CJ, Amyes SG. Mutations responsible for reduced susceptibility to 4 quinolones in clinical isolates of multi resistant Salmonella typhi in India. J Antimicrob Chemother. 1996;37:891–900.
29. Wain J, Hoa NT, Chinh NT, Vinh H, Everett MJ, Deep TS, et al. Quinolone resistant Salmonella typhi from Vietnam: molecular basis of resistance and clinical response to treatment. Clin Infect Dis. 1997;25:1404–10.
30. Griggs DJ, Hall MC, Jin YF, Piddock LJ. Quinolone resistance in veterinary isolates of Salmonella. J Antimicrob Chemother. 1994;33:1173–89.
34. Joshi S, Amarnath SK. Fluoroquinolone resistance in Salmonella typhi and S. paratyphi a in Bangalore, India. Trans R Soc Trop Med Hyg. 2007;101(3):308–10.
35. Britto CD, Dyson ZA, Duchene S, Carter MJ, Gurung M, Kelly DF, et al. Laboratory and molecular surveillance of paediatric typhoidal Salmonella in Nepal: antimicrobial resistance and implications for vaccine policy. PLoS Negl Trop Dis. 2018;12(4):e0006408.
36. Zellweger RM, Basnyat B, Shrestha P, Prajapati KG, Dongol S, Sharma PK, et al. A 23-year retrospective investigation of Salmonella Typhi and Salmonella Paratyphi isolated in a tertiary Kathmandu hospital. PLoS Negl Trop Dis. 2017;11(11):e0006051.
37. Badyal A, Kumar Y, Sharma A, Mani KR. Re-emergence of chloramphenicol sensitive isolates of Salmonella enterica serovar typhi isolates in India during 2013–14. Int J Curr Microbiol App Sci. 2015;4:6.
38. Chand HI, Rijal KR, Neupane B, Sharma VK, Jha B. Re-emergence of susceptibility to conventional first line drugs in Salmonella isolates from enteric fever patients in Nepal. J Infect Dev Ctries. 2014;8(11):1485–7.
39. Khadka S, Shrestha B, Pokhrel A, Khadka S, Joshi RD, Banjara MR. Antimicrobial resistance in Salmonella Typhi isolated from a referral Hospital of Kathmandu, Nepal. Microbiol Insights. 2021;14:1–8.
40. Enriquez R, Abad R, Salcedo C, Vazquez JA. Nalidixic acid disk for laboratory detection of ciprofloxacin resistance in Neisseria meningitidis. Antimicrob Agents Chemother. 2009;53(2):796–7.
41. Bhagra S, Sood A, Singh D, Kang A. Increased resistance to Nalidixic acid and Ciprofloxacin in Salmonella isolates from the Sub Himalayan region. Int J Res Med Sci. 2017;5(9):4025–9.
42. Agrawal P, Tuladhar R, Dahal N. Nalidixic acid susceptibility test for screening Salmonella isolates of reduced susceptibility/Higher minimum inhibitory concentration to ciprofloxacin. Nepal J Sci Technol. 2014;15(2):8.
43. Hopkins KL, Day M, Thrall J, Plasmid-mediated quinolone resistance in Salmonella enterica, United Kingdom. Emerg Infect Dis. 2008;14(2):340–2.
44. Gopal M, Elumalai S, Arumugam S, Durairajapandian V, Kannan MA, Selvam E, et al. GyrA ser83 and ParC trp106 mutations in Salmonella enterica Serovar Typhi isolated from typhoid fever patients in tertiary care hospital. J Clin Diagn Res. 2016;10(7):DC14–8.
45. Renuka K, Kapil A, Kabra SK, Wrig N, Das BK, Prasad VV, et al. Reduced susceptibility to ciprofloxacin and gyrA gene mutation in north Indian strains of Salmonella enterica serotype Typhi and serotype Paratyphi a. Microb Drug Resist. 2004;10(2):146–53.
46. Turner AK, Nair S, Wain J. The acquisition of full fluoroquinolone resistance in Salmonella Typhi by accumulation of point mutations in the topoisomerase targets. J Antimicrob Chemother. 2006;58(4):733–40.
47. Koirala S, Basnyat B, Aryal A, Shilpakar O, Shrestha K, Shrestha R, et al. Gatifloxacin versus ofloxacin for the treatment of uncomplicated enteric fever in Nepal: an open-label, randomized, controlled trial. PLoS Negl Trop Dis. 2013;7(10):e2523.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.