Indian J Med Res 137, April 2013, pp 800-802

Antimicrobial susceptibility of *Salmonella enterica* serovars in a tertiary care hospital in southern India

Ashwini Choudhary, Ram Gopalakrishnan, Senthur Nambi P., Ramasubramanian V., Abdul Ghafur K. & Thirunarayan M.A.*

*Departments of Infectious Diseases & *Microbiology, Apollo Hospitals, Chennai, India*

Received October 11, 2011

***Background & objectives*: *Salmonella enterica* serovars Typhi and Paratyphi are predominantly known to cause enteric fever. Multidrug resistance in *S.* Tphi and *S.* Paratyphi has emerged as a cause of concern. This study was done to evaluate status in antimicrobial susceptibility patterns of *Salmonella enterica* serovar Typhi (*S.* Typhi) and *S.* Paratyphi obtained from blood culture in a tertiary care hospital in south India.**

***Methods*: Blood isolates of *Salmonella* species over a two year period between May 2009 and June 2011 were studied. A total of 322 isolates of *Salmonella* species were tested for antimicrobial susceptibility by Kirby-Bauer disc diffusion method. The MIC of ciprofloxacin was obtained by E-test, and azithromycin MIC was confirmed by agar dilution method for a limited number of isolates.**

***Results*: Of the total of 322 isolates studied, 186 (57.8%) were *S.* Typhi, 134 (41.6%) were *S.* Paratyphi A, and two were *S. P*aratyphi B. Of these, 44(13.66%) were resistant to ciprofloxacin (MIC <0.50 µg/ml) and 296 (91.9%) were nalidixic acid resistant. Of these 296 nalidixic acid resistant isolates, 278 (94%) were susceptible to ciprofloxacin by MIC criteria (<0.5 µg/ml). Of the 262 isolates tested for azithromycin sensitivity, only 120 (46%) were susceptible, whereas 81 (31%) were resistant and 55 (21%) showed intermediate susceptibility. Of the isolates, 322 (90%) were susceptible to ampicillin and (95%) were susceptible to co-trimoxazole. However, all the isolates were susceptible to chloramphenicol and ceftriaxone.**

***Interpretation & conclusions*: Nalidixic acid resistance screening is not a reliable surrogate indicator of ciprofloxacin resistance. Ciprofloxacin MIC should to be routinely done. Azithromycin resistance appears to be emerging. However, isolates showed a high degree of susceptibility to ampicillin, co-trimoxazole and chloramphenicol. Thus, antibiotics like ampicillin and co-trimoxazole may once again be useful for the management of enteric fever in southern India.**

Key words Antimicrobial susceptibility - azithromycin - ciprofloxacin - co-trimoxazole - Salmonella - typhoid

Multidrug resistant (MDR) strains (resistant to chloramphenicol, ampicillin and co-trimoxazole) of *Salmonella enterica* have emerged worldwide in the last two decades[1]. Isolates of *S. enterica* with reduced susceptibility to fluoroquinolones have now appeared in the Indian subcontinent and other regions[2,3]. However, in India the degree of resistance to commonly used antibiotics such as chloramphenicol, ampicillin and

co-trimoxazole in the era of quinolone resistance is not clear[1,4,5]. The present study was undertaken to document the change in the antibiotic susceptibility of *S. enterica* serovar Typhi and *S.* Paratyphi isolates obtained from blood culture during 2009-2011 in a tertiary care hospital in south India.

Material & Methods

All *S. enterica* isolates obtained from blood cultures of clinically suspected cases of enteric fever seen in Apollo Hospital, a tertiary care center in Chennai, south India, from May 2009 to June 2011 were included in the study. The study protocol was approved by the hospital ethics committee.

Antimicrobial susceptibility patterns were determined using commercial antimicrobial disks (Hi-Media, Mumbai): chloramphenicol (30 µg), nalidixic acid (30 µg), ampicillin (10 µg), azithromycin (15 µg), co-trimoxazole (1.25/23.75 µg), ciprofloxacin (5 µg), and ceftriaxone (30 µg). Antimicrobial susceptibility testing was performed in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines by Kirby-Bauer disc diffusion method[6].

Minimum inhibitory concentrations (MICs) for ciprofloxacin were determined using E-test (AB Bipods, Solana, Sweden). At the time of study the MIC for ciprofloxacin was 0.5 µg/ml as per CLSI but this has subsequently been reduced to 0.0625 mg/ml. ATCC *Escherichia coli* 25922 strain was used for quality control[7].

Results & Discussion

Of the total 322 isolates studied, 186 (57.8%) were *S.* Typhi and 134 (41.6%) were *S.* Paratyphi A, two were *S.* Paratyphi B. Of these isolates, 177 (55%) were sensitive to ciprofloxacin (MIC <0.25 mg/ml), 296 (91.9%) were nalidaxic acid resistant. Of the 296 nalidaxic acid resistant isolates, 278 (94%) were susceptible to ciprofloxacin (MIC <0.5 mg/ml). of the 262 isolates tested for azithromycin sensitivity, 120 (46%) were susceptible, 81 (31%) were resistant and 55 (21%) were intermediate. All 322 isolates were sensitive to ceftriaxone and chloramphenicol, 290 isolated (90%) were sensitive to ampicillin and 306 (95%) were sensitive to co-trimoxiazol (Table).

Enteric fever is a major public health problem in India. Various studies document *S.* Typhi as the commonest serovar isolated over the years[8], and our study also showed 57.86 per cent isolates of serovar Typhi while 41.61 per cent were serovar Paratyphi A[9].

Table. Sensitivity rates to various antibiotics

	No. of isolates	Sensitive isolates
Cotrimoxazole	322	306 (95.03%)
Ceftriaxone	322	322 (100%)
Nalidixic acid	322	26 (8.07%)
Ciprofloxacin	322	175 (54.34%)
Ampicillin	322	290(90.68%)
Chloramphenicol	322	322(100%)

In the last decade, there have been some reports of ciprofloxacin resistance in *Salmonella*[10]. It is believed that nalidixic acid resistance is a surrogate marker for ciprofloxacin resistance, as clinical failures have been documented in cases where ciprofloxacin has been used (based on susceptibility) for nalidixic acid resistant strains[11]. In our study, 13.66 per cent of isolates displayed reduced susceptibility to ciprofloxacin (MIC >0.5 µg/ml). However, as many as 94 per cent of nalidixic acid resistant isolates were ciprofloxacin sensitive by MIC testing. Kirby-Bauer disc diffusion assay using currently recommended breakpoints to ciprofloxacin may not be a reliable method, E-test should be the preferred method of choice to determine ciprofloxacin MIC[12,13]. Routine investigation and reporting of ciprofloxacin and azitromycin MICs in patients presenting with invasive *Salmonella* infections, like typhoid fever have been suggested[14,15].

Since its introduction in 1948, chloramphenicol has been the treatment of choice for typhoid fever and remains the standard against which newer antimicrobials are compared. Treatment with chloramphenicol reduces mortality due to typhoid fever from about 20 to 1 per cent and the duration of fever from 14-28 days to 3-5 days[16]. However, chloramphenicol therapy has been associated with the emergence of resistance to chloramphenicol, a high relapse rate, bone marrow toxicity and high mortality rates in a recent study reported from the developing world[17]. Ampicillin and co-trimoxazole could be effective alternative drugs[18]. In our study *Salmonella* sp. remained sensitive to chloramphenicol, ampicillin, and co-trimoxazole (100, 90 & 95%, respectively) over the two year study period as reported earlier[10,19]. These drugs may be preferred for treatment of enteric fever in our region.

Azithromycin has done well in clinical studies for typhoid[3]; however, there have been sporadic reports of azitromycin resistance[20]. All isolates in our study were

sensitive to ceftriaxone in contrast to some studies that reported resistance to ceftriaxone[21,22].

A limitation of our study was that clinical outcomes were not analyzed. Quinolones may remain effective despite *in vitro* resistance and ceftriaxone may be associated with prolonged time to fever resolution despite *in vitro* sensitivity[23].

In conclusion, for optimal interpretation of susceptibility, quinolone MIC is needed in cases of enteric fever where nalidixic acid is reported resistant. Azithromycin resistance is emerging. However, chloramphenicol, co-trimoxazole and ampicillin have re-emerged as valuable oral options and ceftriaxone remains a viable parenteral option for treatment of typhoid in India.

References

1. Madhulika U, Harish BN, Parija SC. Current pattern in antimicrobial susceptibility of *Salmonella* Typhi isolates in Pondicherry. *Indian J Med Res* 2004; *120* : 111-4.
2. Brown JC, Shanahan PM, Jesudason MV, Thomson CJ, Amyes SG. Mutations responsible for reduced susceptibility to 4-quinolones in clinical isolates of multi-resistant *Salmonella* Typhi in India. *J Antimicrob Chemother* 1996; *37* : 891-900.
3. Effa EE, Bukirwa H. Azithromycin for treating uncomplicated typhoid and paratyphoid fever (enteric fever). *Cochrane Database Syst Rev* 2008; (*4)* : CD006083.
4. Menezes GA, Harish BN, Khan MA, Goessens WH, Hays JP. Antimicrobial resistance trends in blood culture positive *Salmonella* Typhi isolates from Pondicherry, India, 2005-2009. *Clin Microbiol Infect* 2012; *18* : 239-45.
5. Harish BN, Menezes GA. Preserving efficacy of chloramphenicol against typhoid fever in a tertiary care hospital, India. *Reg Health Forum* 2011; *15* : 92-6.
6. Clinical and Laboratory Standards Institute (CLSI) *Performance standards for antimicrobial disk susceptibility tests*. Approved Standard - 9th ed., CLSI Document M2-A9. Wayne, PA: CLSI; 2006.
7. European Committee on Antimicrobial Susceptibility Testing Breakpoint tables for interpretation of MICs and zone diameters Version 1.3, 2011. Vaxjo, Sweden. Available from: *http://www.eucast.org/fileadmin/src/media/pdfs/EUCAST-files/Disk-test-documents/EUCAST_breakpoints_vl.3.pdf.pdf.*
8. Manchanda V, Bhalla P, Sethi M, Sharma VK. Treatment of enteric fever in children on the basis of current trends of antimicrobial susceptibility of *Salmonella enterica* serovar typhi and paratyphi A. *Indian J Med Microbiol* 2006; *24* : 101-6.
9. Chandel DS, Chaudhry R, Dhawan B, Pandey A, Dey AB. Drug-resistant *Salmonella enterica* serotype paratyphi A in India. *Emerg Infect Dis* 2000; *6* : 420-1.
10. Nath G, Tikoo A, Manocha H, Tripathi AK, Gulati AK. Drug resistance in *Salmonella* Typhi in north India with special reference to ciprofloxacin. *J Antimicrob Chemother* 2003; *46* : 145-53.
11. Mandal S, Mandal MD, Pal VK. Nalidaxic acid resistance predicting reduced ciprofloxacin susceptibility of *Salmonella enterica* seroval typhi. *Asian Pacific J Trop Dis* 2012; *2* (Suppl 2): S585-7.
12. Crump JA, Barett TJ, Nelson JI, Angulo FJ. Reevaluating fluoroquinolone breakpoints for *Salmonella enterica* serotype Typhi and for non-Typhi salmonellae. *Clin Infect Dis* 2003; *37* : 75-81.
13. Harish BN, Menezes GA. Antimicrobial resistance in typhoidal salmonellae. *Indian J Med Microbiol* 2011; *29* : 223-9.
14. Parry CM, Thuy CT, Dongol S, Karkey A, Vinh H, Chinh NT, *et al*. Suitable disk antimicrobial susceptibility breakpoints defining *Salmonella enterica* serovar Typhi isolates with reduced susceptibility to fluoroquinolones. *Antimicrob Agents Chemother* 2010; *54* : 5201-8.
15. Ray P, Sharma J, Marak RSK, Garg RK. Predictive efficacy of nalidixic acid resistance as a marker of fluoroquinolone resistance in *Salmonella enterica* var Typhi. *Indian J Med Res* 2006; *124* : 105-8.
16. Mirza SH, Beeching NJ, Hart CA. Multi-drug resistant typhoid: a global problem. *J Med Microbiol* 1996; *44* : 317-9.
17. Nagshetty K, Channappa ST, Gaddad SM. Antimicrobial susceptibility of *Salmonella* Typhi in India. *J Infect Dev Ctries* 2010; *4* : 70-3.
18. Parry CM. The treatment of multidrug-resistant and nalidixic acid-resistant typhoid fever in Vietnam. *Trans R Soc Trop Med Hyg* 2004; *98* : 413-22.
19. Tankhiwale SS, Agrawal G, Jalgaonkar SV. A preliminary report on current antibiogram of *Salmonella enterica* serotype Typhi in Nagpur. *Indian J Med Microbiol* 2003; *21* : 292.
20. Pfeifer Y, Matten J, Rabsch W. *Salmonella enterica* Serovar Typhi with CTX-M beta-lactamase, Germany. *Emerg Infect Dis* 2009; *15* : 1533-5.
21. Molloy A, Nair S, Cooke FJ, Wain J, Farrington M, Lehner PJ, *et al*. First report of *Salmonella enterica* serotype paratyphi A arzithromycin resistance leading to treatment failure. *J Clin Microbiol* 2010; *48* : 4655-7.
22. Gokul BN, Menezes GA, Harish BN. Acc-1 β-lactamase–producing *Salmonella enterica* serovar Typhi, India. *Emerg Infect Dis* 2010; *16* : 1170-1.
23. Jog S, Soman R, Singhal T, Rodrigues C, Mehta A, Dastur FD. Enteric fever in Mumbai - clinical profile, sensitivity patterns and response to antimicrobials. *J Assoc Physicians India* 2008; *56* : 237-40.

Reprint requests: Dr Ashwini Choudhary, Department of Infectious Diseases, 21 Greams Lane, Off Greams Road, Apollo Hospitals, Chennai 600 006, India
e-mail: drashwini.tayade@gmail.com