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ANTIBIOTIC SUSCEPTIBILITY PATTERN OF *SALMONELLA ENTERICA* SEROVAR TYPHI AND PARATYPHI A FROM NORTH INDIA: THE CHANGING SCENARIO

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ABSTRACT

Enteric fever prevails as a major public health problem despite the use of antimicrobials due to the emergence of drug resistance. The present study was undertaken to find out the antimicrobial susceptibility pattern of *Salmonella* isolates from our region. Antimicrobial susceptibility testing for six drugs chloramphenicol (30µg), amoxicillin (10µg), cotrimoxazole (1.25/23.75µg), nalidixic acid (30µg), ciprofloxacin (5µg), and ceftriaxone (30µg) was done by the Kirby Bauer disc diffusion method. E-test was performed to calculate the MIC of ciprofloxacin. A total of the 80 *Salmonella* species was isolated which comprised of 51 (63.8%) *Salmonella enterica* serovar Typhi and 29 (36.2%) *Salmonella enterica* serovar Paratyphi A. All the isolates were nearly susceptible to the first line drugs, and none was multi drug resistant.Nalidixic acid resistance was high being 96% in *Salmonella typhi* and 100% in *Salmonella paratyphi* A. In *Salmonella typhi* 17.6% isolates were resistant to ciprofloxacin by the disc diffusion technique whereas 47% were resistant by the E-test. Similarly in *Salmonella paratyphi* A 48.3% were detected resistant by the E- test. Reduced susceptibility to ciprofloxacin indicating higher MIC to ciprofloxacin was seen in 49 % isolates of *Salmonella typhi* and 51.7% in the other serotype. These isolates are undetected by the routine disc diffusion techniques and result in treatment failure cases. It is pertinent to mention that MIC of ciprofloxacin by the E-test is more beneficial to assess the true pattern of resistance.

KEY WORDS: Multidrug resistance, *Salmonella enterica* serovar Typhi, *Salmonella enterica* serovar Paratyphi A, nalidixic acid, ciprofloxacin, ceftriaxone

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INTRODUCTION

Enteric fever is a global public health problem and highly endemic in India. Worldwide 22 million cases of enteric fever occur annually and result in nearly 600,000 deaths. This accounts for the highest concentration in Asia especially the Indian subcontinent.[1] Untreated cases have a high mortality rate of 30% while appropriate treatment drastically reduces it to 0.5%.[2] Infants, children and adolescents in South Eastern and South Central Asia experience the greatest burden of illness. In 1999 Sinha *et al* estimated the incidence of enteric fever as 9.8 per 1000 person years in urban slums of North India.[3] While nearly a decade later Ochiai *et al* found the incidence to be 214.2 per 100,000 years in slums from Eastern India.[4] Enteric fever is an important cause of pyrexia of unknown origin and includes both typhoid and paratyphoid fever. *Salmonella enterica* serovar Typhi and *Salmonella enterica* serovar Paratyphi A are predominant etiological agents of enteric fever in India. Other serovars of *Salmonella, Paratyphi* B and C are rare in our country.[5] *Salmonella enterica* serovar Typhi is known to be amongst the most resistant of all human pathogens. Chloramphenicol was considered as the gold standard for treatment since its introduction in 1948. Thereafter epidemic of chloramphenicol resistant typhoid fever appeared in the year 1972 in Calicut, Kerala. Outbreaks of chloramphenicol resistant strains occurred in Vietnam, Indonesia, Korea, Chile and Bangladesh.[6] Initially these strains were susceptible to amoxicillin and cotrimoxazole. Subsequently *Salmonella* species rapidly gained resistance to all the first line antibiotics chloramphenicol, amoxicillin and cotrimoxazole in the late 1980s and early 1990s and became multidrug resistant (MDR). MDR is defined as simultaneous resistance to all the first line drugs and is endemic in South America, Africa, India and South-east Asia. A highly transmissible plasmid belonging to incompatibility group HI1 was responsible for MDR.[7] Incidence of MDR was reported to be as high as 92% with a declining trend down the years to 1.94%.[8,9] The resurgence of MDR strains in north India in 2002 is a matter of great concern. [10] Recent reports suggest re-emergence of chloramphenicol sensitive strains in previously resistant areas.[11,12,13] To overcome the rising burden of MDR, ciprofloxacin a fluroquinolone was the antibiotic of choice in early 1990's. Nevertheless the indiscriminate use of this drug in human and veterinary therapeutics leads to decreased susceptibility and resistance to ciprofloxacin. Isolates of *Salmonella* with reduced susceptibility to fluoroquinolones have appeared in the Indian subcontinent and other regions.[6,11] Strains exhibiting in vitro resistance to nalidixic acid also exhibit reduced susceptibility to ciprofloxacin, hence nalidixic acid acts as a surrogate marker to detect ciprofloxacin resistance.[14] The problem of nalidixic acid resistant *Salmonella* is escalating in India and is a cause of worry. Ceftriaxone, a third generation cephalosporin has emerged as a suitable alternative in ciprofloxacin treatment failure cases.[11] The emergence of resistance to this group of drugs due to extended spectrum β lactamases in typhoidal *Salmonellae* constitutes a new therapeutic challenge to the clinicians. The present study was aimed to detect the current trends in antibiotic susceptibility pattern of *Salmonella* isolates in a tertiary care hospital in North India. The regional variations in the sensitivity pattern of *Salmonella enterica* serovar Typhi and *Salmonella enterica* serovar Paratyphi A highlight the significance of continuous monitoring of susceptibility pattern to provide appropriate treatment guidelines. This inspired us to study the antimicrobial susceptibility of *Salmonella* isolates in our region and evaluate the changing pattern over the years.

MATERIALS AND METHODS

This was a prospective study conducted in the department of Microbiology over a span of three years from 2010 to 2012. Blood culture samples from hospitalized and out patients with suspected enteric fever, septicaemia and other infections were included in the study. Samples collected with strict aseptic measures in sterile brain, heart infusion broth was incubated at 37° C followed by subculture on Blood and MacConkey agar after 24, 48 hours and on the 7th day. The cultures were

declared negative if no growth was seen after seven days of incubation. The growth of non lactose fermenting colonies was processed for identification by standard techniques.[15] Confirmation of *Salmonella* serotypes was done with slide agglutination using antisera for *Salmonella* polyvalent O, O2, O9 (Central Research Institute, Kasauli). All the *Salmonella* serotypes were subjected to antimicrobial susceptibility testing by the Kirby Bauer disc diffusion method and interpreted as per the current CLSI guidelines.[16]Antimicrobial susceptibility pattern was determined using the following commercial antimicrobial discs (Hi–Media, Mumbai); chloramphenicol (30µg), amoxicillin (10µg), cotrimoxazole (1.25/23.75µg), nalidixic acid (30µg), ciprofloxacin (5µg), ceftriaxone (30µg).The isolates were tested for ESBL production, if any. Minimum inhibitory concentration (MIC) for ciprofloxacin was determined using E-Test strips (Hi–Media, Mumbai). An isolate was considered resistant to ciprofloxacin if MIC is (≥1µg/ml), intermediately susceptible MIC (0.125-0.5 µg/ml) and susceptible if MIC (≤ 0.06 µg/ml) according to CLSI (2012) guidelines .*Escherichia coli* ATCC 25922 strain was used for quality control.

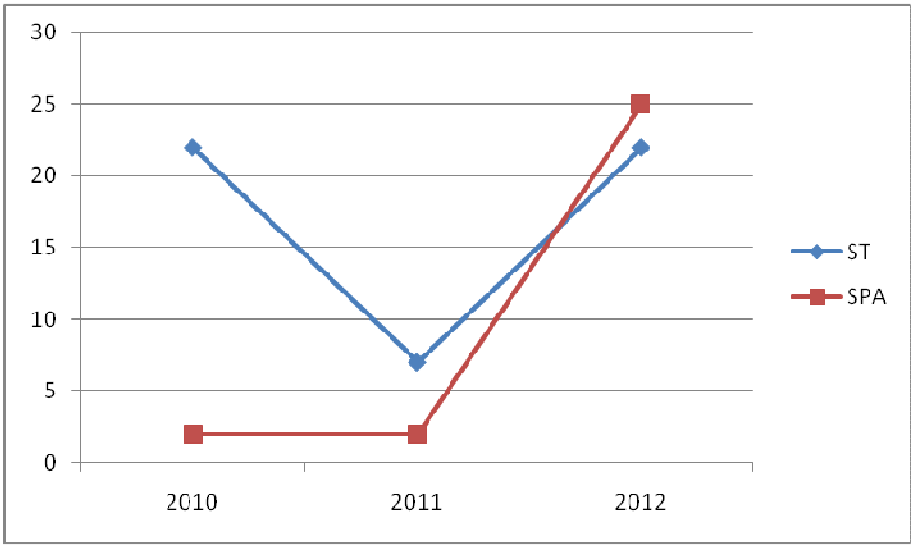
RESULTS

A total of 15,265 blood culture samples was received in the department of Microbiology of these 5321, 4436 and 5508 samples were processed in the years 2010, 2011, 2012 respectively. Of the total specimens 80(0.54%) yielded growth of *Salmonella* species. The mean age was 24.22 years, being more predominant in younger adults in 58.75% cases followed by children in the pediatric age group in 25% cases. The isolation rate was higher in males 56 (70%) as compared to females 24 (30%). Over the last three years *Salmonella typhi* was the predominant serotype 51 (63.8%) followed by29 (36.2%) *Salmonella Paratyphi* A. However an escalation of enteric fever due to *Salmonella Paratyphi* A was seen in 25 out of 47 (53.19%) cases in year 2012. Fig 1 shows year wise distribution of *Salmonella* species. The antimicrobial susceptibility pattern of *Salmonella enterica* serovar Typhi for six antimicrobial agents is shown in table 1. Amongst the first line drugs, all isolates were 100% susceptible to chloramphenicol and amoxicillin while only 3 (5.88%) isolates were resistant to co-trimoxazole. No multidrug resistant serotype Typhi was seen in the present study. Nalidixic acid resistance was higher in 49 (96.07%) isolates. Low level ceftriaxone resistance was seen in 2 (3.92%) isolates; however there was no ESBL producer. Susceptibility testing for ciprofloxacin by the Kirby Bauer disc diffusion method revealed 14 (27.45%) isolates as susceptible, 28 (54.95 %) had intermediate susceptibility and 9 (17.6%) were resistant. On performance of E- test for determining MIC of ciprofloxacin a larger proportion of *Salmonella enterica* serovar Typhi 24 (47.5%) was detected resistant. Table 3 shows the year wise antibiogram of *Salmonella enterica* serovar Typhi. During the three year study period, 100% susceptibility to chloramphenicol was seen throughout, however, susceptibility to cotrimoxazole declined from 100% to 86.36% over the three years. Susceptibility to ciprofloxacin decreased notably from 31.8% in 2010 to 22.72% in 2012.

The antimicrobial susceptibility of *Salmonella enterica* serovar Paratyphi A for the enlisted antimicrobial agents are shown in table 2. Overall, 100% susceptibility was seen for chloramphenicol, while only 1 (3.44%) isolate each was resistant to amoxicillin and cotrimoxazole respectively. Likewise serotype Typhi, none of the isolates of serovar Paratyphi A was MDR. The concomitant reemergence of chloramphenicol susceptibility is attributed to its restricted use, which resulted in with drawl of selection pressure. Nalidixic acid resistance was 100% in *Salmonella paratyphi* A. Susceptibility testing for ciprofloxacin by the Kirby Bauer disc diffusion method depicted 3 (10.34%) isolates as susceptible and 26 (89.65%) had intermediate susceptibility. No isolate was detected ciprofloxacin resistant by the disc diffusion method. On the contrary, E- test detected more isolates of *Salmonella enterica* serovar Paratyphi A 14 (48.27%) as resistant (MIC≥1µg/ml), 15 (51.7%) were moderately susceptible (MIC 0.125-0.5 µg/ml) and none were susceptible (MIC $0.06 \leq$µg/ml) to ciprofloxacin. Nearly all 28 (96.55%) isolates

of *Salmonella enterica* serovar Paratyphi A were susceptible to ceftriaxone and no ESBL production was seen. Table 4 shows the year wise susceptibility pattern of *Salmonella enterica* serovar Paratyphi A. During these three years all isolates were totally susceptible to the first line drugs except in the year 2012 susceptibility for amoxicillin and cotrimoxazole was 92% and 96%respectively. A remarkable decrease in ciprofloxacin susceptibility from 50% in 2010 to 4% in 2012 was seen in serovar Paratyphi A.

Figure 1
Year wise distribution of Salmonella isolates



ST-Salmonella typhi SPA-Salmonella paratyphi A

Table 1
Showing the antimicrobial susceptibility pattern of Salmonella enterica serovar Typhi (n=51)

Kirby Bauer Disc Diffusion method	Susceptible	Intermediate	Resistant
chloramphenicol	51 (100%)	0 (0%)	0 (0%)
amoxicillin	51 (100%)	0 (0%)	0 (0%)
cotrimoxazole	48 (94.11%)	0 (0%)	3 (5.88%)
nalidixic acid	2 (3.92%)	0 (0%)	49 (96.07%)
ceftriaxone	48 (94.11%)	1 (1.96%)	2 (3.92%)
ciprofloxacin	14 (27.45%)	28 (54.9%)	9 (17.6%)
Ciprofloxacin (MIC) µg/ml E-test(CLSI 2012)	2 (3.92%)	25 (49.01%)	24 (47.05%)

Table 2
Showing the antimicrobial susceptibility pattern of Salmonella Enteric serovar Parayphi A(n=29)

Kirby Bauer Disc Diffusion method	Susceptible n (%)	Intermediate n (%)	Resistant n (%)
chloramphenicol	29 (100%)	0 (0%)	0 (0%)
amoxicillin	27 (93.10%)	1 (3.44%)	1 (3.44%)
cotrimoxazole	28 (96.55%)	0 (0%)	1 (3.44%)
nalidixic acid	0 (0%)	0 (0%)	29 (100%)
ceftriaxone	28 (96.55%)	1 (3.44%)	0 (0%)
ciprofloxacin	3 (10.34%)	26 (89.65%)	0 (0%)
Ciprofloxacin (MIC) µg/ml E-test(CLSI 2012)	0 (0%)	15 (51.72%)	14 (48.72%)

Table 3
Antibiogram of S. enterica serovar Typhi (% susceptible)

Year	N=no of isolates	Chl	Amox	Cotri	NA	Cip	Ctri
2010	22	100	100	100	4.5	31.8	100
2011	7	100	100	100	0	28.57	100
2012	22	100	100	86.36	4.5	22.72	86.36

Chl- Chloramphenicol, Amox- Amoxicillin, Cotri- Cotrimoxazole, NA- Nalidixic acid, Cip- Ciprofloxacin, Ctri- Ceftriaxone

Table 4
Antibiogram of S. enterica serovar Paratyphi A. (% susceptible)

Year	N=no of isolates	Chl	Amox	Cotri	NA	Cip	Ctri
2010	2	100	100	100	0	50	100
2011	2	100	100	100	0	50	100
2012	25	100	92	96	0	4	96

Chl- Chloramphenicol, Amox- Amoxicillin, Cotri- Cotrimoxazole, NA- Nalidixic acid, Cip- Ciprofloxacin, Ctri- Ceftriaxone

DISCUSSION

Enteric fever is endemic in developing countries. The predisposing factors are poor sanitation, lack of safe drinking water supply and proper sewerage disposal. It is prevalent in the tropics affecting the young and pediatric age group, being more common in males as they are more engaged in outdoor activities and tend to consume street food. The male to female ratio in our study was 2 : 1 which coincides well with other studies.[17] The etiological agents are *Salmonella enterica* serovar Typhi followed by *Salmonella enterica* serovar Paratyphi A as observed in the present study. Infections due to *Salmonella enterica* serovar Paratyphi B are rare being 0.62% to 1.94 % cases in India.[18´9] However *Salmonella enterica* serovar Paratyphi B is prevalent in Western America, Britian and *Salmonella enterica* serovar Paratyphi C in Eastern Europe and Guyana.[19] Several studies highlight *Salmonella enterica* serovar Typhi as the commonest causative agent over the years [18´20´21] which concurs with our findings where *Salmonella enterica* serovar Typhi was predominant in 63.8% cases followed by 36.3% *Salmonella enterica* serovar Paratyphi A. A previous six years study (2000 to 2006) from our region reflects an upsurge of *Salmonella enterica* serovar Paratyphi A during the year 2003 - 2004 with the resurgence of *Salmonella enterica* serovar Typhi in the subsequent period.[22] In the present study increased occurrence of the *Salmonella enterica* serovar Paratyphi A was seen in year 2012 which coincides with other studies. [23] Increasing trend of isolation of serovar Paratyphi A from 13.46% in year 2004 to 54.16% in 2007 was seen in another report from North India. [24] This may be explained due to the widespread use of monovalent vaccine effective against serovar Typhi which has gradually replaced the bivalent TA vaccine. In our study *Salmonella* species were highly sensitive to the first line drugs i.e. amoxicillin, chloramphenicol and cotrimoxazole being 97.5%, 100% and 95% respectively over the three year study period as reported earlier.[18´24] Since the last decade reports of reemergence of chloramphenicol susceptibility are on the rise and it concurs with our findings where 100% susceptibility was seen.

Strikingly no MDR *Salmonella* species were detected in the present study unlike a previous study from this region where MDR was 10.69%, 13.13% in *Salmonella typhi* and *Paratyphi A* respectively.[22] Resistance to cotrimoxazole was seen in both serovars being more in serovar Typhi to the tune of 5.88%. Resistance to amoxicillin was observed in serovar Paratyphi A being 3.7%. A report from the Eastern region depicts larger number of isolates with combined resistance to cotrimoxazole and amoxicillin and prevalence of MDR in serovar Typhi and Paratyphi A to be 11.9% and 15.6% respectively.[25] A study from a tertiary care hospital in Delhi from year1999 to 2004 revealed a gradual decrease in MDR *Salmonella typhi*. However the proportion of MDR *Salmonella Paratyphi* A increased from 3.5% to 11.6% in the respective years.[26] The scenario was different nearly a decade back as the high prevalence of MDR *Salmonella* up to 83.3% was seen in India.[27] The introduction of fluroquinolones as the drug of choice was a landmark to tackle the problem of multidrug resistance in *Salmonella* species. A declining trend for MDR was seen throughout the country except for rare reports of resurgence.[10] Similar trend in our study may be attributed to the non usage of the first line drugs by the clinicians for the treatment of enteric fever. The indiscriminate use of ciprofloxacin in human and animal therapeutics was responsible for the evolution of fluroquinolone resistant *Salmonella* species. Nalidixic acid resistance has evolved as surrogate marker for to detect ciprofloxacin resistance as clinical failures have been documented in cases where ciprofloxacin has been used based on the susceptibility for nalidixic acid resistant strains. Kirby Bauer disc diffusion assay is not a reliable method; E-test should be the preferred method to determine ciprofloxacin MIC. In our study overall 97.5% *Salmonella* isolates were nalidixic acid resistant. High degree of nalidixic acid resistance was seen in *Salmonella* being 100% in serovar Paratyphi A. This finding is contradictory to another report where nalidixic acid resistance was more in serovar Typhi.[28] Similar pattern of high level nalidixic acid resistance in genus *Salmonella* has been observed in Central India unlike previous reports from our region and others where frequency of nalidixic acid resistance was less ranging from 13.6% to 78% respectively.[29´10] Ciprofloxacin emerged as the drug of choice in 1990's in the era of MDR in *Salmonella* species. Resistance to ciprofloxacin has increased manifold over the years currently being 47.5% presently. This was strikingly in contrast to previous reports from our region where ciprofloxacin resistance was 3.67% to 9.41%.[29´22] Pattern of ciprofloxacin resistance is variable in our subcontinent ranging from 1% to 21.4%.[28´20] Interestingly in a report from South India ciprofloxacin resistant serovar Paratyphi A was nil in contrast to ours where high resistance was seen.[28] A recent study from Delhi revealed 32% isolates of serovar Paratyphi A to be ciprofloxacin resistant.[26]

Disc diffusion is not a reliable method to detect ciprofloxacin resistance. Towards the end of the last decade treatment failures have been reported due to infection with NAR strains which were falsely detected susceptible to ciprofloxacin by the disc diffusion method. These strains with high MIC of ciprofloxacin are detected by E-test. Kirby Bauer disc diffusion method detected lesser proportion of isolates 11.6% to be ciprofloxacin resistant. In comparison more isolates 47.5% were ciprofloxacin resistant on determination of MIC with the E-test. This suggests that strains with higher MIC fail to be detected by the disc diffusion test. It is advocated that MIC of ciprofloxacin should be determined for all nalidixic acid resistant S*almonella* species to avoid false reporting.[18] As per the MIC determined by the E–test 96.07% serovar Typhi and all isolates 100% of serovar Paratyphi A either had reduced susceptibility or resistance according to the current CLSI guidelines. Similarly in another study 95.5% *Salmonella typhi*, 97.6% Salmonella *Paratyphi* A had reduced susceptibility to ciprofloxacin MIC (0.125-to 0.5) ug/ml.[28] Third generation cephalosporin, ceftriaxone has emerged as a suitable alternative in cases with treatment failure due to ciprofloxacin. In the present study 3.96% isolates of *Salmonella typhi* were resistant to ceftriaxone and 3.44% isolates of *Salmonella Paratyphi* A exhibited decreased

susceptibility to this drug. Our findings corroborate with a multicenter study, where less than 4% ceftriaxone resistance was documented.[30] In the Eastern region ceftriaxone resistance in serovar Typhi and Paratyphi A was 3% and 4.69% respectively.[25] Majority of the reports denote 100% sensitivity to ceftriaxone.[18,9] However there are occasional reports of high resistance to ceftriaxone where CTX-M-15 and SHV-12 ESBL's were detected.[31] Fortunately in our study there was no ESBL producer, though sporadic reports of high level resistance to ceftriaxone have emerged in Bangladesh, Pakistan, Phillipines.[32,33,34] These studies warn us to use ceftriaxone as a reserve drug and not for empirical treatment.

CONCLUSION

The present study highlights the declining trend of MDR in our region. The first line drugs may still have a role to play in the treatment of enteric fever as suggested by the reemergence of chloramphenicol susceptibility, except for the side effects like bone marrow toxicity, high relapse rate. However the higher level resistance to ciprofloxacin and emergence of nalidixic acid resistant *Salmonella* species has posed a challenge for the medical fraternity. Detection of ciprofloxacin resistance by the E-test based on the MIC values has proved to be more effective than the disc diffusion method. *Salmonella* isolates showed higher susceptibility to a third generation cephalosporin, ceftriaxone which is a suitable alternative in patients non responsive to ciprofloxacin therapy. However, reports of ESBL producers in *Salmonella enterica* serovar Typhi and *Salmonella enterica* serovar Paratyphi A are on the rise in the Indian subcontinent. This emphasizes the role of continuous antimicrobial surveillance coupled with molecular analysis of fluoroquinolone and ceftriaxone resistant typhoidal *Salmonellae* to reconfirm novel and established the molecular basis of resistance. It is the need of the hour that the clinicians should be well aware of the judicious use of antibiotics, to tackle the problem of drug resistance in enteric fever.

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