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Study on recent trends in antibiogram of enteric fever pathogens in a tertiary care centre in South India

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Abstract
Enteric fever is a systemic infection caused by Salmonella Typhi (S.Typhi), Salmonella Paratyphi A (S.Paratyphi A), Salmonella Paratyphi B and Salmonella Paratyphi C. It is endemic in India and is a major public health problem in the country. Treatment with Ciprofloxacin which was considered the drug of choice for multi drug resistant Salmonellae, is now showing high rates of failure clinically. A total of 33 isolates (S.Typhi -19, S. Paratyphi A – 14) obtained from blood and bone marrow were tested for antimicrobial susceptibility by Kirby Bauer disc diffusion method. The minimum inhibitory concentrations of Ciprofloxacin & Ceftriaxone were determined by broth dilution method. Majority of the patients were young adults falling in the age group 16-35 years (57.7%).Only 2 isolates of S.Typhi were resistant to all three first line drugs (Ampicillin, Cotrimoxazole and Chloramphenicol) and classified as multi drug resistant S.Typhi (MDRST). One strain of S.Paratyphi A was resistant to Ampicillin. 90.9% of isolates were susceptible to Azithromycin. None of the isolates showed full susceptibility to Ciprofloxacin. 7 isolates showed intermediate susceptibility with an MIC of 0.5μg/mL. The MIC of Ceftriaxone showed an upward trend though still within the susceptible limits. 69.7% of the 33 isolates had an MIC of 0.06 μg/mL and 30.3% of isolates had an MIC of 0.12μg/mL. There is reemergence of susceptibility to the first line drugs. Presence of fluoroquinolone resistance and the rise in MIC of Ceftriaxone is a concern. Antibiotic stewardship has to be implemented urgently.

Introduction
Enteric fever is a global public health problem especially in the developing countries. It is endemic in India and is an important cause of mortality and morbidity.

The syndrome of enteric fever is most often caused by *Salmonella enteric* serovar Typhi (*S*.Typhi) and less commonly by *Salmonella enteric* serovar Paratyphi A (*S*. Paratyphi A),

S*almonella enteric* serovar Schottmulleri (*S*. Schottmulleri) and *Salmonella enteric* serovar Hirschfeldii (*S*. Hirschfeldii).

High fever, toxaemia, constipation during first week of fever, diarrhoea during second week, mild splenomegaly and leukopenia, complicated by encephalopathy, intestinal haemorrhage and perforation during third week of fever are the typical manifestations of typhoid fever. But

atypical clinical presentations make an early diagnosis difficult.

In the preantibiotic era, the enteric fever case-fatality rate was 10-30%. Treatment with effective antimicrobial agents has reduced the rate to less than 1%. But enteric fever continues to be a major health problem in the developing world despite availability of antibacterial drugs. Infection with a multi-drug resistant strain & delayed antimicrobial therapy increase the risk of death.

Chloramphenicol was the drug of choice until resistance to the drug developed in mid-1970s. Alternative drugs for typhoid fever were Ampicillin and Trimethoprim. But strains resistant to Ampicillin and Trimethoprim in addition to Chloramphenicol emerged in late 1980s.

Because antimicrobial resistance to several classes of traditional first-line drugs had emerged, the quinolone antimicrobial agents, particularly the fluoroquinolones, became the drugs of choice. However, Nalidixic-acid-resistant (NAR) strains exhibiting reduced susceptibility to Ciprofloxacin have become endemic in several geographical areas of the Indian subcontinent[1]. The emergence of complete resistance to Ciprofloxacin in salmonellae severely limits the choice of antimicrobials available for treating enteric fever.

Extended-spectrum cephalosporins are recommended as alternatives in such a setting. However, outbreaks or cases of infections caused by *Salmonellae* resistant to extended-spectrum cephalosporins have also been reported[2].

The antimicrobial susceptibility pattern of salmonellae is changing now with re-emergence of sensitivity to Chloramphenicol but rising resistance to Ciprofloxacin. The irrational use of antibiotics in treating human infections, especially of Ciprofloxacin, could be the probable reason for emergence of antibiotic-resistant strains of bacteria.

For patients with typhoid fever, it is imperative to begin administration of an effective antibiotic as soon as a clinical diagnosis is made. Given the variation in the susceptibility patterns reported for Salmonellae, it is important to constantly monitor it so as to provide suitable guidelines for treatment. The present study was conducted to analyse the antibiogram of recent clinical isolates of enteric fever causing salmonellae, with a special focuson the increasing resistance to fluoroquinolones & re-emergence of sensitivity to the first line drugs.

Materials and Methods

A one year prospective study was conducted. The study protocol was approved by the institutional ethics committee.

All *S. enterica* isolates obtained from blood cultures of clinically suspected cases of enteric fever were included in the study. The isolates identified biochemically as *S.*Typhi or *S.*Paratyphi were confirmed by serotyping using salmonella agglutinating antisera (Remel Europe Ltd, UK)

Antibiotic susceptibility testing was performed using commercial antibiotic discs. (Hi-Media Laboratories Ltd, Mumbai) by Kirby Bauer disc diffusion method on Mueller Hinton Agar.The disc strengths and zone size interpretation were in accordance with Clinical Laboratory Standards Institute (CLSI) guidelines: Ampicillin (10μg), Cotrimoxazole (Trimethoprim 1.2μg/ Sulphamethoxazole 23.8 μg), Chloramphenicol (30μg), Nalidixic acid (30μg), Azithromycin (15μg), Ciprofloxacin (5μg) and Ceftriaxone (30μg).

Escherichia coli ATCC 25922 was used as the control strain

Isolates resistant to Ampicillin, Chloramphenicol, and Cotrimoxazole were defined as MDR *Salmonella.*

Minimum inhibitory concentration (MIC) of Ciprofloxacin and Ceftriaxone were determined for all isolates by broth macrodilution method (Hi-Media Laboratories Ltd, Mumbai).

The interpretation of results were done as per CLSI guidelines 2016.

Ciprofloxacin - Sensitive - ≤0.06μg/mL

Intermediate –0.12 – 0.5μg/mL

Resistant - ≥1μg/mL.

Ceftriaxone - Sensitive - ≤1µg/mL,
Intermediate – 2µg/mL
Resistant - ≥4µg/mL

Results

A total of 33 *Salmonella* isolates were obtained from patients with suspected enteric fever during the study period of one year.

19 isolates belonged to the serotype *Salmonella* Typhi constituting 57.6%, and the remaining 14 isolates were *Salmonella* Paratyphi A constituting 42.4%.

Majority of the patients were young adults falling in the age group 16-35 years (57.7%). 3 patients constituting 9.1% were in the paediatric age group (Table 1).

Drug susceptibility of the 33 isolates were done using Kirby Bauer disc diffusion method.

None of the isolates were susceptible to Ciprofloxacin.

Two isolates of *S.* Typhi were resistant to Ampicillin, Chloramphenicol & Cotrimoxazole and were classified as multi drug resistant (MDR). One strain of *S.*Paratyphi A was resistant to Ampicillin.

3 isolates were found to be resistant to Azithromycin- one isolate of *S.*Typhi and two isolates of *S.*Paratyphi A. (Table 2).

MIC of Ciprofloxacin was determined for all 33 isolates. No isolate of *S.*Typhi and *S.*Paratyphi A were fully susceptible to Ciprofloxacin.

7 isolates showed intermediate susceptibility with an MIC of 0.5µg/mL.

The highest MIC obtained for *S.*Typhi was 64µg/mL, and for *S.*Paratyphi A was 4µg/mL.(Table 3).

MIC of Ceftriaxone was also determined for the 33 isolates. All isolates of *S.*Typhi and *S.*Paratyphi A had MIC of Ceftriaxone well below the CLSI breakpoint. 69.7% of the 33 isolates had an MIC of 0.06 µg/mL and 30.3% of isolates had an MIC of 0.12µg/mL.(Table 4).

Table 1 Age distribution of culture confirmed cases of enteric fever

Age group	No. of cases(%)
≤15years	3 (9.1%)
16-25years	11 (33.3%)
26-35years	8 (24.2%)
36-45years	7 (21.2%)
≥46years	4 (12.1%)

Table 2 Antimicrobial susceptibility results of the isolates (by disc diffusion)

Antimicrobial	Total - 33		*S.* Typhi - 19		*S.* Paratyphi A- 14	
	S[a] No. (%)	R[b] No. (%)	S[a] No. (%)	R[b] No. (%)	S[a] No. (%)	R[b] No. (%)
Ampicillin	30 90.9)	3 (9.1)	17 (89.5)	2 (10.5)	13 (92.9)	1 (7.1)
Cotrimoxazole	31 93.9)	2 (6.1)	17 (89.5)	2 (10.5)	14 (100)	0
Chloramphenicol	31 (93.9)	2 (6.1)	17 (89.5)	2 (10.5)	14 (100)	0
Azithromycin	30 90.9)	3 (9.1)	18 (94.7)	1 (5.3)	12 (85.7)	2 (14.3)
Ciprofloxacin	0	33 (100)	0	19 (100)	0	14 (100)
Ceftriaxone	33 (100)	0	19 (100)	0	14 (100)	0

Footnotes –
a. Sensitive
b. Resistant

Table 3 MIC of Ciprofloxacin in µg/mL

MIC values	0.5 (µg/mL) No. (%)	1 (µg/mL) No (%)	2 (µg/mL) No (%)	4 (µg/mL) No (%)	8 (µg/mL) No (%)	16 (µg/mL) No. (%)	32 (µg/mL) No (%)	64 (µg/mL) No. (%)
	7 (21.2)	8 (24.2)	9 (27.3)	5 (15.2)	2 (6.1)	0	1 (3)	1 (3)
S.Typhi	5 (26.3)	3 (15.8)	6 (31.6)	1 (5.9)	2 (10.5)	0	1 (5.3)	1 (5.3)
S.Paratyphi A	2 (14.3)	5 (35.7)	3 (21.4)	4 (28.6)	0	0	0	0

Table 4 MIC of Ceftriaxone in µg/mL

	0.06 µg/mL No. (%)	0.12 µg/mL No. (%)
Total	23 (69.7)	10 (30.3)
S.Typhi	14 (73.7)	5 (26.3)
S.Paratyphi A	9 (64.3)	5 (35.7)

Discussion

Salmonella enterica is a major therapeutic concern for physicians in developing countries where typhoid and paratyphoid fevers are endemic. In the present study 33 *Salmonella* isolates were obtained from clinically suspected cases of enteric fever over a period of one year.

19 isolates (57.6%) were *S.*Typhi and 14isolates (42.4%) were *S.*Paratyphi A. *S.*Paratyphi B and *S.*Paratyphi C were not isolated in this study. Worldwide the ratio of paratyphoid fever: typhoid fever is 1:10, but there are several reports of increased incidence of infection with *S.*Paratyphi A in India[3,4]. Paratyphi A had been less frequently isolated in our institution till 2002. In 2001 the percentage of *S.*Paratyphi A isolates was 11.1% but it abruptly increased to 59.6% in 2002 and to 59% in 2003.This emergence of *S.*Paratyphi A as a major cause of enteric fever could be due to withdrawal of the trivalent TAB vaccine and introduction of typhoid vaccines, oral Ty21a and Vi, which confer protection only against *S.* Typhi. Changes in pathogenicity and virulence of *S.* Paratyphi A could also be a reason. An increase in prevalence of paratyphoid fever is a matter of concern for travellers visiting the region, in the absence of an effective vaccine against *S.*Paratyphi serotypes.

In a country like India where sanitation and hygiene are a mixture of poor and good, outbreaks of enteric fever may involve all age groups. 19 patients (57.5%) in the study group were young adults in the age group 16-35 years. Paratyphoid fever is reported to be more common in adults and the increased occurrence of patients in the adult age group in this study may be attributed to the increased prevalence of *S.*Paratyphi A[5].

Only two isolates of *S.*Typhi were MDR strains (resistant to Ampicillin, Cotrimoxazole and Chloramphenicol) accounting for 6.1% of the total isolates. Sensitivity to Chloramphenicol and Cotrimoxazole in the present study was 93.9%. Re-emergence of sensitivity to Chloramphenicol in *S.*Typhi has been reported in India since 1996[6]. In this institution, prevalence of MDRST was 100% in 1998 which subsequently decreased to 56.25% in 2001 and to 6.25% in 2003. 10.5% of *S.*Typhi were MDRST in the present study which shows that sensitivity to the first line drugs has remained fairly unchangedin our area for over 10yrs.There are several studies from India which report a re-emergence of sensitivity to the first line drugs, especially Chloramphenicol[6,7]. However study done by Surinder Kumar et al in New Delhi had found 80.5% of the isolates from enteric fever cases to be *S.*Typhi of which 52.9% were MDR[8]. Of the 14 isolates of *S.* Paratyphi A obtained, only one isolate was resistant to Ampicillin. All other isolates were sensitive to Ampicillin, Cotrimoxazole and Chloramphenicol. Multi-drug

resistance in *S*.Paratyphi A has always been low in our institution. It was 3.6% in 2002 compared to 21.05% among *S*.Typhi isolates, and was not detected in any isolate in 2003.

The replacement of Chloramphenicol with Ciprofloxacin for empirical treatment of typhoid fever has probably resulted in this re-emergence of sensitivity due to the withdrawal of selection pressure. Moreover Chloramphenicol is very rarely used by physicians for treating other infections as well.

All isolates in this study were resistant to Ciprofloxacin by disc diffusion method. The highest MIC detected in S.Typhi was 64µg/mLand in S.Paratyphi A was 4µg/mL. Majority of isolates showed an MIC of 2µg/mL (27.3%). High level resistance to Ciprofloxacin in southern India was first reported in a strain of *S*.Paratyphi A (MIC 8µg/mL) from JIPMER in 2004 by Harish et al[9]. This was soon followed by a report of *S*.Typhi with an MIC of 16µg/mL from AIIMS in 2005[10]. Since then enteric fever caused by salmonellae with high level resistance to Ciprofloxacin has been reported in several studies[11,12]. MICs as high as 512µg/mL have been reported from India and Bangladesh[13,14]. Ciprofloxacin is used in the treatment of a broad range of clinical conditions as well as in veterinary medicine. Indiscriminate use of Ciprofloxacin by medical practitioners as well as availability of Ciprofloxacin over-the-counter has resulted in such high level resistance in the developing nations. Incomplete treatment may also be a factor contributing to development of resistance.

30 isolates were found to be sensitive to Azithromycin (90.9%) in this study. *S*.Paratyphi A showed a higher prevalence of resistance (14.2%) compared to *S*.Typhi (5.2%). Injudicious out-patient use of this drug for enteric and other unrelated infections is a major risk factor for increase in resistance to this drug too. A study from Bangladesh by Islam et al in 2008had reported 100% resistance in both *S*.Typhi and *S*.Paratyphi A isolates[15].

However, Azithromycin has been shown to be effective in vivo even if resistance was detected in vitro[16]. Azithromycin is highly effective in removing intracellular salmonellae with rapid effervescence and is a potential alternative in paediatric patients. Large scale in vivo and in vitrostudies are needed to assess the clinical efficacy and potential as a drug of choice for treating enteric fever.

All isolates were sensitive to Ceftriaxone by disc diffusion as well as by MIC determination. Majority of the isolates (69.7%) had MIC 0.06µg/mL and the remaining 10 isolates (30.3%) had MIC 0.12µg/mL. Study by Capoor et al in 2007, reports that majority of isolates in 2001 had an MIC of ≤0.03 while in 2005-2006 most isolates had an MIC of 0.125µg/mL[13]. In our study, 26.3% of *S*.Typhi and 35.7% of *S*.Paratyphi A had an MIC of 0.12µg/mL. Though the value falls much below the CLSI criteria for sensitivity, this rise in MIC is of grave concern. There are several reports on resistance to Ceftriaxone in salmonellae, but has not been observed in our institution as yet. Ceftriaxone resistance was first reported in Bangladesh in 1999[17]. There are sporadic reports from India of *S*.Typhi showing resistance to Ceftriaxone. Study by Capoor et al in AIIMS in 2006 and another by Ketan Kulkarni et al in PGIMER in 2009 have reported *S*.Typhi isolates resistant to Ceftriaxone[2,18.] But recent studies from India report 100% sensitivity to Ceftriaxone [7,19,20].

Third generation cephalosporins are now widely used in the treatment of enteric fever due to Ciprofloxacin resistant isolates. However, high cost and need for parenteral administration are the disadvantages of cephalosporin therapy. Moreover, there is also a concern that their extensive use inthe outpatient setting will select *ß*-lactamases, ashas been observed in the hospital setting.

Continued surveillance on antimicrobial susceptibility pattern from different parts of the country will help in proper selection of antibiotics for therapeutic cure.

Conclusions

Paratyphoid fever occurs in almost the same frequency as typhoid fever. Hence future vaccination strategies should include bivalent vaccines with protection capacities against both *S.*Typhi as well as *S.*Paratyphi A.

Considering the high prevalence of resistance, Ciprofloxacin can no longer be considered the drug for treating enteric fever.

Chloramphenicoland Cotrimoxazolemay be reconsidered for empiric therapy of enteric fever as resistance to both drugs has been persistently low over last several years.

The importance of Azithromycin as a promising alternative is increasing, especially in the setting of Ciprofloxacin-resistant and ESBL-producing salmonellae. Judicious use of the drug is warranted to prevent more widespread resistance.

With Ceftriaxone emerging as the sole defence against highly drug resistant *Salmonellae,* usage of this drug in empiric therapy should be discouraged and should be instituted only in the event of resistance or non-responsiveness to the other available drugs.

There is an urgent need for national surveillance of antimicrobial use in order to promote rational usage of antimicrobial agents.

Acknowledgements

References

1. Maskell D. Salmonella Infections: Clinical, Immunological and Molecular Aspects. Cambridge University Press; 2006.
2. Capoor MR, Nair D, Hasan AS, Aggarwal P, Gupta B. Typhoid fever: narrowing therapeutic options in India. Http wwwtmmahidolacthseameo200637618-3738pdf 2006.
3. Bhutta ZA. Current concepts in the diagnosis and treatment of typhoid fever. BMJ 2006;333:78–82.
4. M SK. Comparison of Salmonella typhi and Paratyphi A Occurrence in a Tertiary Care Hospital. J ClinDiagn Res 2013. doi:10.7860/JCDR/2013/6551.3743.
5. Walia M, Gaind R, Paul P, Mehta R, Aggarwal P, Kalaivani M. Age-related clinical and microbiological characteristics of enteric fever in India. Trans R Soc Trop Med Hyg 2006;100:942–8. doi:10.1016/j.trstmh.2006.02.015.
6. Bhatia JK, Mathur AD, Arora MM. Reemergence of chloramphenicol sensitivity in enteric fever. Med J Armed Forces India 2007;63:212–214.
7. Gupta V, Singla N, Bansal N, Kaistha N, Chander J. Trends in the Antibiotic Resistance Patterns of Enteric Fever Isolates–a Three Year Report from a Tertiary Care Centre. Malays J Med Sci MJMS 2013;20:71.
8. Kumar S, Rizvi M, Berry N. Rising prevalence of enteric fever due to multidrug-resistant Salmonella: an epidemiological study. J Med Microbiol 2008;57:1247–50. doi:10.1099/jmm.0.2008/001719-0.
9. Harish BN, Madhulika U, Parija SC. Isolated high-level ciprofloxacin resistance in Salmonella enterica subsp. enterica serotype Paratyphi A. J Med Microbiol 2004;53:819–819.
10. Renuka K, Sood S, Das BK, Kapil A. High-level ciprofloxacin resistance in Salmonella enterica serotype Typhi in India. J Med Microbiol 2005;54:999–1000. doi:10.1099/jmm.0.45966-0.
11. Al DSC et. Drug-Resistant Salmonella enterica Serotype Paratyphi A in India - Volume 6, Number 4—August 2000 - Emerging Infectious Disease journal - CDC n.d. doi:10.3201/eid0604.000420.

12. Harish BN, Menezes GA, Sarangapani K, Parija SC. A case report and review of the literature: Ciprofloxacin resistant Salmonella entericaserovarTyphi in India. J Infect Dev Ctries 2008;2. doi:10.3855/jidc.229.

13. Capoor MR, Nair D, Deb M, Aggarwal P. Enteric fever perspective in India: emergence of high-level ciprofloxacin resistance and rising MIC to cephalosporins. J Med Microbiol 2007;56:1131–2. doi:10.1099/jmm.0.47170-0.

14. Saha SK, Darmstadt GL, Baqui AH, Crook DW, Islam MN, Islam M, et al. Molecular Basis of Resistance Displayed by Highly Ciprofloxacin-Resistant Salmonella entericaSerovarTyphi in Bangladesh. J ClinMicrobiol 2006;44:3811–3. doi:10.1128/JCM.01197-06.

15. Islam M, Das K, Sharmin N, Hasan M, Azad AK. Antimicrobial susceptibility of Salmonella serovars isolated from blood. J Innov Dev Strategy 2008;2:22–27.

16. Butler T, Sridhar CB, Daga MK, Pathak K, Pandit RB, Khakhria R, et al. Treatment of typhoid fever with azithromycin versus chloramphenicol in a randomized multicentre trial in India. J AntimicrobChemother 1999;44:243–50. doi:10.1093/jac/44.2.243.

17. Saha SK, Talukder SY, Islam M, Saha S. A highly ceftriaxone-resistant Salmonella typhi in Bangladesh. Pediatr Infect Dis J 1999;18:387.

18. Kulkarni K, Singh M, Kumar R. Is drug-resistant Salmonella typhi an emerging threat? BMJ Case Rep 2009;2009. doi:10.1136/bcr.04.2009.1783.

19. 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 2016;5. doi:10.1186/s13756-016-0121-8.

20. PorwAl A, BhAt S. Antibiotic Resistance among Enteric Fever Pathogens in a Tertiary Care Centre. Natl J Lab Med 2016;5:MO15–MO18.