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Original Article

Trends in antimicrobial susceptibility of *Salmonella* Typhi from North India (2001-2012)

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Abstract

Purpose: Enteric fever is endemic in India with *Salmonella* Typhi being the major causative agent. Antibiotic therapy constitutes the mainstay of management. The present study was undertaken to find the susceptibility profile of *Salmonella enterica* var Typhi (*S.* Typhi) blood isolates in a tertiary care hospital between January 2001 and December 2012. **Materials and Methods:** A retrospective analysis of laboratory records was carried out. Conventional blood culture method was used until 2009; from January 2010 onwards BACTEC 9240 system has been in use. *Salmonella* were confirmed by serotyping using group and type specific antisera. Antibiotic susceptibility was performed using the disk diffusion method. In addition 116 isolates were subjected to minimum inhibitory concentration testing for chloramphenicol, ciprofloxacin, amoxicillin and nalidixic acid (NA) using agar dilution and for ceftriaxone and azithromycin using E-strips (Biomerieux). **Result:** A total of 1016 typhoidal *salmonellae* were obtained. The predominant serotype obtained was *S.* Typhi (852, 83.8%) followed by *Salmonella enterica* var Paratyphi A (164, 16.2%). We observed a re-emergence of susceptibility to first line antibiotics and a notable decline in multidrug resistant (MDR) strains. We also found all recent isolates resistant to NA and susceptible to third generation cephalosporins and 84.5% of isolates having decreasing ciprofloxacin susceptibility using revised criteria as per Clinical and Laboratory Standards Institute 2012 guidelines. **Conclusion:** There has been re-emergence of susceptibility to first line antibiotics and a notable decline in MDR strains of *S.* Typhi. We have a very high resistance to NA and decreasing susceptibility to ciprofloxacin. Third generation cephalosporins and azithromycin seem to be effective therapeutic options. Judicious use of these antibiotics is mandatory to prevent emergence of resistant strains.

Key words: *Decreased ciprofloxacin susceptibility, minimum inhibitory concentration, revised zone diameters, Salmonella Typhi*

Introduction

Enteric fever is endemic in India with *Salmonella enterica* var Typhi (*S.* Typhi) and *Salmonella enterica* var Paratyphi A (*S.* Paratyphi A) being the major causative agents. These human restricted pathogens are transmitted by the faeco-oral route in regions with poor standards of hygiene and sanitation accounting for high morbidity and mortality. Antibiotic therapy constitutes the mainstay of management of enteric fever; mortality being as high as 30% in untreated cases, which falls to <1% with appropriate antibiotic therapy. Failure to treat an infection properly leads to prolonged illness, thus increasing the chance of developing a carrier state in which persons are contagious and able to spread the resistant strain to others. In the last few decades, the emergence of multidrug resistant (MDR) salmonellae (resistant to ampicillin, chloramphenicol and co-trimoxazole) has led to widespread use of fluoroquinolones and third-generation cephalosporins as the first-line drugs.[1,2]

In recent years, both changes in the epidemiology and drug resistance profile of enteric fever have been noted by various workers. Firstly, many of the researchers have reported an increasing trend of *S.* Paratyphi A over the last decade in India.[3] Secondly, re-emergence of susceptibility to conventional first-line antibiotics (ampicillin, co-trimoxazole and chloramphenicol) and emergence of reduced susceptibility towards ciprofloxacin among salmonellae have been reported from different parts of India.[4,5] Furthermore, *S.* Typhi resistant to third-generation cephalosporins, though low at present (1%) is also emerging in India.[2]

Considering the changing trends in the susceptibility patterns for *S.* Typhi along with the high endemicity of typhoid fever in India, continual monitoring of drug resistance is imperative. The present study was undertaken to find out the epidemiology and susceptibility profile of

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S. Typhi isolates in an 1800 bedded tertiary care super specialty hospital in North India, which also caters to a large out-patient population for providing suitable guidelines for the treatment of this potentially fatal disease.

Materials and Methods

Salmonella isolates obtained from blood cultures received for routine bacterial culture sensitivity at the bacteriology laboratory between January 2001 and December 2012 were included in the study. A retrospective analysis of laboratory records was carried out over these 12-years. Only one isolate per patient was included. Conventional blood culture method was used until 2009.

From January 2010 onwards all the blood culture samples were processed by the automated blood culture system-BACTEC 9240 (Becton Dickinson). Identification of salmonellae isolates was performed by conventional methods and was confirmed by using *Salmonella* spp. polyvalent O, O9 and H: d antisera (Murex Biotech, Dartford, UK).

Antibiotic susceptibility was performed using the Kirby-Bauer disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines for the corresponding years[6] using commercially available disks (Hi-media Laboratories, Maharashtra, India) of ampicillin (10 μg), chloramphenicol (30 μg), co-trimoxazole (1.25/23.75 μg), ciprofloxacin (5 μg), cefotaxime (30 μg) and nalidixic acid (NA) (30 μg). *Escherichia coli* ATCC 25922 was used as the quality control strain. Isolates with intermediate levels of resistance in disk diffusion were included in the percentage of resistant organisms for final analysis. NA susceptibility testing was done from 2005 onwards.

The isolates obtained after October 2011 had been preserved at −20°C in 10% glycerol brain heart infusion. Of these, 116 randomly selected isolates were subjected to minimum inhibitory concentrations (MIC) testing for chloramphenicol, ciprofloxacin, amoxicillin and NA using agar dilution method (Hi-media, Mumbai) and for ceftriaxone and azithromycin using E-strips (Biomerieux). As CLSI does not mention MIC breakpoint for azithromycin against *Salmonella*, the British Society for Antimicrobial Chemotherapy guidelines of MIC breakpoint of ≤16 μg/ml were considered as sensitive and >16 μg/ml as resistant.[7]

Results

During the 12 year study period, a total of 1038 cases of enteric fever were culture-confirmed. A total of 1016 (98%) Typhoidal and 22 (2%) non-Typhoidal salmonellae were obtained. Among Typhoidal salmonellae, the predominant serotype obtained was *S.* Typhi (852, 83.8%) followed by *S.* Paratyphi A (164, 16.2%). The ward to outpatient department ratio was 2:1. Majority of patients were males (67%) with a male-to-female ratio of 2:1. Most of the patients were in pediatric age group (<12 years) (452 [53%]) with: 5-12 years (247 [29%]), 1-5 years (170 [20%]), 1 month-1 year (25 [2.9%]) and neonates (9 [1.1%]). Although 358 (42%) cases were in the age group of 12-45 years; the least cases occurred amongst those >45 years (42[4.9%]). Typhoid fever cases occurred in all months throughout the year, however they peaked during the months of July-September (rainy seasons) followed by April-June each year.

The pattern of antimicrobial resistance of the 852 *S.* Typhi isolates is shown in Figure 1. On dividing the study period into two parts of 6 years each (i.e. 2001-06 and 2007-12), a statistically significant decrease ($P < 0.001$) was observed over the years in resistance to chloramphenicol, ampicillin and co-trimoxazole with *S.* Typhi having at present a susceptibility of >95% towards each of these first-line antibiotics. The percentage MDR isolates per year decreased over time with a present rate of around 1%. Resistance to NA was found to be highest amongst all the antibiotics; it has been rising since 2005 and is presently 100%. Ciprofloxacin resistance was relatively stable over the time period studied with a drastic increase from 5.8% in 2008 to 10% in 2009, since then it has increased in 2011-12 to 18.2% (5.7% resistant and 12.5% intermediate susceptible) using the CLSI 2011 guidelines.[6] On applying the revised zone sizes as per CLSI 2012 guidelines[8] on the same 2011-2012 isolates, the resistance increased to 97.7% (18.2% resistant and 79.5% intermediate susceptible). Sensitivity to third generation cephalosporins remained high during the study period with no resistance noted. The MIC of 116 isolates tested in the study is shown in Table 1.

Discussion

In our study, several interesting trends were observed. Over the study period, Typhi outnumbered Paratyphi A with almost 5 times higher rate of isolation in our region.

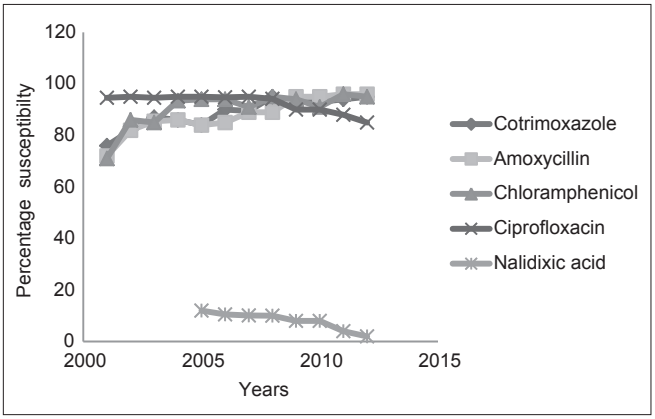

Figure 1: Percentage susceptibility of 852 *Salmonella enterica* var Typhi blood isolates over 12 years using Clinical and Laboratory Standards Institute gudelines of corresponding years

Table 1: MIC and susceptibility pattern of 116 isolates

	MIC_{50} (MIC_{90})					
	Chloro	Cipro	Ceftri	Amoxi	NA	Azi
Pediatric (*n*=44)	4 (8)	0.5 (4)	0.094 (0.125)	2 (4)	1024 (>1024)	3 (6)
Adult (*n*=72)	4 (8)	0.5 (8)	0.094 (0.125)	2 (4)	1024 (>1024)	3 (8)
Outdoor (*n*=75)	4 (8)	0.5 (8)	0.094 (0.125)	2 (4)	1024 (>1024)	4 (8)
Indoor (*n*=41)	4 (8)	0.5 (0.5)	0.094 (0.125)	2 (4)	1024 (>1024)	3 (6)
Salmonella Typhi (*n*=84)	4 (8)	0.5 (8)	0.094 (0.125)	2 (4)	1024 (>1024)	3 (6)
MIC range (*n*=84)	1->32	≤03125-16	0.023-0.5	0.25->8	128->1024	1-12
Resistance rate %	0.9	98.3	0	0.9	100	0

MIC: Minimum inhibitory concentration, Chloro: Chloramphenicol, Cipro: Ciprofloxacin, Ceftri: Ceftriaxone, Amoxi: Amoxicillin, Azi: Azithromycin

Similar to many previous studies we have also observed an increased isolation particularly during the rainy season (July-September) probably due to the higher chances of water contamination[9] with children being most susceptible, probably because adults develop immunity from recurrent infection and sub-clinical cases.[1,4] A significant decrease ($P < 0.001$) over the years in resistance to chloramphenicol, ampicillin and co-trimoxazole was noticed with all drugs having susceptibility >95% at present using the routine disk susceptibility. Similar fall in resistance to first line drugs due to decreased use has been documented in literature.[4] Of the 116 isolates tested for MIC to chloramphenicol, ampicillin and co-trimoxazole, the susceptibility was found to be >98% for each. The incidence of MDR *Salmonella* Typhi (MDRST) has been reported to vary from 25% to 55% from different parts of India with various studies revealing a gradual decrease to less than 5% MDRST.[9] MDRST was first encountered in 1990 in our laboratory. In the fourth quarter of the same year, all the *S.* Typhi isolates were MDR.[10] The resistance dropped to 26% in 2004 and thereafter has shown a downward trend with almost 1% MDR isolates at present. The low frequency of MDRST isolated has important therapeutic implication since these drugs could once again be used for the treatment of typhoid fever. This may be partway due to lack of antibiotic pressure as these drugs are not being used routinely. In addition as suggested by Dutta *et al*., this re-emergence of susceptibility to these drugs may be a result of the emergence of *de novo* susceptible strains or the loss of high molecular weight self-transmissible plasmids.[5]

We found a high NA resistance (~86% in 2005), which has been increasing during the last 6 years (100% at present). All the 116 isolates tested for MIC by agar dilution were 100% resistant to NA (nalidixic acid resistant *S.* Typhi) (MIC range 128 to >1024 μg/mL). In contrast, though on a rise when compared to previous years, we found 18.2% (5.7% resistant and 12.5% intermediate susceptible) isolates being resistant to ciprofloxacin by disk diffusion using CLSI 2011 guidelines.[6] We had previously reported that NA susceptibility is a good marker for fluoroquinolone susceptibility, but NA resistance had a poor predictive value for ciprofloxacin resistance.[10] Using the routine disc diffusion tests, isolates which test sensitive to ciprofloxacin and resistant to NA may have decreased ciprofloxacin susceptibility (DCS), which may not be detected unless MIC testing is performed leading to suboptimal response or treatment failures. Further, on performing MIC testing and applying the revised breakpoints for ciprofloxacin testing as recommended by CLSI 2012,[8] we found 13.8% resistance and 84.5% of isolates having characteristic intermediate or DCS i.e. ciprofloxacin MIC 0.12-1.0 μg/mL. This corroborates well with the disk diffusion results using the CLSI 2012 guidelines (as stated above in results) and may aid in better depicting the ciprofloxacin susceptibility at places where MIC determination is not accessible.

Earlier studies have shown that a single mutation in chromosomal gyr A gene (encoding for subunit of DNA gyrase) located in the quinolone resistance determining region (QRDR) are found in most of such isolates, whereas multiple mutations in QRDR confer high level of resistance to fluoroquinolone (MIC ciprofloxacin ≥ 4.0 μg/mL) and to NA. Plasmid mediated quinolone resistance and chromosomal gyr B mutation results in isolates with DCS and modest elevation of NA MIC.[11,12] Although we did not determine the molecular mechanism of quinolone resistance in the present study but, from MIC values for ciprofloxacin and NA, it appears that these isolates may be harbouring the chromosomal mutation in gyr A gene (DCS with NA resistance) in 84.5% of isolates and multiple mutations (chromosomal gyr A and gyr B gene with ciprofloxacin MIC ≥ 4 μg/mL and NA resistance) in 13.8% of isolates. Decreasing susceptibility of *S.* Typhi to ciprofloxacin has been well documented in several studies[4] and is indicative of the effects of indiscriminate use of this group of antibiotics.

S. Typhi resistant to third generation cephalosporins have been long reported from our neighbouring countries[13] and though very low at present (1%), such strains are also emerging in India.[2] However, we found no resistance

during the study period based on disk diffusion testing and *E*-test. This emphasises the importance of this group of antibiotic as a reserve drug for treating MDR and ciprofloxacin resistant cases.

Trials have demonstrated that azithromycin compares favourably with ceftriaxone in terms of clinical and microbiological cure rates with ease of administration and lower relapse rate for the treatment of uncomplicated typhoid fever.[14,15] Studies on human volunteers have shown that neutrophil concentrations of azithromycin are 100 times more than the serum concentration with a long half-life (2-3 days).[16] The efficacy of azithromycin during treatment is related to this tissue concentration rather than the serum concentration. The MIC_{50} (MIC_{90}) of azithromycin as observed by various workers are 8 (16) µg/mL by Butler *et al*. and 16 (24) µg/mL by Capoor *et al*.[14,15] We found a low MIC_{50} (MIC_{90}) of 3 (8) µg/mL in the present study, which may be attributed to the relatively lower antibiotic consumption in our region. This however warrants further trials to know the exact role of azithromycin, which is an orally effective drug in the endemic areas in view of non-uniform susceptibility pattern noticed in various studies.

Conclusion

Over the last decade there has been the re-emergence of susceptibility to ampicillin, chloramphenicol and co-trimoxazole in *S.* Typhi and a notable decline in MDR strains. We have a very high resistance to NA and decreasing susceptibility to ciprofloxacin, which requires MIC testing for detection along with judicious use to avoid selection of existing resistant subpopulations. Third generation cephalosporins and azithromycin seem to be effective therapeutic options in our region though judicious use is mandatory to prevent emergence of resistant strains.

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