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Resistance to Fluoroquinolones and Other Antimicrobials in Culture-Positive *Salmonella typhi* Isolates in Gulbarga, South India

Kavita Nagshetty[1*], N. G. Manjula[2], Girish C. Math[1], Arali Sagar Mohan[1], C. T. Shivannavar[1], S. M. Gaddad[1]

[1]Department of P. G. Studies and Research in Microbiology, Gulbarga University, Gulbarga, Karnataka, India
[2]Department of Microbiology, School of Basic and Applied Sciences, Dayananda Sagar University, Bengaluru, Karnataka, India
Email: *kavita_nagshetty@yahoo.com

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Abstract

Background: Typhoid fever is a major public health concern in developing countries. The upsurge in the occurrence of bacterial isolates that are resistant to nalidixic acid; with reduced susceptibility to ciprofloxacin in typhoidal *Salmonellae* constitutes a challenge to the clinician. **Methods:** In order to better understand the epidemiology of *Salmonella* infections in South India, *Salmonella typhi* isolates were screened from various healthcare centers. *Salmonella* isolates were identified by using standard phenotypic, serological, antibiotic susceptibility and molecular methods. **Results:** Among a total of 100 *S. typhi* isolates 9% were found to be multidrug resistant and 30% were nalidixic acid resistant. Isolates with reduced susceptibility to ciprofloxacin displays single base mutations in the gyrA gene. A very low rate of 1% resistance was found to ciprofloxacin. The only one isolate with ciprofloxacin MIC ≥ 4 µg/ml also showed single mutation in the QRDR of the gyrA gene in *S. typhi* (GenBank accession no. HQ176349-HQ176368). **Conclusions:** A very low rate of nalidixic acid resistance with reduced susceptibility to ciprofloxacin was observed in comparison to other endemic areas in isolates of *S. typhi* from Gulbarga, South India, with steadily increasing NAR *S. typhi* but decreasing MDR isolations over the study period. This is most likely due to an increased use of ciprofloxacin as a first line drug of choice over more traditional antimicrobial agents for the treatment of typhoid fever.

Keywords

Salmonella typhi, Fluoroquinolones, MDR, NAR, gyrA, QRDR

1. Introduction

Typhoid fever etiological agents, *Salmonella typhi* and Paratyphi A, are causing diverse clinical manifestations worldwide. Estimation of typhoid fever episodes over a decade period (2004-2014) was found to be 13.5 - 21.1 million cases [1]. Various factors influence the incidence of enteric fever. Transmission routes, personal hygiene management, emerging of multidrug resistance strains, prolonged carrier stages are governing factors for the emerging risk of the enteric infections. Though, the enteric fever is now less common in developed countries, many developing countries with limited resources are still facing this menace [2]. Globally, 217,000 deaths are attributable to typhoid fever annually with the highest disease burden in South central and Southeast Asia. Infants, children and adolescents are more likely to get typhoid fever in these regions. Nearly, 80% of the total typhoid fever cases have been reported in eight South Asian countries (Bangladesh, China, India, Indonesia, Laos, Nepal, Pakistan and Vietnam), suggesting typhoid fever to be a major health risk in the region [3] [4].

Untreated enteric fever carries with a mortality rate of 30%, with appropriate antimicrobial treatment reduces the mortality rate to as low as 0.5% [5]. Sometimes it is often necessary to commence treatment with immediate effect to reduce the severity of infections. Fluoroquinolone, ciprofloxacin are choice of drugs for treatment, especially when treatment miscarries with traditional antimicrobial agents comprising chloramphenicol, ampicillin and trimethoprim-sulfamethoxazole (cotrimoxazole) [5]. Multidrug-resistant typhoid fever (MDRTF) is defined as typhoid fever caused by *S. typhi* strains which are resistant to all the three first-line recommended drugs for treatment, *i.e.*, chloramphenicol, ampicillin and trimethoprim-sulfamethoxazole (cotrimoxazole) [6]. However, this switch to ciprofloxacin has led to a subsequent increase in the occurrence of *S. typhi* isolates resistant to this antimicrobial agent across the globe, including in India [7] [8].

In *Salmonellae*, quinolone resistance is usually associated with mutations in the quinolone resistance-determining region (QRDR) of the A subunit of DNA gyrase, though the presence of plasmid-mediated quinolone resistance *qnr* genes and *aac*(6')-*Ib-cr* has also been described in quinolone-resistant non-Typhoid *Salmonella* [9]. The association of quinolone resistance with mutations in the genes encoding for DNA gyrase, causes reduced drug accumulation, either by a decreased uptake or by an increased efflux. The exact mechanism of this DNA gyrase-mediated resistance in *S. typhi* is not fully understood, though various studies have found that single point mutations in this region confer resistance to nalidixic acid and hence reduced susceptibility to Fluoroquinolones [10]. Worldwide there are sporadic reports of high level cephalosporin resistance in typhoidal *Salmonellae* [11] [12].

A number of cultural, social and environmental factors are associated with the occurrence of typhoid in different endemic settings of which poor quality of life, inadequate provision of safe water and sanitation are found to be the major

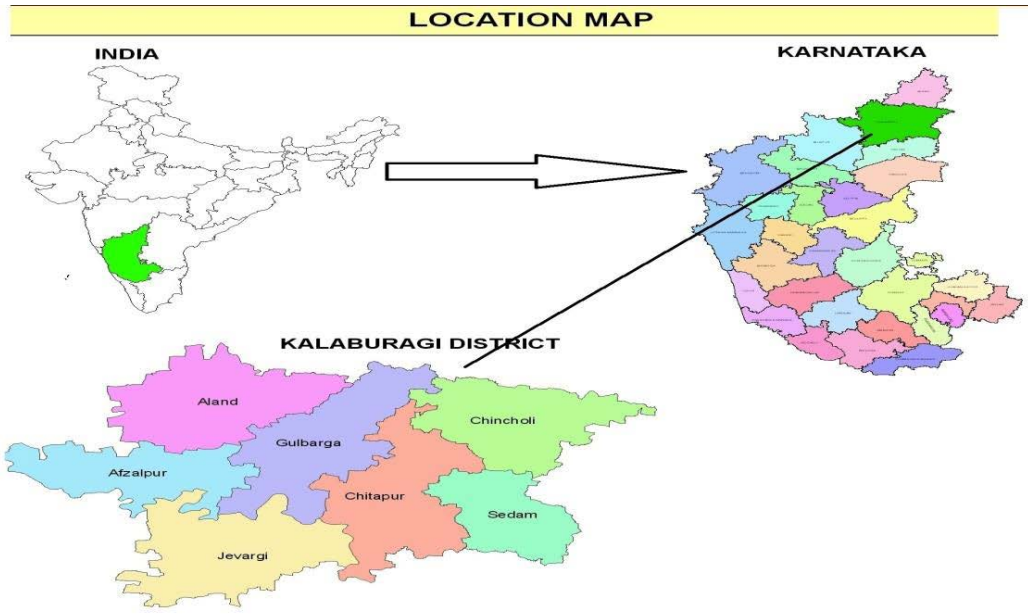
causes [13]. Gulbarga region, falling under the Hyderabad Karnataka area, is considered to be socio-economically and educationally backward and Typhoid is endemic in this region occurring at regular intervals (Picture 1).

Therefore, this study was undertaken to characterize trends in antimicrobial resistance in clinically relevant *S. typhi* isolates and environmental isolates originating from Gulbarga, South India, to help clinicians in constituting a more effective treatment regimen.

2. Materials and Methods

Bacterial culture and identification

S. typhi isolates from a total of 1200 clinical samples like Blood, CSF and Urine samples from patients presenting with fever at the outpatient clinics or were admitted in the private and government hospitals of this region and 50 environmental samples from sewage, water and food during the period from August 2006 to September 2009 were included in the study. The patients ranged in age from 2 years to 75 years (median, 40 years). Blood samples were cultured by clot and blood culture methods, while the cerebral spinal fluid (CSF) samples were cultured directly by plating and the urine and stool samples were cultured by using Selenite F broth (Hi-Media Laboratories Ltd, Mumbai). All the biological samples were collected from the same area of the study and the samples were based on the positive Widal test result of the patient, irrespective of the organ affected. Colonies were identified as *S. typhi* on Wilson and Blair bismuth sulphite agar medium and Xylose-lysine deoxycholate agar (XLD) (Hi-media Laboratories Pvt Ltd, Mumbai, India) using standard biochemical methods [14], and confirmed using *Salmonell*a polyvalent O, O9 and H: d antisera procured from King Institute of Preventive Medicine, Guindy, Chennai, South India.



Picture 1. Study area map of Gulbarga district.

Antimicrobial susceptibility testing

Isolates were tested for susceptibility to antibiotics using the Kirby Bauer disk diffusion method. Mueller Hinton agar plates were inoculated with a standardized inoculum of 0.5 McFarland (approximately 10^8 CFU/ml) over the entire surface. Antibiotic disks were dispensed to the agar surface with the forceps and incubated at 37˚C for 16 to 18 hours in ambient air and diameter of inhibition zones were measured and results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2013) [15]. Reduced susceptibility to ciprofloxacin was determined as a minimum inhibitory concentration (MIC) of 0.125 - 1.0 μg/ml. The breakpoint for ciprofloxacin is, ≤0.5 μg/ml (susceptible) and >1 μg/ml (resistant) according to the CLSI guidelines (CLSI, 2013) [15]. Escherichia coli ATCC 25922 were used as a standard control. Nalidixic acid susceptibility was used as a screening test for reduced susceptibility to ciprofloxacin [16]. MICs were determined by agar dilution for ampicillin, chloramphenicol, co-trimoxazole, ceftriaxone, ceftazidime, cefotaxime, ceftizoxime, ciprofloxacin and nalidixic acid separately.

Molecular analysis of quinolone resistance

Molecular analysis of quinolone resistance was performed for 20 nalidixic acid resistant (1 ciprofloxacin resistant and 19 ciprofloxacin susceptible) isolates. The molecular mechanism of quinolone resistance was determined by investigating mutations in the QRDRs of DNA gyrase (gyrA and gyrB) genes, according to the previously described protocols [17] [18].

PCR amplification of gyrA gene

Amplification of gyrA gene was performed with primers Forward 5'-ATG AGC GAC CTT GCG AGA GAA ATT ACA CCG-3' and Reverse 5'- TTC CAT CAG CCC TTC AAT GCT GAT GTC TTC-3' [17].

The cycling conditions include Preheating at 94˚C for 5 min for 1 cycle, and Denaturation at 94˚C for 30 sec, Primer Annealing at 55˚C for 30 sec and Elongation at 72˚C for 45 sec for 35 cycles. Lastly final elongation was done at 72˚C for 10 min. The size of amplicon was 620 bp by using ABI (Applied Biosystems) PCR.

Electrophoresis

PCR products were loaded on to 1% agarose gel along with 500 bp ladder using 1XTBE Buffer containing Ethidium Bromide for 20 to 30 minutes at 130 volts and observed using UV transilluminator (UV-Tech).

3. Results

Trends in antimicrobial susceptibility of *S. typhi* isolates

A total of 100 *S. typhi* strains isolated during the period 2006-2009 from various health care settings. All *Salmonella typhi* isolates were from blood samples and Environment samples such as contaminated water samples. However, environmental samples are all susceptible for all antibiotics used in this study. 40% (40/100) were fully susceptible to all the antibiotics tested; 9% (9/100) were MDR; 30% (30/100) were nalidixic acid resistant (NAR); and 1% (1/100) was

both MDR and NAR (MDR being defined as resistance to all the first line antimicrobials *i.e.* ampicillin, cotrimoxazole and chloramphenicol). The results are summarized in Table 1. Only two isolates (2%) were resistant to ciprofloxacin (MIC- ≥ 1 μg/ml) (CLSI Guidelines, 2017) (Table 4). There was a steady decline in the number of MDR isolates over the 4 year study period, as well as a parallel increase in NAR (non-MDR) isolates (Table 1 and Table 2).

All 30 nalidixic acid resistant isolates were performed for MIC for Nalidixic acid. 20 NAR isolates were chosen for ciprofloxacin MIC randomly, and a single isolate possessed an MIC of ≥4 μg/ml for ciprofloxacin with the corresponding agar dilution method. Ciprofloxacin MICs for nalidixic acid resistant isolates ranged between 0.125 - 4 μg/ml. A correlation was observed between reduced ciprofloxacin susceptibility and nalidixic acid resistance (19/20 isolates with ciprofloxacin MICs ranging from 0.125 - 1 μg/ml were NAR). The value of R is 0.1602 for Pearson correlation test. Although technically a positive correlation, the relationship between two variables is weak correlation (Figure 1).

All isolates were found to be susceptible to cephalosporins though interestingly there was a gradual increase in MIC values for ceftizoxime and ceftriaxone during the study period from 0.125 - 1 μg/ml (Table 3). All environmental isolates were found to be susceptible to all the antibiotics tested.

Molecular analysis of quinolone resistance

PCR amplification of gyrA gene, nucleotide changes within DNA gyrase (gyrA) gene and their relationship between ciprofloxacin MIC investigated for 20 NAR isolates, with different resistance profiles are shown in Figures 2(a)-(c) and Table 4 respectively. Comparatively, 18 NAR isolates found, with reduced susceptibility to ciprofloxacin (MIC 0.125 - 1 μg/ml) (GenBank accession no. HQ176349-HQ176357 and HQ176359-HQ176366 and HQ176368). Remaining two NAR isolates exhibit exceptional features. One strain (ST-54) with higher MIC ≥ 4 μg/ml for ciprofloxacin has a single gyrA mutation within the QRDR of gyrA, at position Ser83. Sequence of gyrA submitted to NCBI and its GenBank accession no. HQ176358. Another one (ST-77) having reduced ciprofloxacin susceptibility (0.125 μg/ml; GenBank accession no. HQ176367) does not show any mutation at Ser83 in QRDR region of the gyrA.

Table 1. Distribution of antimicrobial resistance phenotypes among *S. typhi* isolates.

Period of Isolation	No (%) of susceptiblea	No (%) single resistanceb	No (%) paired resistancec	No (%) multiple resistanced	No (%) MDRe	Total No.	
2006	15/44 (34.09)	12/44 (27.27)	02/44 (4.54)	10/44 (22.72)	5/44 (11.36)	44	(44)
2007	07/28 (25)	10/28 (28.57)	01/28 (3.57)	07/28 (25)	3/28 (10.71)	28	(28)
2008	09/17 (52.94)	04/17 (23.52)	0	03/17 (17.64)	1/17 (5.88)	17	(17)
2009	09/11 (81.81)	01/11 (9.09)	0	1/11 (9.09)	0	11	(11)
Total	40 (40)	27 (27)	03 (3)	21 (21)	09 (09)	100	(100)

[a]Susceptible to all the antibiotics; [b]Single resistance—resistance to only one antimicrobial; [c]Paired resistance—resistance to two antimicrobials; [d]Multiple resistance—resistance to three and more than three antimicrobial; [e]MDR—defined as resistance to ampicillin, chloramphenicol and cotrimoxazole.

Table 2. Trends in resistance to quinolones among *S. typhi* isolates during the study period.

Period of Isolation	No. (%)	No. (%)
	NAR^R	CIP^R
2006	9 (20.45)	0
2007	8 (28.57)	1 (1)
2008	7 (41.17)	0
2009	6 (54.54)	0
Total	30 (30)	1 (1)

CIP^R—Ciprofloxacin resistance; NAR^R—Nalidixic acid resistance.

Table 3. MIC values for Cephalosporins.

Cephalosporins	No of Isolates	MIC values (mg/ml)
CTX	3	0.125
(N = 5)	2	0.62
CTR	5	0.62
(N = 6)	1	0.32
CTZ	5	0.62
(N = 6)	1	0.25
CAZ	8	1
(N = 12)	4	0.62

Table 4. Relationship between ciprofloxacin MIC, point mutations within the QRDRs of DNA gyrase gene for 20-seleced serovar Typhi isolates.

Isolate	Source	MIC (μg/ml)		% Similarity with the Wild Strain Acc.No: AE014613	Nucleotide change and Amino acid substitution in DNA *gyrase* (gyrA)	
		Nalidixic acid	Ciprofloxacin		Aminoacid Substitution	Nucleotide Change
ST 11	Blood	128	1	97%	Ser-83 → Tyr	TCC → TAC
ST 12	Blood	128	0.125	99%	Ser-83 → Phe	TCC → TTC
ST 13	Blood	128	0.125	99%	Ser-83 → Tyr	TCC → TAC
ST 18	Blood	128	0.125	99%	Ser-83 → Tyr	TCC → TAC
ST 29	Blood	128	0.125	99%	Ser-83 → Phe	TCC → TTC
ST 33	Blood	128	0.125	99%	Ser-83 → Phe	TCC → TTC
ST 34	Blood	128	0.125	99%	Ser-83 → Tyr	TCC → TAC
ST 45	Blood	64	0.125	99%	Ser-83 → Phe	TCC → TTC
ST 51	Blood	64	0.125	99%	Ser-83 → Phe	TCC → TTC
ST 54	Blood	128	4	98%	Ser-83 → Phe	TCC → TTC
ST 55	Blood	128	0.125	98%	Ser-83 → Phe	TCC → TTC
ST 56	Blood	128	0.125	98%	Ser-83 → Tyr	TCC → TAC
ST 59	Blood	128	0.125	98%	Ser-83 → Phe	TCC → TTC
ST 60	Blood	128	0.125	94%	Ser-83 → Tyr	TCC → TAC
ST 61	Blood	128	0.125	98%	Ser-83 → Phe	TCC → TTC
ST 66	Blood	128	0.125	99%	Ser-83 → Tyr	TCC → TAC
ST 70	Blood	64	0.125	99%	Ser-83 → Phe	TCC → TTC
ST 73	Blood	64	0.125	99%	Ser-83 → Phe	TCC → TTC
ST 77	Blood	64	0.125	99%	No Change	No Change
ST 80	Blood	128	0.125	98%	Ser-83 → Phe	TCC → TTC

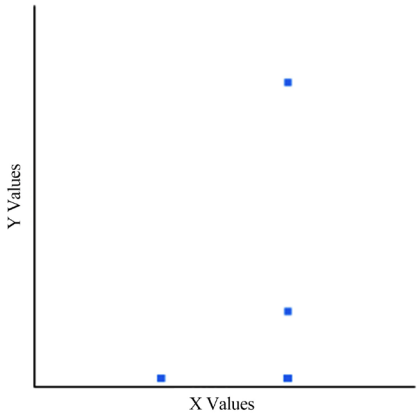
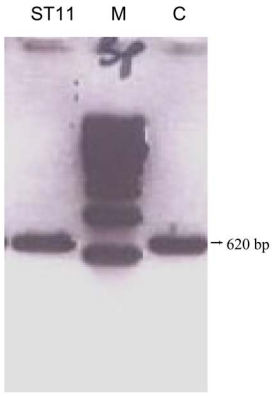
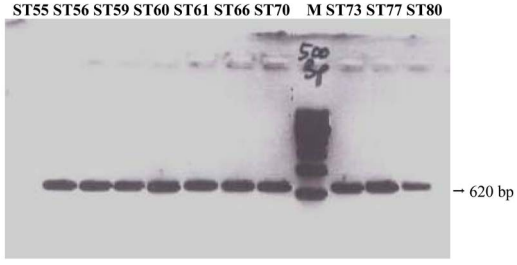


Figure 1. Pearson correlation. The value of R is 0.1602. Although technically a positive correlation, the relationship between the variables is weak. The value of R2, the coefficient of determination, is 0.0257. Where X Value for reduced ciprofloxacin susceptibility, Y Value for Nalidixic acid resistance.



(a)

(b)



(c)

Figure 2. (a) PCR amplification of gyrA gene, M-500 bp DNA Ladder, ST11: (*S. typhi* Isolate) and C: Control (*S. typhi* MTTC: 734); (b) PCR amplification of gyrA gene, M-500 bp DNA Ladder, ST12-ST54: *S. typhi* Isolates; (c) PCR amplification of gyrA gene, M-500 bp DNA Ladder, ST55-ST80: *S. typhi* Isolates.

4. Discussion

Salmonella typhi accounts for a major proportion of enteric fever in developing countries such as India. In the present study, a gradual decline observed in the number of *S. typhi* MDR isolates. Similar reports found from other regions of South India [7]. 30% *S. typhi* isolates were resistant to NAR and showed reduced susceptibility to ciprofloxacin. This finding is most likely due to decreased prescribing of traditional antimicrobial agents, and an increasing reliance on ciprofloxacin as the first line treatment for *S. typhi* in Gulbarga, which is in accordance with Rudresh *et al.* [8]. All environment samples are susceptible to all antibiotic used. Environment is a media of transmission for typhoid fever [19].

Indeed, only 2% of *S. typhi* isolates from positive cultures in Gulbarga were found to be resistant to ciprofloxacin (MIC ≥ 1 and 4 μg/ml), and the emergence of such resistant bacteria is a cause for concern. In recent years, there are some sporadic reports of high-level ciprofloxacin resistance in *Salmonella typhi* [8]. Capoor *et al.*, in 2007 reported ciprofloxacin resistance rate of 1% in *Salmonella typhi* isolates [20]. However, isolates with reduced susceptibility to ciprofloxacin (≥0.125; NAR-isolates) have a less favorable response to ciprofloxacin [5].

In our study, one *S. typhi* isolate ST-54 with a ciprofloxacin MIC ≥ 4 μg/ml, is found to have a single mutation in gyrA (ser 83 to phe) and other strain with lower MICs (0.125 μg/ml - 1.0 μg/ml) also exhibits single point mutations. Our report was contrary to the earlier reports [19] [20] who had found complete resistance to ciprofloxacin (MIC ≥ 4 μg/ml) due to a double mutation in the QRDR region. Menezes *et al.* (2012), studies focus on NAR isolates with a comparatively reduced susceptibility to ciprofloxacin (MIC 0.125 - 0.5 mg/L), possessed a single gyrA mutation (either at Ser83 or Asp87) only. Resistance mutations of gyrA have been clustered in a region of the gene product between amino acids 67 and 106 termed the QRDR. Gyrase appears to be the primary target for quinolones in gram-negative bacteria since missense mutations in gyrA genes are sufficient to render quinolone resistant in these organisms [21]. gyrA and Par C mutations in QRDR region render quinolone and fluoroquinolones resistance. However, the single mutation in gyrA Ser83 to Phe, Try, Ala, is most common mechanism, for NAR isolates show reduced susceptibility to ciprofloxacin [22]. In the present study there is a single mutation in Ser 83 to Phe, it supports the MIC studies of *S. typhi* isolate ST54 for Ciprofloxacin resistance.

There was a gradual increase in MICs against ceftriaxone, ceftazidime, cefotaxime and ceftizoxime (a 3rd generation cephalosporin) during the study period, from a MIC value of 0.125 μg/ml in 2006 to 1 μg/ml in 2009, though still well within the susceptible range. This type of resistance to quinolones, and more recently, increase in MIC levels to third and fourth-generation cephalosporins, re-emphasize the importance of continued surveillance in the treatment of enteric fever [23].

5. Conclusions

In our study we found that typhoid fever is endemic in this region. In our expe-

rience, nalidixic acid disk diffusion testing is a good indicator of decreased ciprofloxacin susceptibility. A high-level ciprofloxacin resistance (MIC ≥ 4 μg/mL) in single *S. typhi* isolate is mediated by a single mutation in gyrA. There is a slow emergence of ciprofloxacin resistant typhoidal *Salmonellae* in this part of south India. A low level of ciprofloxacin resistance was observed, with steadily increasing NAR, but decreasing MDR isolations over the study period. This is most likely due to an increased use of ciprofloxacin as a first line drug of choice for enteric fever. Furthermore all typhoidal *Salmonellae* were found to be susceptible to third-generation cephalosporin.

Hence in order to better manage and prevent the spread of antimicrobial resistance, both clinicians and governments require accurate epidemiological information. At the present moment, this information tends to be lacking, especially in countries with large populations and unrestricted "over the counter" prescription policies, such as India. The spread of nalidixic acid resistant *S. typhi* with reduced susceptibility to ciprofloxacin necessitates a change towards "evidence-based" treatment practices for the treatment of typhoid fever, not least in Southern India.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Buckle, G.C., Walker, C.L.F. and Black, R.E. (2012) Typhoid Fever and Paratyphoid Fever: Systematic Review to Estimate Global Morbidity and Mortality for 2010. *Journal of Global Health*, **2**, Article ID: 10401. https://doi.org/10.7189/jogh.01.010401

[2] Yan, M.Y., Li, X.L., Liao, Q.H., Li, F., Zhang, J. and Kan, B. (2016) The Emergence and Outbreak of Multidrug-Resistant Typhoid Fever in China. *Emerging Microbes & Infections*, **5**, 1-6. https://doi.org/10.1038/emi.2016.62

[3] Al-Emran, H.M., Eibach, D., Krumkamp, R., Ali, M., Baker, S., Biggs, H.M. and Cruz Espinoza, L.M. (2016) A Multicountry Molecular Analysis of *Salmonella enterica* Serovar Typhi with Reduced Susceptibility to Ciprofloxacin in Sub-Saharan Africa. *Clinical Infectious Diseases*, **62**, S42-S46. https://doi.org/10.1093/cid/civ788

[4] Behl, P., Gupta, V., Sachdev, A., Guglani, V. and Chander, J. (2017) Patterns in Antimicrobial Susceptibility of *Salmonellae* Isolated at a Tertiary Care Hospital in Northern India. *Indian Journal of Medical Research*, **145**, 124-128. https://doi.org/10.4103/ijmr.IJMR_862_14

[5] Harish, B.N. and Menezes, G.A. (2011) Preserving Efficacy of Chloramphenicol against Typhoid Fever in a Tertiary Care Hospital, India. *Regional Health Forum*, **15**, 92-96.

[6] Kumar, S., Rizvi, M. and Berry, N. (2008) Rising Prevalence of Enteric Fever Due to Multidrug-Resistant *Salmonella*: An Epidemiological Study. *Journal of Medical Microbiology*, **57**, 1247-1250. https://doi.org/10.1099/jmm.0.2008/001719-0

[7] Choudhary, A., Gopalakrishnan, R., Senthur, N.P., Ramasubramanian, V., Ghafur, K.A. and Thirunarayan, M.A. (2013) Antimicrobial Susceptibility of *Salmonella en-*

terica Serovars in a Tertiary Care Hospital in Southern India. *The Indian Journal of Medical Research*, **137**, 800-802.

[8] Rudresh, S.M. and Nagarathnamma, T. (2015) Antibiotic Susceptibility Pattern of *Salmonella* Enterica Serovar Typhi and *Salmonella* Enterica Serovar Paratyphi A with Special Reference to Quinolone Resistance. *Drug Development and Therapeutics*, **6**, 70-73.

[9] Xia, S.L., Hendriksen, R.S., Xie, Z.Q., Huang, L.L., Zhang, J., Guo, W.S., Xu, B.L., Ran, L. and Aarestrup, F.M. (2009) Molecular Characterization and Antimicrobial Susceptibility of *Salmonella* from Infections in Humans in Henan Province, China. *Journal of Clinical Microbiology*, **47**, 401-409. https://doi.org/10.1128/JCM.01099-08

[10] Gaind, R., Paglietti, B., Murgia, M., Dawar, R., Uzzau, S., Cappuccinelli, P., Deb, M., Aggarwal, P. and Rubino, S. (2006) Molecular Characterization of Ciprofloxacin-Resistant *Salmonella enterica* Serovar TYPHI and Paratyphi a Causing Enteric Fever in India. *Journal of Antimicrobial Chemotherapy*, **58**, 1139-1144. https://doi.org/10.1093/jac/dkl391

[11] Capoor, M.R. and Nair, D. (2010) Quinolone and Cephalosporin Resistance in Enteric Fever. *Journal of Global Infectious Diseases*, **2**, 258-262. https://doi.org/10.4103/0974-777X.68529

[12] Rajni, S. (2010) Ceftriaxone Resistance in *Salmonella typhi*—Myth or a Reality! *Indian Journal of Pathology and Microbiology*, **53**, 389. https://doi.org/10.4103/0377-4929.64321

[13] Robert, J.C., Ashraf, M.D. and Masahiro, H. (2013) Modelling Typhoid Risk in Dhaka Metropolitan Area of Bangladesh: The Role of Socio-Economic and Environmental Factors. *International Journal of Health Geographics*, **12**, Article No. 13. https://doi.org/10.1186/1476-072X-12-13

[14] Collee, J.G., Miles, R.S. and Watt, B. (1996) Mackie & McCartney Practical Medical Microbiology. 14th Edition, Churchill Livingstone, London.

[15] Clinical and Laboratory Standards Institute (2013) Performance Standards for Antimicrobial Susceptibility Testing. CLSI Document M100-S23, Clinical and Laboratory Standards Institute, Wayne.

[16] Hakanen, A., Kotilainen, P., Jalava, J., Scitonen, A. and Huovinen P. (1999) Detection of Decreased Fluoroquinolone Susceptibility in *Salmonellas* and Validation of Nalidixic acid Screening Test. *Journal of Clinical Microbiology*, **37**, 3572-3577. https://doi.org/10.1128/JCM.37.11.3572-3577.1999

[17] Brown, J.C., Shanahan, P.M.A., Jesudason, M.V., Thomson, C.J. and Amyes, S.G.B. (1996) Mutations Responsible for Reduced Susceptibility to 4-Quinolones in Clinical Isolates of Multi-Resistant *Salmonella typhi* in India. *Journal of Antimicrobial Chemotherapy*, **37**, 891-900. https://doi.org/10.1093/jac/37.5.891

[18] Renuka, K., Kapil, A., Kabra, S.K., Wig, N., Das, B.K., Prasad, V.V., Chaudhry, R. and Seth, P. (2004) Reduced Susceptibility to Ciprofloxacin and *gyrA* Gene Mutation in North Indian Strains of *Salmonella enterica* Serotype Typhi and Serotype Paratyphi A. *Microbial Drug Resistance*, **10**, 146-154. https://doi.org/10.1089/1076629041310028

[19] Akullian, A., Ng'eno, E., Matheson, A.I., Cosmas, L., Macharia, D., Fields, B. and Montgomery, J.M. (2015) Environmental Transmission of Typhoid Fever in an Urban Slum. *PLoS Neglected Tropical Diseases*, **19**, e0004212. https://doi.org/10.1371/journal.pntd.0004212

[20] Capoor, M.R., Nair, D., Aggarwal, P., Mathys, V. and Bifani, P. (2007) *Salmonella*

enterica Serovar Typhi: Molecular Analysis of Strains with Decreased Susceptibility and Resistant to Ciprofloxacin in India from 2001-2003. *The Brazilian Journal of Infectious Diseases*, **11**, 423-425. https://doi.org/10.1590/S1413-86702007000400011

[21] Gokul, B.N., Menezes, G.A. and Harish, B.N. (2010) Emergence of ACC-1 β-Lactamase-Producing *Salmonella enterica* Serover Typhi. *Emerging Infectious Diseases*, **16**, 1170-1171. https://doi.org/10.3201/eid1607.091643

[22] Dutta, S., Das, S., Mitra, U., Jain, P., Roy, I., Ganguly, S.S., Ray, U., Dutta, P. and Paul. D.K. (2014) Antimicrobial Resistance, Virulence Profiles and Molecular Subtypes of *Salmonella enterica* Serovars Typhi and ParatyphiA Blood Isolates from Kolkata, India during 2009-2013. *PLoS ONE*, **9**, e101347. https://doi.org/10.1371/journal.pone.0101347

[23] Rushdy, A.A., Mabrouk, M.I., Abu-Sef, F.A.H., Kheiralla, Z.H., Abdel-All, S.M. and Saleh, N.M. (2013) Contribution of Different Mechanisms to the Resistance to Fluoroquinolones in Clinical Isolates of *Salmonella enterica*. *The Brazilian Journal of Infectious Diseases*, **17**, 431-437. https://doi.org/10.1016/j.bjid.2012.11.012