

Antimicrobial susceptibility of *Salmonella* Typhi in India

Kavita Nagshetty¹, Shivannavar T. Channappa¹ and Subhashchandra M. Gaddad²

¹Department of Microbiology, Gulbarga University, Gulbarga-585106, Karnataka, India

²Department of Post Graduate Studies and Research in Microbiology, Gulbarga University, Gulbarga-585106, Karnataka, India

Abstract

Background: Typhoid fever continues to remain a major public health problem, especially in regions such as Gulbarga, due to poor sanitation and personal hygiene. Gulbarga region is often prone to enteric fever outbreaks and is an endemic region of typhoid fever. Enteric fever caused by *Salmonella* Typhi has not been adequately explored in this region.

Methodology: A total of 95 isolates of *S. Typhi* collected from different clinical and environmental sources were tested for antimicrobial susceptibility according to the CLSI guidelines. MIC of resistant isolates to various antibiotics was performed by agar dilution method.

Results: Of the total isolates studied, 10% were found to be multidrug resistant (MDR) (defined as resistance to ampicillin, chloramphenicol and co-trimoxazole). There was a decrease in the susceptibility to ciprofloxacin of *S. Typhi* with MIC showing an upward trend (0.125-4µg/mL). Concurrently, there has been an increase in the number of isolates sensitive to all antibiotics except nalidixic acid.

Conclusion: MDR *S. Typhi* continues to be an important public health issue in Gulbarga. Presence of quinolone resistance and associated low-level ciprofloxacin resistance is a concern and requires further study.

Key words: typhoid, multidrug resistance, *Salmonella* Typhi

J Infect Dev Ctries 2010; 4(2):070-073.

(Received 25 May 2009 – Accepted 12 December 2009)

Copyright © 2010 Nagshetty *et al.* This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Introduction

Enteric fever is a global public health problem. Almost 80% of the cases and deaths are in Asia and the rest occur mostly in Africa and Latin America [1]. It is estimated that there are 22 million new cases of enteric fever annually, with 200,000 deaths [2]. Regions with the highest incidence of enteric fever (> 100 cases per 100,000 persons per year) are South Central Asia and Southeast Asia. Regions of moderate incidence (10-100 cases per 100,000 persons per year) include the rest of Asia, Africa, Latin America and the Caribbean and Oceania, except for Australia and New Zealand. In Delhi, India, the incidence of enteric fever is 9.8 cases per 1,000 person-years [3]. *Salmonella enterica* serovar Typhi and Paratyphi A are the predominant types of etiological agents responsible for enteric fever in India, particularly during summer [4].

The emergence of antimicrobial resistance, especially the multidrug resistance to ampicillin, chloramphenicol and co-trimoxazole, has further complicated the treatment and management of enteric fever [4,5]. In India, antibiotic resistance among *S. Typhi* has been reported since 1960, and the first

outbreak of multidrug resistant *S. Typhi* (MDRST) was reported in Calicut [6]. Since then MDRST has appeared throughout the world, especially in South America, the Indian subcontinent, Africa and Southeast Asia [5]. The incidence of multidrug resistant (MDR) *S. Typhi* has been reported to be as high as 60% but then declined in Pune (1999), Nagpur (2001), Delhi (2004) and Calcutta (2000) [7-10]. However, resurgence of resistant strains in Ludhiana in 2002 is of concern [11]. A study of imported strains that was based in the United States [12] noted an increase in the number of MDR and nalidixic acid resistant *S. Typhi* globally (NARST), although all isolates remained sensitive to ciprofloxacin and ceftriaxone. In Bangladesh there has been a reported decrease in MDR isolates with no corresponding increase in sensitive strains [13]. For ciprofloxacin there has been an increase in MIC strains imported into the United Kingdom [14], Bangladesh [15], and India [16,17,18]. These observations with variations in the sensitivity patterns reported for *S. Typhi* and *S. Paratyphi A* stress the significance of continuous monitoring of antibiotic sensitivity patterns to provide suitable guidelines for treatment. Gulbarga is

Table 1. Number of *S. Typhi* isolates resistant to antibiotics by disc diffusion method.

Antibiotics Screened	No of isolates	Percentage
Nalidixic acid	30	31.57%
Ampicillin	28	29.47%
Chloramphenicol	27	28.42%
Co-trimoxazole	17	17.89%
Ciprofloxacin	4	4.21%
Ceftriaxone	6	6.31%
MDR*	9	10%

*Defined as resistance to ampicillin, chloramphenicol and co-trimoxazole

a socio-economically deprived region where both personal and community hygiene are minimal. According to records of the public and private hospitals, enteric fever is a major infectious disease occurring at high fluctuating incidences. Since there are no reports on current sensitivity patterns of *S. Typhi* isolates from this region, this study assesses the multidrug resistance among *S. Typhi* isolates with emphasis on susceptibility to ciprofloxacin.

Materials and Methods

Bacterial Culture

The study included 84 *S. Typhi* isolates from blood cultures of patients suffering from suspected typhoid fever who attended the outpatient clinics or were admitted in the private and government hospitals of the region during August 2006 to September 2007. Eleven additional environmental isolates were obtained from sewage, water and food. A total of 1,200 blood samples and 50 environmental samples were screened for *S. Typhi*.

Bile salt broth (broth culture) [19] and streptokinase broth (clot culture) [20] blood samples were used for enrichment as well as Selenite F-broth for environmental samples. The enriched samples after visible turbidity were streaked on Mac-Conkey, XLD and Wilson Blair media. The isolates producing characteristic colonies were identified by conventional biochemical tests using API20E and confirmed by agglutination with *Salmonella* O9, Vi specific and Hd antisera procured from King Institute of Preventive Medicine Guindy, Chennai.

Antimicrobial Susceptibility Testing

The antibiotic susceptibility testing was done by Kirby-Bauer disk diffusion method according to National Committee for Clinical Laboratory Standards (NCCLS) guidelines [21] using ampicillin (10 µg/disk), chloramphenicol (30 µg/disk), co-trimoxazole (1.25-23.75 µg/disk), ciprofloxacin (5

µg/disk), ceftriaxone (5 µg/disk), nalidixic acid (30 µg/disk) and imipenem (10 µg/disk) [23]. *Escherichia coli* ATCC 25922 was used as a negative control and *S. Typhi* MTCC 734 was used as a positive control for the effectiveness of the antibiotic disks. Commercially available six mm disks (Himedia Laboratories, Mumbai) were used.

MICs of isolates resistant to chloramphenicol, ampicillin and nalidixic acid were determined by agar dilution test [22] using purified antibiotic powders (Himedia Laboratories, Mumbai). MIC of ciprofloxacin were determined for only 20 randomly selected nalidixic acid resistant isolates. Isolates resistant to ampicillin, chloramphenicol and co-trimoxazole were termed MDR.

Result

A total of 95 *S. Typhi* isolates were obtained from 1,250 samples included in the study, indicating an incidence rate of 7.6% with highest incidence in environmental (11 isolates) samples (22%) followed by that in blood (84 isolates) samples (7%).

Antibiogram of these isolates revealed that all the isolates of *S. Typhi* were sensitive to imipenem. Highest resistance was observed against nalidixic acid (31.57%) closely followed by ampicillin (29.47%) and chloramphenicol (28.42%). The *S. Typhi* isolates showed low-level resistance against the majority of the remaining antibiotics (Table 1).

Approximately 10% (N = 9) of the isolates were resistant to multiple antibiotics. Of the antibiotics tested, 40% (N = 38) of the isolates had sensitive responses. MDR isolates were mainly resistant to three antibiotics: ampicillin, chloramphenicol and co-trimoxazole.

Among the 28 ampicillin resistant *S. Typhi* isolates, eight isolates showed an MIC of 64 µg/ml while 16 isolates showed MIC of 128 µg/ml, and for the remaining four isolates, the MIC was 256 µg/ml. Among the 27 chloramphenicol resistant *S. Typhi*

Table 2. MIC value of resistant *S. Typhi* to various antibiotics.

Antibiotic	No	Range ($\mu\text{g/mL}$)
Nalidixic acid (N = 30)	15	128
	13	64
	2	32
Ciprofloxacin (N = 20)	18	0.125
	1	1
	1	4
Ampicillin (N = 28)	4	256
	16	128
	8	64
Chloramphenicol (N = 27)	3	256
	24	32

*20 Nalidixic acid resistant isolates were tested for ciprofloxacin MIC

isolates, 24 have shown MIC of 32 $\mu\text{g/ml}$ while the remaining three isolates have shown MIC of 256 $\mu\text{g/ml}$.

Among the 20 nalidixic acid resistant isolates chosen for ciprofloxacin MIC, 18 showed an MIC of 0.125 $\mu\text{g/ml}$ and two had MIC of 1 $\mu\text{g/ml}$ and 4 $\mu\text{g/ml}$ (Table 2).

Discussion

Due to a combination of factors including poor sanitation and health care infrastructure, typhoid fever remains a major public health problem in most resource-poor countries such as India.

This is the first report of *S. Typhi* antimicrobial susceptibility from Gulbarga. According to public and private hospital records, enteric fever is a major infectious disease occurring at high fluctuating incidences in this region.

The data presented in our study highlights that MDR, although small, exists in this region. The presence of MDR (i.e resistance to ampicillin, chloramphenicol and co-trimoxazole) was 10% for the year 2006-2007. This finding is in accordance with recent reports from some regions where the incidence of MDR *S. Typhi* isolates appeared to have decreased [23-25]. The low frequency of MDR *S. Typhi* isolated is remarkable, since these drugs could once again be used for the treatment of enteric fever [10,25,26].

Nalidixic acid resistance is a marker for predicting low-level resistance to ciprofloxacin among *S. Typhi* and also an indicator of treatment failure to ciprofloxacin [10,15,25]. Hence it is now recommended by CLSI (previously NCCLS) that all *S. Typhi* isolates should be screened for nalidixic acid resistance along with ciprofloxacin [22]. Any isolate that shows resistance to nalidixic acid should be

reported as intermediately susceptible to ciprofloxacin [18]. Such strains have been found to be endemic in different parts of the world including India [26]. In our study nalidixic acid resistance was observed in 31.5% and was associated with increase in MIC to ciprofloxacin.

In conclusion, the findings of the present study indicated that first-line antibiotics might be an effective component in the treatment of enteric fever. Also, increasing resistance to quinolones is alarming and of particular concern is the rise in MIC levels to ciprofloxacin.

Acknowledgements

Thanks are due to N. V. Sherikar, Sr. Technician, and Shankar Gouda Patil, Department of Microbiology, M. R. Medical College, Gulbarga, for rendering their help in isolation of *S. Typhi*.

References

1. The World Health Report, Report of the Director General WHO (1996) World Health Organisation: Geneva.
2. Crump JA, Luby SP, Mintz ED (2004) The global burden of typhoid fever. *Bull World Health Org* 82: 346-53.
3. Sinha A, Sazawal S, Kumar R *et al.* (1999) Typhoid fever in children aged less than 5 years *Lancet* 354: 734-737.
4. Jesudason MV, John TJ (1992) Plasmid mediated multidrug resistance in *Salmonella typhi*. *Ind J Med Res* 95: 66-7.
5. Mourad AS, Matwally M, Nour Ei Deen A, *et al.* (1993) Multiple drug-resistant *Salmonella typhi*. *Clin Infect Dis* 17: 135-6.
6. Agarwal SC (1962) Chloramphenicol resistance of *Salmonella* species in India, 1956-61. *Bull Wld Hlth Orgn* 17: 331-5.
7. Sanghavi SK, Mane MP, Niphadkar KB (1999) Multidrug resistance in *Salmonella* serotypes. *Ind J Med Microbiol* 17: 88-90.
8. Chande C, Shrikhande S, Kapale S, Agarwal S, Fule RP (2002) Change in antimicrobial resistance pattern of *Salmonella typhi* in central India. *Ind J Med Res* 115: 248-50.

9. Saha MR, Dutta P, Niyogi SK, Dutta S, Mitra U, Ramamurthy T, *et al.* (2002) Decreasing trend in the occurrence of *Salmonella enterica* serotype *typhi* amongst hospitalized children in Kolkata, India during 1990-2000. *Ind J Med Res* 115: 46-8.
10. Walia M, Gaiind R, Mehta R, Paul P, Aggarwal P, Kalaivani M *et al.* (2005) Current perspectives of enteric fever: a hospital based study from India. *Ann Trop Paediatr* 25: 161-174.
11. Kumar R, Aneja KR, Roy P, Sharma M, Gupta R, Ram S (2002) Evaluation of minimum inhibitory concentration of quinolones and third generation cephalosporins to *Salmonella typhi* isolates. *Ind J Med Sci* 56: 1-8.
12. Ackers ML, Puhf ND, Tauxe RV, Mintz ED (2000) Laboratory-based surveillance of *Salmonella* serotype *typhi* infections in the United States: antimicrobial resistance on the rise. *JAMA* 283: 2668-73.
13. Rahman M, Ahmad A, Shoma S (2002) Decline in epidemic of multidrug resistant *Salmonella typhi* is not associated with increased incidence of antibiotic susceptible strain in Bangladesh. *Epidemiol Infect* 129: 29-34.
14. Threlfall EJ, Ward LR (2001) Decreased susceptibility to ciprofloxacin in *Salmonella enterica* serotype *typhi*, United Kingdom. *Emerg Infect Dis* 7: 448-50.
15. Asna SM, Haq JA, Rahman, M (2003) Nalidixic acid resistant *Salmonella enterica* serovar *typhi* with decreased susceptibility to ciprofloxacin caused treatment failure: a report from Bangladesh. *Jpn J Infect Dis* 56: 32-33.
16. Baliga S, Shenoy S, Vidyalaxmi K, Pereira P (1999) Ciprofloxacin-resistant *Salmonella typhi*. *Natl Med J India* 12: 138.
17. Jesudason MV, Malathy B, John TJ (1996) Trend of increasing levels of minimum inhibitory concentration of ciprofloxacin to *Salmonella typhi*. *Ind J Med Res* 103: 247-9.
18. Harish BN, Madhulika U, Parija SC (2004) Current Pattern in antimicrobial susceptibility of *Salmonella typhi* isolates in Pondichery. *Ind J Med Res* 120: 111-4.
19. Watson KC (1954) Clot culture in typhoid fever *J Clin Pathol* 7:305-307.
20. Watson KC (1978) Laboratory and clinical investigation of recovery of *Salmonella typhi* from blood *J Clin Microbiol* 7: 122-126.
21. Performance Standards for Antimicrobial disk Susceptibility Tests. CLSI, Wayne, PA, USA, 2005. NCCLS document M7-A.
22. Wayne, PA. Clinical and Laboratory Standards Institute (2006) *Clinical and Laboratory Standards Institution: Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*. Approved standard, CLSI document M7-A7.
23. Nath G, Tikoo A, Manocha H, Tripathi AK, Gulati AK. (2003) Drug resistance in *Salmonella typhi* in North India with special reference to ciprofloxacin. *J Antimicrobial Chemother* 46: 145-53.
24. Tankhiwale SS, Agrawal G, Jalgaonkar SV (2003) A Preliminary Report on Current Antibiogram of *Salmonella enterica* serotype *typhi* in Nagpur. *Ind J Med Microbiol* 21: 292
25. Rodrigues C, Shennai S, Mehta A (2003) Enteric fever in Mumbai, India: the good news and the bad news. *Clin Infect Dis* 36: 535.
26. Mandal S, Mandal MD, Kumar NP (2004) Reduced minimum inhibitory concentration of chloramphenicol for *Salmonella enterica* serovar *typhi*. *Ind J Med Sci* 58: 16-23.

Corresponding Author

Prof. Subhashchandra M Gaddad
 Department of P. G. Studies and Research in Microbiology
 Gulbarga University
 Gulbarga-585106,
 Karnataka,
 India
 E-mail: smgaddad@gmail.com
 Telephone: 08472-9342352882

Conflict of interest: No conflict of interest is declared.