Research Article

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Antimicrobial resistance pattern of Salmonella enterica servars in Southern Delhi

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ABSTRACT

Background: Enteric fever is predominantly caused by serovars Typhi and Paratyphi of *Salmonella enterica*. Recently upsurge in multidrug resistant typhoid fever has become a major public health concern in developing countries. This study was conducted to determine the sensitivity pattern of *S.* Typhi and *S.* Paratyphi in Southern Delhi.

Methods: A total of 98 Salmonella species were isolated over a period of two years between March 2012 – March 2014. Diagnosis of patients was based on clinical features, blood culture and serology. Blood samples were collected and incubated in BacT alert 3D system. Identification was done and antibiotic susceptibility was done by conventional methods and cross checked by Vitek 2 compact system.

Results: Of the 98 isolates 87 (88.7 %) were *S.* Typhi and 11 (11.22%) were *S.* Paratyphi. Out of 87 samples from which *S.* Typhi was isolated (68) 78% were male and (19) 21.8% were female patients and they belonged to all age groups. Fever was present in all patients. In this study high resistance was found for both *S.* Typhi and *S.* Paratyphi respectively for third generation cephalosporins (35.6%, 54.5%), good sensitivity for macrolides (90.8% & 100%) and average sensitivity for ofloxacin (81.6% & 63.7%). Ampicillin and chloramphenicol shows lesser resistance i.e. (11.5% & 18.1%) and (12.6% and 27.2%). 3.5% and 0% resistance was found for imipinem and tigecycline was found 100% sensitive for both *S.* Typhi and *S.* Paratyphi, used rarely in treatment of typhoid fever. None of the strains were found sensitive to all drugs.

Conclusion: Typhoid bacilli keeps changing its antimicrobial susceptibility pattern. Currently newer drugs like tigecyclline, imipenem and macrolide are showing great response. Primary drugs are showing less resistance may be due to their infrequent usage whereas cephalosporins are showing high resistance towards typhoid bacilli. For better treatment it is important to know the recent susceptibility trend of the area.

Keywords: Antibiotic sensitivity, Salmonella Typhi, Enteric fever

INTRODUCTION

Enteric fever continues to be a huge health problem in developing countries inspite of antibiotic use and the development of newer drugs. In India *S.* Typhi is the predominant serotype of Salmonella responsible for enteric fever in and has rapidly gained resistance to ampicillin, chloramphenicol, cotrimoxazole and to previously effective drugs like ciprofloxacin. ¹⁻³ Sporadic

cases of resistance to chloramphenicol in *S*. Typhi were reported as early in 1950 and later in 1962 and 1965.⁴ The first major epidemic by multidrug resistant strain was reported in Mexico.⁵ *S*. Typhi of Vi phage type DI-N was isolated from Calicut.⁶ Since then, many cases were reported from India⁷ and other tropical countries, indicating the broad distribution of chloramphenicol resistant *S*. Typhi.

Drug resistant strains of S. Typhi have posed a vast problem in the treatment of typhoid patients. Enteric fever due to multidrug resistant (MDR) S. Typhi is frequently associated with increased morbidity and mortality.8 MDR S. Typhi has been prevalent in India since 1989. During 1990-1992 these strains were resistant the three commonly used antibiotics chloramphenicol, ampicillin and cotrimoxazole. Subsequently during 1993-1997, 30-35% regained susceptibility to these drugs9 and ciprofloxacin, a fluroquinolone emerges as the drug of choice for the treatment of MDR typhoid fever in India as it shortened the illness by three days. 10 However, due to their injudicious use reports of clinical failure and decreased efficacy of quinolones came in although they showed in vitro susceptibility. Subsequently ciprofloxacin third generation cephalosporins like ceftriaxone were started to be used for the empirical therapy of suspected typhoid fever anticipating resistance to chloramphenicol. Gradually reports of resistance emergence started coming in from various parts of worlds for these drugs too. Currently newer drugs like macrolides, imipenem, tigecyclline and others are in use. Also discontinuation of chloramphenicol therapy is expected to relieve the selection pressure paving the way for reemergence of S. typhi isolates sensitive to chloramphenicol. 11 From 2000, there have been reports of high susceptibility for chloramphenicol in India.¹² Emergence of resistance and reemergence of sensitivity make us to keep an eye on the sensitivity of this organism. However there is no general consensus among the practitioners regarding the choice of drug for the treatment of enteric fever. This all prompted us to undertake the present study with the objective to observe any change in the sensitivity pattern of S. typhi especially to quinolones, cephalosporins and chloramphenicol and some newer drugs.

METHODS

Blood culture samples were collected in BacT/ALERT 3D culture bottles (BioMerieux, France) from OPD and IPD patients over a period of 2 years (March 2012-March 2015). Bottles were then incubated in BacT/ALERT 3D system (BioMerieux) until microbial growth was detected or maximum for five days continuously. BacT/ALERT 3D bottles that indicated growth underwent gram staining and microscopy, and were plated onto Sheep Blood agar and MacConkey agar and further incubated at 37°C. The isolates were identified as Salmonella enterica serotype Typhi, Paratyphi A and Paratyphi B by standard biochemical methods⁶ and confirmed by slide agglutination with specific antisera (Salmonella agglutinating serum, Himedia, Mumbai). Sensitivity was done by Kirby bauers methods as per CLSI guidelines. 41 Some strains, in case of doubts were tested on VITEK 2 Compact System too (BioMerieux). VITEK 2 Compact is an automated microbial identification and antibiotic susceptibility testing system, which gives identification and antibiotic susceptibility of bacteria within 8-18 h.

The antibiotic susceptibility testing was carried out by the Kirby-Bauer disc diffusion method using CLSI breakpoints 2013. ⁴¹ The antimicrobial agents tested were Ampicillin (10 μg), Ceftriaxone (30 μg), Cefepime (30 μg), Imipenem (10 μg), Meropenem (10 μg), Chloramphenicol (30 μg), Ciprofloxacin (5 μg), Cotrimoxazole (25 μg) and Azithromycin (30 μg), Ciprofloxacin (30 μg), Ofloxacin (30 μg), Tigecyclline (30 μg), Nalidixic acid (30 μg). A standard strain of *E. coli* ATCC 25922 was included as quality control. Isolates resistant to Ampicillin, Chloramphenicol and Cotrimoxazole were defined as multi-drug resistant *S. typhi* (MDRST).

RESULTS

Of the 98 isolates 87 (88.7%) were S. Typhi and 11 (11.22%) were S. Paratyphi. Out of 87 samples from which S. Typhi was isolated 68 (78%) were from male and 19 (21.8%) were from female patients whereas isolation rate of S. Paratyphi was 72.7% from males and 27.3% from females and they belonged to all age groups. Fever was present in all patients. In this study high resistance was found for both S. Typhi and S. Paratyphi, respectively for third generation cephalosporins (35.6%, 54.5%), good sensitivity for macrolides (90.8% & 100%) and average sensitivity for ofloxacin (81.6% & 63.7%). Ampicillin and chloramphenicol shows lesser resistance i.e. (11.5% & 18.1%) and (12.6% and 27.2%). 3.5% and 0% resistance was found for imipinem and tigecycline was found 100% sensitive for both S. Typhi and S. Paratyphi, used rarely in treatment of typhoid fever. None of the strains were found sensitive to all drugs.

DISCUSSION

Enteric fever caused by *Salmonella enterica*, is a systemic infection with high rate of morbidity and mortality and have been a major public health problem in developing countries due to poor sanitation, overcrowding and factors like health care infrastructure.¹³ In our study, total of 98 strains were found over a period of two years. Male to female ratio was nearly 3:1 in concordance with other reports.^{14,15} which may be due to the more involvement of males in outdoor activities and also as males are more likely to report in hospitals.

Majority was in the age group of 6 to 30 yrs. It may be due to more mobility and high consumption of unhygienic food and water in schools or colleges. Health education may help and bring this number down. These figure are consistent with other studies. 15-18

Proper treatment with antibiotics can reduce the mortality from 30% to 0.5%. ¹⁹ But constant rise in antibiotic resistance in *S. enterica* has become a therapeutic concern for clinicians in endemic regions for patients and travelers who visit these regions and are not vaccinated.

Prevalence rate of 0-61% has been documented by different studies. 20-23

Several developing countries reporting emergence and spread of multidrug resistance (MDR) in *S. enterica* are busy in frantic search of effective and inexpensive alternative drugs to reduce the morbidity and mortality of enteric fever.²⁴

In 1948 chloramphenicol was introduced for the treatment of typhoid fever would reduce the duration of fever from 14-28 days to 3-5 days and mortality to 1%. Where chloramphenicol could not be used ampicillin and cotrimoxazole were used as alternative drug. But due to its rampant use chloramphenicol resistant S. typhi was emerged in UK within two years of its successful use. In India it was first reported in Kerela in 1972. Subsequently reports of sporadic cases of transferable resistance of chloramphenicol with other drugs started coming in. In 1990s rapid emergence MDR S. typhi was reported in many parts of India due to the acquisition of R-plasmid encoding acetyl transferase inactivating chloramphenicol and loss of OMP.25 Dihydrfolate reductase VII and TEM-1 beta lactamase were found responsible for conferring resistance to ampicillin and cotrimoxazole.26 In India, MDR was first reported in Mumbai in 1990, an outbreak came to known as dombivalli fever.²⁷

In our study, chloramphenicol was found to be sensitive in 87.4% strains which is consistent with other studies²⁸ where higher resistance of have also been reported. Rise in resistance was reported in 2002-2004.²⁹⁻³⁰

Since 1989, emergence of strains with resistance to ampicilin, chloramphenicol and cotrimoxazole leads to wider use of fluoroquinolones as ciprofloxacin and ofloxacin.

Moderate cost, advantage of oral intake, tolerability convenient dosage schedule of fluoroquinolones led to rampant use and subsequent decrease in its sensitivity and clinical efficacy which prompted physician to use third generation cephalosporins. Nalidixic acid resistance is used as marker for predicting low level resistance to ciprofloxacin among *S.* Typhi and also an indicator of treatment failure to ciprofloxacin. St-37 In our study, nalidixic resistance was found in 51.8% strains and was associated with increase in MIC to ciprofloxacin. Ciprofloxacin was found resistant in 26.4% strains, where this study showed similar results with others.

Drug resistance has been observed on rise for quinolones with emergence of Nalidixic acid resistance (NAR) *S. enterica* i.e. 51% in 2006 to 87.8% and even higher.³⁸⁻⁴²

This resistance to quinolones is caused by amino acid substitutions in the quinolone resistance–determining region of the DNA gyrase, subunit gyrA, gyrB or DNA topoisomerase IV (parC, parE) which are key targets of quinolones. ¹⁶ Single mutation in gyrA is said to be

responsible for decreased susceptibility to ciprofloxacin whereas combination of 2 or more mutations in gyrA, gyrB, parC and parE makes them resistant.¹⁷ Ciprofloxacin is concentrated in human monocytes and increases their bactericidal activity against intracellular bacteria which may explain why it is still effective in achieving clinical cure in patients with salmonella infections, which is intracellular. But plasmid mediated quinolone resistance has already been reported.⁴³ Third and fourth generation cephalosporins, carbapenems are reported to be a good alternative to quinolone.

All the isolates were sensitive to tigecyclline in our study and 10.4% resistance were seen for cefepime. However cefepime, ceftriaxone and cefxime have shown good results. Only cefexime can be administered orally while ceftriaxone and cefepime have parentral routes of administration. *S.* Typhi has been found to produce a wide variety of ESBL types as TEM, SHV, PER, CTXM enzymes. This is a new challenge and has become a matter of concern Amp C beta lactamase producing *S.* Typhi has also been reported. ²⁶

Treatment option are becoming limited with increasing resistance for third and fourth generation cephalosporin.

Azithromycin can be a drug of choice. In our study, 90.8% strains were sensitive similar to other reports.²⁴ It has negligible relapse rate and a favourable outpatient outcome, defervesence is rapid and represents potential alternative in paedetric population for whom quinolone are contraindicated.²⁶

Non availability of breakpoint concentration of azithromycin for Salmonella in most standard antibiotic guideline make the laboratory interpretation difficult.³⁸

Carbapenems can be given and found effective. In our study, 96.5% strains were sensitive where other studies have reported sensitivity of 100%.⁴⁶

CONCLUSION

Antibiotic resistance in typhoidal, Salmonella is a matter of concern for clinicians. The management depends on understanding of local patterns of antimicrobial pattern and on result of antimicrobial susceptibility testing. Though the azithromycin, tigecycline, and carbapenem are not recommended by CLSI. It is required in cases of quinolone resistant and ESBL producing Salmonella so it is advisable to control the disease by vaccination identification and treatment of carrier and improvement in sanitation.

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REFERENCES

- 1. Saberwal U, Chaudhary U, Saini S. Multi drug resistant Salmonella Typhi in Haryana in 1980-90. Indian J Med Res. 1992;95:12-3.
- Jesudason MV, John TJ, Plasmid mediated multidrug resistance in Salmonella Typhi. Indian J Med Res. 1992:95:66-7.
- 3. Butt T, Ahmad RN, Mahmood A, Zaidi S. Ciprofloxacin treatment failure in typhoid fever case, Pakistan. Emerg Infect Dis. 2003:9:1621-2.
- Murti RB, Rajyalakshami K, Bhaskaran CS. Resistance to salmonella typhi to chloramphenicol. Part 1A, Prelimnary report. J Clin Pathol. 1962;15:544-6.
- 5. Anderson ES, Smith HR. Chloramphenicol resistance in the typhoid bacillus. Br Med J. 1972;3:329-31.
- 6. Pariker CK, Vimla KN. Transfrable chloramphenicol reistance in Salmonella typhi. Nature. 1972;239:109-10.
- 7. Salmonella and Shigella incidence data from Medical Colleges in India. Bull NICD. 1977; Apriljune and July- September.
- 8. Kumar R, Aneja KR, Punia AK, Roy P, Sharma M, et al. Changing pattern of biophage and drug resistance of Salmonella typhi in Ludhiyana during 1980-99. Indian J Med Res. 2001;113:175-80.
- 9. Saha MR, Dutta S, Niyogi SK, Dutta S, Mitra U, et al. Decreasing trend in the occurrence of Salmonella enterica serovar Typhiamongst the hospitalize children in Kolkata, India during 1990 -2000. Indian J Med Res. 2002;115:46-8.
- Mathai D, Kudvu GC, Keystone JS, Jesudasson MV, Lalitha MK. Short Course ciprofloxacin therapy for enteric fever. J Assoc Physicians India. 1993;41:428-30.
- 11. Madhulika U, Harish BN, Parija SC. Current pattern in antimicrobial susceptibility of salmonella Typhi isolate in Pondicherry. Indian J Med Res. 2004;120: 111-4.
- 12. Sen B, Dutta S, Sur D, Munna B, Deb AK, et al. Phage typing, biotyping and antimicrobial resistance profile of Salmonella enteric serovar Typhi from Kolkata. Indian J Med Res. 2007;125:685-8.
- 13. Roy P, Rawat D, Malik S. A case of extended spectrum beta-lactamase producing Salmonella enterica serotype paratyphi A from India. Indian J Pathol Microbiol. 2015;58:113-4.
- 14. Sudeepa Kumar M, Vijaykumar GS, Prakash R, Prashanth HV, Raveesh PM, et al. Salmonella typhi and paratyphi A in Tertiary Care. J Clin Diagn Res. 2013;7(12):2724-6.
- 15. Hafsa A, Hussain M, Fakruddin M. Prevalence and antibiotic susceptibility patterns of blood stream

- Salmonella infections in a tertiary care hospital, Dhaka. J Med Sci. 2013;13(5):363-6.
- 16. Parande MA, Patil CG, Rayate MV, Lukde MU. Epidemiological profile of enteric fever cases admitted in scsmgh, solapur. Natl J Community Med. 2011;2(1):91–5.
- 17. Walia M, Gaind R, Paul P, Mehta R, Aggarwal P, Kalaivani M, et al. Age related clinical and microbiological characteristics of enteric fever in India. Trans R Soc Trop Med Hyg. 2006;100:942-48.
- 18. Kanungo S, Dutta S, Sur D. Epidemiology of Typhoid and paratyphoid fever in India. J Infect Developing Countries. 2008;2(6):454-60.
- 19. Cooke FJ, Wain J. The emergence of antibiotic resistance in typhoid fever. Travel Med Infect Dis. 2004;2:67-74.
- Basnyat B, Maskey A, Zimmerman M. Emerging trends in enteric fever in Nepal—9124 cases confirmed by blood culture 1993—2003. 9th Congress of the International Society of Travel Medicine, Lisbon, Portugal, 2005 (abstract No. FC01.02).
- 21. Rao RS, Sundararaj R, Subramanian S, Shankar V, Morti SA, Kapoor SC. A study of drug resistance among Salmonella typhi and Salmonella paratyphi A in an endemic area 1977—79. Trans R Soc Trop Med Hyg. 1981;75:21—4.
- 22. Pato-Mesola VV, Donaldo MES. Antimicrobial susceptibility of Salmonella typhi isolates from government and private hospitalsin Cebu City. Phil J Microbiol Infect Di.s 1997;26:5—8.
- 23. Rao PS, Rajasekhar V, Verghese GK, Shivananda PG. Emergence of multidrug-resistant Salmonella typhi in rural Southern India. Am J Trop Med Hyg. 1993;48:108—11.
- 24. Manish Chandey and A.S. Multani, A Comparative Study of Efficacy and Safety of Azithromycin and Ofloxacin in Uncomplicated Typhoid Fever. J Clin Diagnc Res. 2012;6(10):1736-9
- 25. Toro CS, Lobos SR, Calderon I, Rodriguez M, Mora GC. Clinical isolate of a porinless Salmonella typhi resistant to high levels of chloramphenicol. Antimicrob Agents Chemother. 1990;34:1715-9.
- Jaspal Kaur, Resistance and Therapeutics in Typhoidal Salmonellae. J Clin Diagnc Res. 2013;7(3):576-5
- 27. Deodhar L, Bhave S, Agarwal A. 'Bacteriophage typing of multi drug resistant Salmonella Typhi in paediatric patients'. Bombay Hospital Journal. 1993;35:114-15.
- 28. Nagshetty K, Channappa ST, Gaddad SM. Antimicrobial susceptibility of Salmonella Typhi in India. J Infect Dev Ctries. 2010;4(2):070-3.
- 29. Gautam V, Gupta N, Chaudhary U and Uma D.R.2002. Sensitivity pattern of salmonella serotypes in Northern India. Braz J Infect Dis. 6(6).Salvodar Dec.
- 30. Momtaz O, Wasfy R, Tharwat f, Ismail f, Mansour H, Joseph l, et al. Trends of multiple drug resistance

- among salmonella serotype typhi Isolates a 14 year period in Egypt. Clin Infect dis. 35:1265-8.
- 31. Bhart Kishore G, Andrade At, Karadesai SG, Hemashettar BM, Patil S. Antimicrobial susceptibility of Salmonella Typhi to quinolones and cephalosporins. Indian J Med Res. 1998;107:247-51.
- 32. Bhattacharya SS, Das Usha. Occurrence of Salmonella Typhi infection in Rourkela, Orissa. Indian J Med Res. 2000:111:75-6.
- 33. Chowta MN, Chowta NK. Study of clinical profile and antibiotic response in typhoid. Indian J Med Microbiology. 2005;23:125-7.
- 34. Sood S, Kapil A, Das B, Jain Y, Kabra SK. Reemergence of chloramphenicol sensitive Salmonella Typhi. The Lancet. 1999;53:1241-2.
- 35. Bhave S, Agarwal A. 'Bacteriophage typing of multi drug resistant Salmonella Typhi in paediatric patients'. Bombay Hospital Journal. 1993;35:114-5.
- Asna SM, Haq JA, Rahman M. Nalidixic acid resistant Salmonella enterica serover typhi with decreased susceptibility to ciprofloxacin caused treatment failure: a report from Bangladesh. Jpn J Infect Dis. 2003;56:32-3.
- 37. Rodrigues C, Shennai S, Mehta A. Enteric fever in Mumbai, India: the good news and the bad news. Clin nfect Dis. 2003;36:535.
- 38. Rai S, Jain S, Prasad KN, Ghoshal U, Dhole TN. Rationale of azithromycin prescribing practices for enteric fever in India. Indian J Med Microbiol. 2012;30:30-3.
- Capoor MR, Nair D, Hasan AS, Aggarwal P, Gupta B. Typhoid fever: Narrowing therapeutic options in India. Southeast Asian J Trop Med Public Health. 2006;37:1170-4.
- 40. Gupta V, Kaur J, Chander J. An increase in enteric fever cases due to Salmonella Paratyphi A in and

- around Chandigarh. Indian J Med Res. 2009;129:95-8
- 41. Krishnan P, Stalin M, Balasubramanian S. Changing trends in antimicrobial resistance of Salmonella enterica serovar Typhi and Salmonella enterica serovar Paratyphi A in Chennai. Indian J Pathol Microbiol. 2009;52:505-8.
- 42. Kapil A, Renuka, Das B. Nalidixic acid susceptibility test to screen ciprofloxacin resistance in Salmonella Typhi. Indian J Med Res. 2002;115:49-54.
- 43. Girish R, Kumar A, Khan S, Dinesh KR, Karim S. Revised Ciprofloxacin Breakpoints for Salmonella: Is it Time to Write an Obituary? J Clin Diagn Res. 2013;7(11):2467-9.
- 44. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. CLSI document (M100-S16) 26, Clinical and Laboratory Standard Institute: Wayne, Pennsylvania. 2007.
- 45. Menezes GA, Harish BN, Khan MA, Goessens WHF, Hays JP. Antimicrobial resistance in Salmonella Typhi. Clin Microbiol Infection. 2012;18(3).
- 46. Pokharel BM, Koirala J, Dahal RK, Mishra SK, Khadga PK, Tuladhar NR. Multidrug-resistant and extended-spectrumbeta-lactamase (ESBL)-producing Salmonella enterica (serotypes Typhi and Paratyphi A) from blood isolates in Nepal: surveillance of resistance and a search for newer alternatives. Int J Infect Dis. 2006;10:434—8.

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