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Lower Susceptibility to Fluoroquinolones and a *gyrA* Gene Mutation in *Salmonella* Typhi Isolates from Enteric Fever Patients

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ABSTRACT

Background: Enteric fever remains a major cause of morbidity and mortality in Nepal. The emergence of multi-drug resistant *Salmonella* is a challenge to the clinician to care for patients with enteric fever. This study assessed the antibiotic susceptibility of *Salmonella* Typhi isolated from enteric fever and the presence of *gyrA* gene mutation at ser83 of *S.* Typhi.

Methods: Blood samples (n = 834) from suspected enteric fever patients were collected and cultured to identify *Salmonella* Typhi. Antimicrobial sensitivity test was performed by the modified Kirby Bauer disc diffusion method. The minimum inhibitory concentration (MIC) tests for ofloxacin and ciprofloxacin were examined by the agar dilution method. The *gyrA* gene was amplified by PCR and restriction enzyme digestion was performed to evaluate the ser83 mutation.

Results: Among 824 blood samples analyzed, 5.1% (42/824) were culture positive for *S.* Typhi. First-line antibiotics chloramphenicol and co-trimoxazole showed higher *in-vitro* efficacy compared to amoxicillin. Macrolides (azithromycin) and third-generation cephalosporins (ceftriaxone, cefixime, and cefotaxime) were highly effective against *S.* Typhi. Nalidixic acid resistance (NAR) was observed in 95.2% (40/42) isolates, among them, all (40/40) isolates harbored mutant *gyrA* gene at ser83. However, none of the nalidixic acid-sensitive *Salmonella* isolates was positive for *gyrA* mutation at ser83.

Conclusions: This study showed decreased susceptibility to fluoroquinolones and the presence of *gyrA* mutation at ser83 position in majority of *S.* Typhi isolates which highlights the importance of alternate drugs as empirical therapy for the treatment of enteric fever patients. So, the clinician should focus on prescribing conventional first-line antibiotics for the treatment of typhoid patients after higher cohort and extended follow-up studies.

Keywords: Antibiotics susceptibility; fluoroquinolones; gyrA gene; nalidixic acid; Salmonella Typhi

INTRODUCTION

Enteric fever is highly prevalent in Nepal. Because isolates with higher nalidixic acid resistance and reduced susceptibility to fluoroquinolones have been reported, it's vital to look into the genetic features of local serovars of S. Typhi, the causative agent of enteric fever.[1,2] Recent reports of fully fluoroquinolone-resistant S. Typhi[3] are of great concern. Such strains prolong fluoroquinolone treatment and limit the therapeutic options.[4] The antibiotic susceptibility pattern of bacteria can fluctuate spatially and temporally.[5] Since the available treatment options for enteric fever are shrinking, monitoring the antibiotic resistance of S. Typhi is critical to guiding treatment policies in Nepal. Therefore, this study intended to determine the antibiotic susceptibility pattern (AST) and the presence of ser83 point mutation in S. Typhi isolated from patients with suspected enteric

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fever attending a tertiary care hospital in Kathmandu, Nepal.

METHODS

Participants included in the study were patients defined by physicians as having a probable case of enteric fever (with fever ≥ 38 °C) that has lasted for at least three days and showing clinical signs and symptoms of enteric fever.[6] Blood samples with incorrect labeling, insufficient blood volume, improper collection and transportation, and repeated specimens were all rejected.

A hospital-based cross-sectional study was carried out among the patients attending Kathmandu Model Hospital from June to August 2018. A total of 824 blood samples from suspected enteric fever patients were included in this study from eligible and interested participants.

Demographic data and clinical history of individuals were recorded using a structured questionnaire. Approximately 3 ml of pediatric blood and 5 ml of adult blood were aseptically collected and inoculated into Brain-heart Infusion Broth (BHI) with blood/BHI ratios of 1: 5 and 1:10, respectively. And bottles were incubated at 37°C for 7 days to observe the growth until discarded.[7] Subculture was performed on xylose lysine deoxycholate (XLD) agar and MacConkey agar (MA) every 24h. *Salmonella* Typhi isolates were identified by colony morphology, Gram`s staining, and a series of biochemical tests (catalase test, oxidase test, sulfide motility and indole (SIM) test, methyl-red and Voges-Proskauer (MR-VP) test, citrate utilization test, triple sugar iron (TSI) agar test, and urea hydrolysis test). Serotyping by agglutination with specific antisera [8] for O, H, and Vi antigens were performed following the manufacturer's instruction (Denka Seiken Co. Ltd, Tokyo, Japan) to identify and confirm the serotype.

Antibiotics susceptibility test (AST) was performed by modified Kirby Bauer disc diffusion method on Mueller-Hinton agar (MHA) using the guidelines and interpretive criteria of the Clinical and Laboratory Standard (CLSI).[9] The antibiotic discs used were amoxicillin (10 µg), nalidixic acid (30 µg), ofloxacin (5 µg), ciprofloxacin (5 µg), azithromycin (15 µg), chloramphenicol (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), levofloxacin (5 µg), cefixime (5 µg) and cotrimoxazole (25 µg) (HiMedia, India).

The MIC of ciprofloxacin and ofloxacin (HiMedia, India) for isolated S. Typhi were determined by the agar dilution method as suggested by Clinical and Laboratory Standards Institute (CLSI) guideline (CLSI 2018, M07).[10] Dilution range 0.03-256 µg/mL was used for both ciprofloxacin and ofloxacin.

Genomic DNA of *S.* Typhi was extracted by phenol: chloroform: isoamyl alcohol method as described elsewhere.[11] Briefly, a loopful of bacterial suspension was transferred to a tube containing 0.5 mL distilled water and centrifugation for 10 min at 12,000 rpm. The pellet was resuspended in 700 µL of lysis buffer, treated with 10 µL of proteinase K and 2 µL of RNase, and submerged for 2 h in a water bath at 37ºC. Then, 712 µL of phenol: chloroform: isoamyl alcohol (25:24:1 ratio) was added and centrifuged for 10 min at 13,000 rpm. The aqueous portion was transferred to a fresh tube and washed with chloroform. Two volumes of isopropanol were added to the supernatant and the suspension was centrifuged for 10 min at 13,000 rpm. The bacterial DNA was precipitated with 2 volumes of ice-cold ethanol (70%) and was centrifuged for 10 min at 13,000 rpm. The DNA pellet was air-dried and redissolved in 50 µL TE buffer and stored at -20°C until further use.

Previously described 2000, to April, 2003, in a North Indian hospital.[12] A total of 422 culture-positive cases of enteric fever were reported to the hospital during the period of study, of which S. Typhi was isolated from 350 cases and S. Paratyphi A from 72 cases. The antimicrobial susceptibility of these strains was determined by disk diffusion and agar dilution method according to NCCLS guidelines, and E-test method. A total of 140 randomly selected strains, isolated during the years 1993-1999, that were available from the laboratory stocks were also studied to compare with the present strains. To study the quinolone susceptibility, the strains were divided into nalidixic acid sensitive (NAS primer pair (F: 5'-ATG AGC GAC CTT GCG AGA GAA ATT ACA CCG-3' and R: 5'-TTC CAT CAG CCC TTC AAT GCT GAT GAT GTC TTC-3') was used for amplification of *gyrA* gene, yielding an amplicon size of 630bp. A 25 µL reaction mixture of was prepared to consist of three µL of template DNA, seven µL of nuclease-free water, 13 µL of master mix (Thermo Fisher Scientific, Waltham, MA, USA), and one µL each of forward and reverse primers (Eurofins India, Eurofins Scientific). After the initial denaturation at 94°C for 10 min, the target gene was amplified by a total of 30 cycles, each cycle consisting of denaturation at 94°C for 35 sec, annealing at 60°C for 90 sec, and extension at 72 °C for one min and final extension at 72°C for 10 min.

Two µL of bovine serum albumin and two µL of restriction buffer were added to 15 µL of the amplified product, followed by the addition of 10 U of *Hin*fI (Promega Corp. USA), and gently mixed. The digestion was allowed for

1h at 37ºC and was resolved by two percent agarose gel electrophoresis at 120 volts for 45 min and visualized under an ultra-violet illuminator. Wild type *S.* Typhi with no mutation at a target site (ser83) of *gyrA* was expected to reveal four bands of fragments 244bp, 149bp, 138bp, and 99bp, while the *S.* Typhi with the mutation was expected to have three bands of 343bp, 149bp and 138bp.[13]

All the collected data were entered into and analyzed with the R-programming (v 1.2.5033, The R Foundation) and statistical package for the social sciences (SPSS 21.0, IBM NY, USA). A chi-squared (χ^2), a non-parametric test was used to evaluate apparent differences for significance. Results were considered significant if $P<0.05$*.

This study was approved by the institutional review committee of public health concern trust (PHECT-Nepal), IRC No. MT 09-2018. The study was carried out without any potential bias in compliance with the Helsinki Declaration. Written informed consent was obtained from the patients, or their parents/guardians for patients younger than 16 years.

RESULTS

Among 824 blood specimens cultured from clinically suspected enteric fever patients, 42 were found to be culture positive for *Salmonella* Typhi. Among 42 culture positives, 22 isolates were from males. There was no significant association between the incidence of enteric fever and the gender of the patient (χ^2 p-value=0.77) (Figure 1A). The mean age of 42 patients was 19.07±6.10 years. The highest (50%) percentage of growth was observed in the age group 11-20 years, while 7.1% of positive cultures for *S.* Typhi were from patients ≤10 years (χ^2 p-value=0.00023*) (Figure 1B). Most of the *Salmonella* isolates were isolated in July (n=20) and which corresponds to the peak of the rainy season in Nepal. The least number of *Salmonella* isolated were recovered during August (n=9) and September (n=13) (χ^2 p-value=0.093) (Figure 1C).

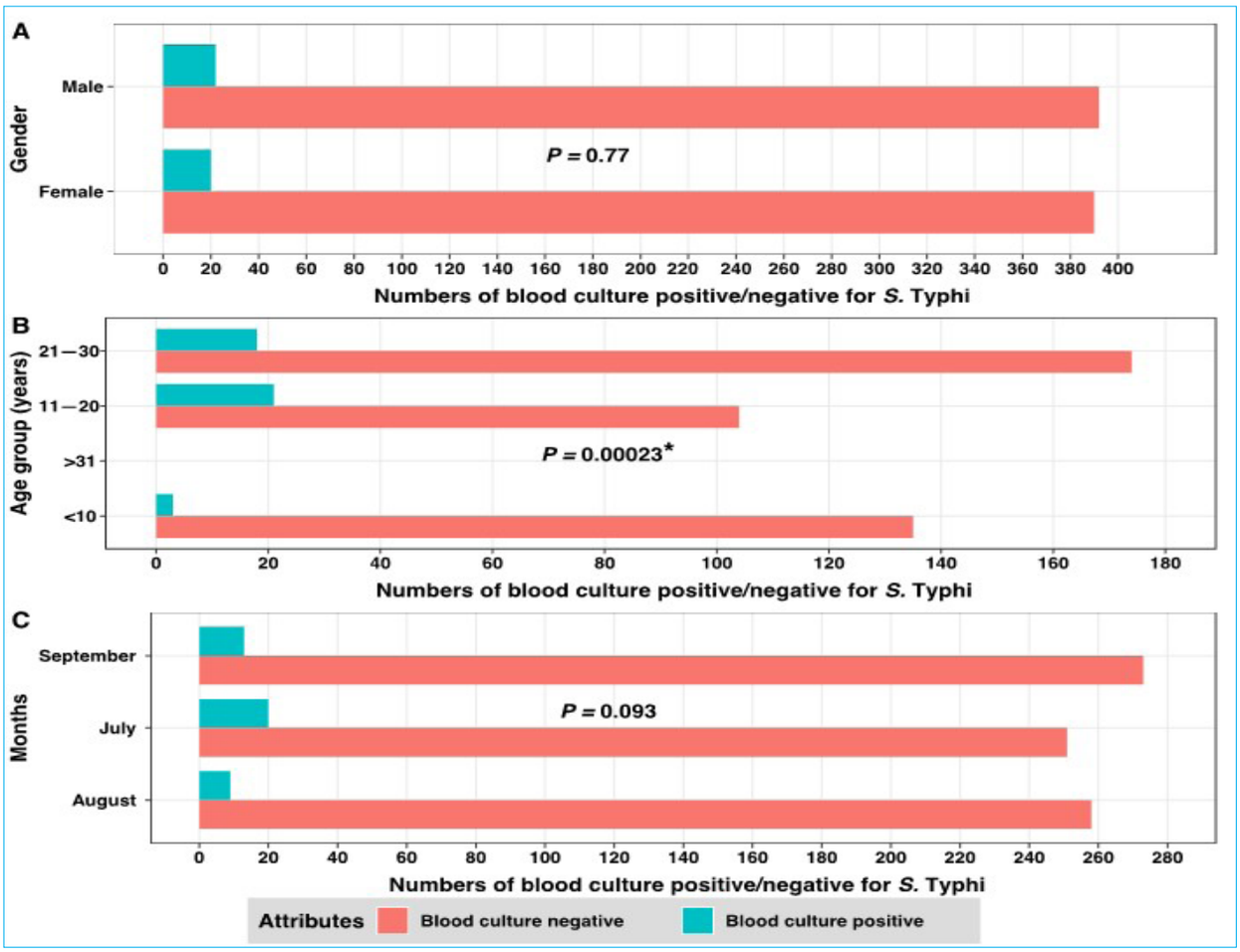

Figure 1. Distribution of S. Typhi according to the gender (A) and age (B) of enteric fever suspected patients, and months of sample collection (C).

Table 1. Susceptibility pattern of *Salmonella* Typhi to routine antibiotics by disk diffusion method.

Antibiotics	Generations	Class of antibiotics	Mode of action	Antibiotic susceptibility pattern (n=42)		
				Sensitive, n(%)	Intermediate, n(%)	Resistant, n(%)
Amoxicillin (10 µg)	-	Aminopenicillins	Cell wall synthesis inhibitors	36 (85.8)	3 (7.1)	3 (7.1)
Cefixime (5µg)	3rd	Cephalosporins		41 (97.6)	0 (0.0)	1 (2.4)
Cefotaxime (30 µg)				39 (92.9)	0 (0.0)	3 (7.1)
Ceftriaxone (30 µg)				42 (100.0)	0 (0.0)	0 (0.0)
Chloramphenicol (30 µg)	-	Chloramphenicol	Protein synthesis inhibitors	38 (90.5)	0 (0.0)	4 (9.5)
Azithromycin (15 µg)	-	Macrolides		38 (90.5)	0 (0.0)	4 (9.5)
Cotrimoxazole (25µg)	-	Sulfonamides	Nucleic acids and proteins biosynthesis inhibitors	39 (92.9)	0 (0.0)	3 (7.1)
Levofloxacin (5 µg)	2nd	Fluoroquinolones	DNA synthesis inhibitors	7 (16.7)	30 (71.4)	5 (11.9)
Nalidixic acid (30 µg)	1st			2 (4.8)	0 (0.0)	40 (95.2)
Ciprofloxacin (5 µg)	2nd			5 (11.9)	25 (59.5)	12 (28.6)
Ofloxacin (5 µg)	2nd			7 (16.7)	35 (83.3)	0 (0.0)

Table 2.Comparison of disk diffusion and MIC of Ciprofloxacin and Ofloxacin.

Antibiotics	Disk-diffusion			MIC		
	Sensitive n(%)	Intermediate n(%)	Resistance n(%)	Sensitive n(%)	Intermediate n(%)	Resistance n(%)
Ciprofloxacin	5 (11.9)	25 (59.5)	12 (28.6)	2 (4.7)	33 (78.6)	7 (16.7)
Ofloxacin	7 (16.7)	35 (83.3)	0 (0.0)	3 (7.1)	33 (78.6)	6 (14.3)

All of the isolates were susceptible to ceftriaxone and >90% of susceptibility was observed against cefixime, cefotaxime, cotrimoxazole, azithromycin, and chloramphenicol. About 95% of isolates were resistant to nalidixic acid. The non-susceptibility to ciprofloxacin was observed in 88.1% (intermediate-59.5%, resistant 28.6%) of the isolates (Table 1).

For ciprofloxacin, 25 isolates had a MIC value of 0.25 µg/mL, six isolates had 16 µg/mL, and five isolates had 0.5 µg/mL MIC value but for ofloxacin, 27 isolates had a MIC value of one µg/mL, six isolates had 16 µg/mL and five isolates had 0.5 µg/mL MIC value (Figure 2, Table 2).

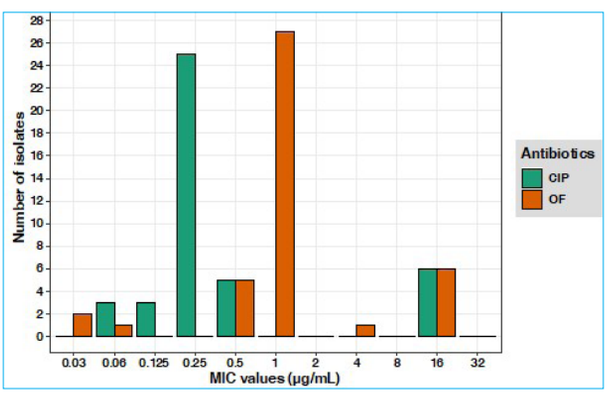

Figure 2. MIC of ciprofloxacin and ofloxacin against *S.* Typhi.

Interpretative range - CIP susceptibility: ≤ 0.06 µg/mL, resistant: ≥ 1 µg/mL; OF susceptibility: ≤ 0.12 µg/mL, resistant: ≥ 2µg/mL; CIP: Ciprofloxacin, OF: Ofloxacin [10].

The numbers of ciprofloxacin-resistant isolates recovered from the disk-diffusion test were found higher than in the MIC test. The MIC test revealed a larger frequency of ofloxacin-resistant isolates (14.3%) while none of the isolates was found resistant to ofloxacin in the disk-diffusion test (Table 2).

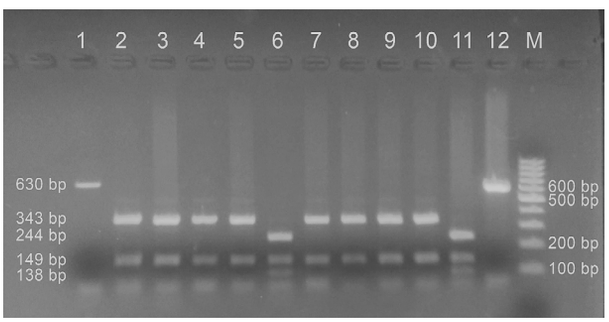

Figure 3. A representative photograph of agarose gel electrophoresis of *Hinf*I restriction digested PCR product, Lane 1 and 12 PCR product of 630bp *gyrA* gene, Lane 2 to 5 and lane 7-10 digested products revealing mutation at *gyrA* ser83, Lane 6 and 11 digested products revealing no mutation at ser83. Lane M: 100bp DNA marker.

The PCR amplified *gyrA* gene fragment of 630 bp consists of three *Hinf*I restriction sites. The restriction digestion of the *gyrA* PCR product showed that 40 S. Typhi isolates were positive for ser83 mutation. All the isolates with ser83 point mutation were nalidixic acid-resistant isolates (Figure 3).

DISCUSSION

Enteric fever is still a common cause of febrile disease in Nepal, and instances are frequently linked to inadequate sanitation and sewage pollution of food and drinking water.[14] *Salmonella* serovars are regarded as the most common etiological agent of bloodstream infection in febrile patients in Nepal.[15] This study examined the mutated *gyrA* gene at ser83 and fluoroquinolones resistance *Salmonella enterica* serovar Typhi from the patients with suspected enteric fever. Only 5% of the analyzed specimens in this study were culture positive for S. Typhi. Although we did not survey the prior antibiotic use by the patients, the lower culture positivity in this study could be attributable to the prior antibiotic usage and small blood volume (3 mL for children) utilized for culture. Britto et al 2018,[16] however, isolated *Salmonella* only from 1.1% of cultured blood in a hospital-based study in Nepal. Furthermore, apart from *Salmonella*, other pathogens are also associated with febrile illness in Nepal.[16-18] Interestingly, we did not recover S. Paratyphi during this study which might be due to the dominance of S. Typhi over S. Paratyphi, which is reported in multiple studies from Nepal even though S. Paratyphi is also a noticeable cause of enteric fever in Nepal.[16,17,19,20] This dominance can be attributed to the water-borne transmission of S. Typhi which requires smaller inoculum compared to S. Paratyphi is primarily transmitted through contaminated food requiring larger inoculum. [7]

We recovered more S. Typhi from male patients compared to females. However, there was no significant difference (p-value>0.05) between the gender of patients and culture positivity. Most of the S. Typhi (92.9%) were isolated from the age group 11-30 years. This apparently higher culture-positive enteric fever among males and adults may be associated with their eating habits who frequently consume contaminated food and water from restaurants,[19] and in essence, males have a less positive attitude towards hygiene compared to females.[21] Good sanitation and hygiene practices can limit the transmission cycle of enteric fever.

Coherent with previous studies from Nepal,[5,16,19] most of the isolates from this study were sensitive to first-line antibiotics (ampicillin, chloramphenicol, cotrimoxazole). As reported by Khanal et al 2017,[22] a few of the isolates were MDR (resistant to first-line drugs). Although the MDR S. Typhi has been reported previously in Nepal, [22]recent literature reveals the decreasing trend of MDR S. Typhi serovar in Nepal.[5,16] As hinted by this study, previous studies[23,24] have also reported the reemergence of S. Typhi susceptibility to first-line drugs in previously resistant areas, suggesting the possibility to shift to these first-line drugs for the treatment of enteric fever caused by FQ resistant[16] S. Typhi serovars. This reemergence of susceptibility could be due to a decline in the usage of first-line antibiotics against *Salmonella*.[23] However, due to the circulation of plasmid-mediated MDR S. Typhi,[25] the probability of reemergence of MDR *Salmonella* cannot be neglected[16,20] with reversion to the prescription of first-line drugs.

Even though this study did not observe cephalosporin non-susceptibility to a considerable extent among our isolates, it is possible that they could emerge when the plasmid-encoded extended-spectrum beta-lactamase genes are acquired, as observed among S. Typhi isolates from the neighboring country India.[26] For quinolone-resistant S. Typhi and S. Paratyphi A, the World Health Organization recommends azithromycin, ceftriaxone, or cefixime, [7] however, a high rate of treatment failure has been reported with cefixime in a trial study from Nepal,[20] making azithromycin or ceftriaxone a good option for culture-confirmed enteric fever.[20,27]

An alarming rate of nalidixic acid resistance (95.2%) was reported in this study, however, others have reported relatively lower nalidixic acid resistance[5,22] from Nepal. Britto et al. (96% of S. Typhi)[16] and Andrews et al. (99% of typhoidal *Salmonella*)[17] reported a relatively higher rate of non-susceptibility to ciprofloxacin than observed in our study (88%). The introduction of NAR strains and the indiscriminate use of FQs as the empirical antimicrobial agent might have contributed to increasing incidences of isolates with reduced resistance to ciprofloxacin and ofloxacin.[16] Due to the higher prevalence of FQs non-susceptibility, fluoroquinolones would not make a better empirical treatment option in Nepal.[27] Most of S. Typhi isolates in this study had a ciprofloxacin-MIC of 0.25 µg/mL, however, Khanal et al 2017[22] found a majority of S. Typhi (51.4%) had a MIC of 0.06 µg/mL for ciprofloxacin. The advent of H58 strains having mutation in *gyrA* and *parC* genes is associated with a rise in MIC[27] and with nalidixic acid resistance.[4] A recent report suggests that an H58 S. Typhi has been introduced into Nepal[27] and is associated with longer fever clearance times (FCTs) and treatment failure in patients treated with the FQ, gatifloxacin.[20,27] In the current study, most of the S. Typhi

isolates (95.2%) had ser83 mutation. All the isolates with ser83 point mutation were nalidixic acid-resistant. The most common mutations associated with quinolone resistance in the *gyrA* gene are located in amino acids serine-83 or aspartic acid-87.[28,29] In *Salmonella enterica* serovar Typhi and Paratyphi, mutations in the *gyrA* gene that cause quinolone resistance and lower sensitivity to fluoroquinolones are clinically significant because they increase the probability of treatment failure and poor response to treatment. [30]

This research only reported the *gyrA* gene mutations in *S.* Typhi while other genes are also found conferring resistance to the arrays of antibiotics. Likewise, the study was conducted in a single hospital for a shorter duration of time, and isolates were not characterized using *16S* rRNA sequencing due to the cost and unavailability of sophisticated tools. Therefore, to overcome the present shortcomings, in the future, a longitudinal study should be carried out in several hospitals for a longer period and multiple genes of *S.* Typhi should be studied to trace the association with the decreased susceptibility to the antibiotics.

CONCLUSIONS

The increasing trend of fluoroquinolones resistance by *S.* Typhi and *S.* Paratyphi A combined with mutation in the ser83 position of gyrA gene challenge treatment of typhoid fever. This study reaffirmed that *S.* Typhi with reduced susceptibility to fluoroquinolones is still a prevalent phenotype in central Nepal. Our findings suggest reintroducing chloramphenicol and cotrimoxazole antibiotics for the treatment of typhoid fever after higher cohort and extended follow-up studies as the proportion of classical multi-drug resistant *S.* Typhi has continued to decline.

CONFLICTS OF INTEREST

The authors declare no competing financial interest.

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REFERENCES

1. Le TAH, Fabre L, Roumagnac P, Grimont PAD, Scavizzi MR, Weill FX. Clonal expansion and microevolution of quinolone-resistant Salmonella enterica serotype typhi in Vietnam from 1996 to 2004. J Clin Microbiol [Internet]. 2007 Nov 1 [cited 2021 Mar 4];45(11):3485–92. Available from: http://www.ncbi.nlm.nih.gov
2. Weill F-X, Tran HH, Roumagnac P, Fabre L, Minh NB, Stavnes TL, et al. Clonal reconquest of antibiotic-susceptible Salmonella enterica serotype Typhi in Son La Province, Vietnam. Am J Trop Med Hyg [Internet]. 2007 Jun;76(6):1174–81. [PubMed]
3. Parry CM, Ho VA, Phuong LT, Van Be Bay P, Lanh MN, Tung LT, et al. Randomized controlled comparison of ofloxacin, azithromycin, and an ofloxacin-azithromycin combination for treatment of multidrug-resistant and nalidixic acid-resistant typhoid fever. Antimicrob Agents Chemother [Internet]. 2007 Mar 1 [cited 2021 Mar 4];51(3):819–25. Available from: http://aac.asm.org/
4. Holt KE, Baker S, Dongol S, Basnyat B, Adhikari N, Thorson S, et al. High-throughput bacterial SNP typing identifies distinct clusters of Salmonella Typhi causing typhoid in Nepalese children. BMC Infect Dis [Internet]. 2010 May 31 [cited 2021 Feb 16];10(1):1–9.[Article]
5. Acharya D, Trakulsomboon S, Madhup SK, Korbsrisate S. Antibiotic Susceptibility Pattern and the Indicator of Decreased Ciprofloxacin Susceptibility of Salmonella enterica Serovar Typhi Isolated from Dhulikhel Hospital, Nepal. Jpn J Infect Dis [Internet]. 2012 [cited 2021 Feb 16];65(3):264–7.[Article]
6. World Health Organization (WHO). Typhoid and other invasive salmonellosis (Vaccine-Preventable Diseases). p. 1–13.
7. WHO. Background document: the diagnosis, treatment and prevention of typhoid fever [Internet]. 2003. [Google Scholar]
8. Farooqui BJ, Khurshid M, Ashfaq MK, Ata Khan M. Comparative yield of Salmonella typhi from blood and bone marrow cultures in patients with fever of unknown origin. J Clin Pathol [Internet]. 1991 [cited 2021 Mar 18];44(3):258–9. [PubMed]
9. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. Wayne, PA; 2018. (M100). Report No.: 28th ed.
10. CLSI. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically [Internet]. Wayne, PA; 2018 [cited 2021 Mar 18]. (M07). Report No.: 11th ed. [Link]
11. Adi PJ, Naidu JR, Matcha B. Multiplex quantification of Escherichia coli, Salmonella typhi and Vibrio cholera with three DNA targets in single reaction assay. Microbial pathogenesis. 2017 Sep 1;110:50-5.[Article]
12. Renuka K, Kapil A, Kabra SK, Wig N, Das BK, Prasad VVSP, 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 [Internet]. 2004 Jun 25 [cited 2021 Mar 18];10(2):146–53.[Article]

13. 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. Journal of Antimicrobial Chemotherapy. 1996 May 1;37(5):891-900.[Article]
14. Lewis MD, Serichantalergs O, Pitarangsi C, Chuanak N, Mason CJ, Regmi LR, et al. Typhoid fever: A massive, single-point source, multidrug-resistant outbreak in Nepal. Clin Infect Dis [Internet]. 2005 Feb 15 [cited 2021 Mar 4];40(4):554–61. [Article]
15. Murdoch DR, Woods CW, Zimmerman MD, Dull PM, Belbase RH, Keenan AJ, et al. The etiology of febrile illness in adults presenting to Patan hospital in Kathmandu, Nepal. Am J Trop Med Hyg [Internet]. 2004 Jun;70(6):670–5. [PubMed]
16. 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. Ryan ET, editor. PLoS Negl Trop Dis [Internet]. 2018 Apr 23 [cited 2021 Feb 16];12(4):e0006408.[Article]
17. Andrews JR, Vaidya K, Bern C, Tamrakar D, Wen S, Madhup S, et al. High Rates of Enteric Fever Diagnosis and Lower Burden of Culture-Confirmed Disease in Peri-urban and Rural Nepal. J Infect Dis [Internet]. 2018 Nov 10 [cited 2021 Feb 16];218(suppl_4):S214–21.[Article]
18. Pokhrel A, Rayamajhee B, Khadka S, Thapa S, Kapali S, Pun SB, et al. Seroprevalence and Clinical Features of Scrub Typhus among Febrile Patients Attending a Referral Hospital in Kathmandu, Nepal. Trop Med Infect Dis [Internet]. 2021 May 13;6(2):78.[Article]
19. 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. [Article]
20. Thompson CN, Karkey A, Dongol S, Arjyal A, Wolbers M, Darton T, et al. Treatment Response in Enteric Fever in an Era of Increasing Antimicrobial Resistance: An Individual Patient Data Analysis of 2092 Participants Enrolled into 4 Randomized, Controlled Trials in Nepal. Clin Infect Dis [Internet]. 2017 Jun 1 [cited 2021 Feb 16];64(11):1522–31.[Article]
21. Johnson HD, Sholcosky D, Gabello K, Ragni R, Ogonosky N. Sex differences in public restroom handwashing behavior associated with visual behavior prompts. Percept Mot Skills [Internet]. 2003 Dec 31 [cited 2021 Feb 9];97(3 I):805–10. [Article]
22. Khanal PR, Satyal D, Bhetwal A, Maharjan A, Shakya S, Tandukar S, et al. Renaissance of Conventional First-Line Antibiotics in Salmonella enterica Clinical Isolates: Assessment of MICs for Therapeutic Antimicrobials in Enteric Fever Cases from Nepal. Biomed Res Int. 2017;2017.[Article]
23. Shrestha KL, Pant ND, Bhandari R, Khatri S, Shrestha B, Lekhak B. Re-emergence of the susceptibility of the Salmonella spp. isolated from blood samples to conventional first line antibiotics. Antimicrob Resist Infect Control [Internet]. 2016 May 25 [cited 2021 Mar 5];5(1):22.[Article]
24. 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 [Internet]. 2021 Jan 10 [cited 2022 Apr 26];14:117863612110563. [Article]
25. Wong VK, Baker S, Pickard DJ, Parkhill J, Page AJ, Feasey NA, et al. Phylogeographical analysis of the dominant multidrug-resistant H58 clade of Salmonella Typhi identifies inter-and intracontinental transmission events. Nat Genet [Internet]. 2015 May 27 [cited 2021 Mar 4];47(6):632–9.[Article]
26. Rodrigues C, Kapil A, Sharma A, Devanga Ragupathi NK, Inbanathan FY, Veeraraghavan B, et al. Whole-Genome Shotgun Sequencing of Cephalosporin-Resistant Salmonella enterica Serovar Typhi. Genome Announc [Internet]. 2017 Mar 9 [cited 2021 Mar 18];5(10).[Article]
27. Thanh DP, Karkey A, Dongol S, Thi NH, Thompson CN, Rabaa MA, Arjyal A, Holt KE, Wong V, Thieu NT, Vinh PV. A novel ciprofloxacin-resistant subclade of H58 Salmonella Typhi is associated with fluoroquinolone treatment failure. Elife. 2016 Mar 14;5:e14003.. [Article]
28. Giraud E, Brisabois A, Martel JL, Chaslus-Dancla E. Comparative studies of mutations in animal isolates and experimental in vitro- and in vivo-selected mutants of Salmonella spp. suggest a counterselection of highly fluoroquinolone-resistant strains in the field. Antimicrob Agents Chemother [Internet]. 1999 [cited 2021 Mar 19];43(9):2131–7.[Article]
29. Hakanen A, Kotilainen P, Jalava J, Siitonen A, Huovinen P. Detection of decreased fluoroquinolone susceptibility in salmonelias and validation of nalidixic acid screening test. J Clin Microbiol [Internet]. 1999 [cited 2021 Mar 18];37(11):3572–7.[Article]
30. Crump JA, Barrett TJ, Nelson JT, Angulo FJ. Reevaluating fluoroquinolone breakpoints for Salmonella enterica serotype typhi and for non-typhi salmonellae [Internet]. Vol. 37, Clinical Infectious Diseases. Clin Infect Dis; 2003 [cited 2021 Mar 19]. p. 75–81.[PubMed]