Antibiotic resistance of *Salmonella enterica* serovar Paratyphi A in India: Emerging and reemerging problem

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**ABSTRACT**

**Background:** Antibiotic resistance pattern and R-plasmid of *Salmonella enterica* serovar Paratyphi A isolates from Kolkata, India are not well documented.

**Aims:** To determine the trend of antibiotic resistance of *S. paratyphi* A isolates.

**Settings and Design:** A retrospective study was carried out using blood culture isolates of *S. paratyphi* A (1991 to 2005) obtained from patients of enteric fever from Asansol and Kolkata and its suburbs (India).

**Materials and Methods:** Antibiotic susceptibility pattern, using seven antibiotics, for the isolates was determined following agar dilution and disk diffusion methods. Transferability of multidrug resistance to ampicillin (Am), chloramphenicol (Chl), cotrimoxazole (Cot) and tetracycline (Tet) among the isolates was determined by in vitro conjugation. The multi-drug resistant (MDR) and antibiotic susceptible *S. paratyphi* A strains and the trans-conjugants were screened for the presence of plasmid.

**Statistical Analysis Used:** The t test was used to compare the difference between mean minimum inhibitory concentration values of ciprofloxacin (Cp) for nalidixic acid (Nx)-resistant and Nalidixic acid (Nx)-susceptible isolates.

**Results:** Among 13 outbreak causing isolates in 1991, 9 (69.23%) showed AmChlCotTet-resistance, while 4 (30.77%) Cot-resistance only. During 1992-1994, all 13 isolates were susceptible to Am, Chl, Cot and Tet. During 1995-2005, isolates demonstrated different resistance patterns and emergence of nalidixic acid (Nx)-resistance. A transferable plasmid conferring AmChlCotTet-resistance was detected among MDR isolates. All the isolates were susceptible to ceftriaxone (Ctx) and ciprofloxacin (Cp). Association between Nalidixic acid (Nx)-resistance and reduced susceptibility to ciprofloxacin (Cp) among 59 *S. paratyphi* A isolates was noticed ($P<0.001$).

**Conclusion:** Vigilance for R-plasmid and surveillance of antibiotic susceptibility among *S. paratyphi* A isolates in and around Kolkata, India, are mandatory in order to combat antibiotic resistance of the isolates in this part of the world.

**KEY WORDS:** *Salmonella enterica* serovar *S. paratyphi* A, multidrug resistance, minimum inhibitory concentration, R-plasmid.

The most common *Salmonella* species that cause enteric fever include *Salmonella enterica* serovar Typhi and *Salmonella enterica* serovar *S. paratyphi* A.[1-3] Emergence of multidrug resistant (MDR) although there are several Indian reports citing the drug resistance developed by *S. Typhi* isolates, such data regarding *S. paratyphi* A is scanty.[4-6] Therefore, the present study was undertaken to assess the antibiotic resistance of *S. paratyphi* A isolates obtained during 1991 to 2005 were used in the present study. The patients were from different places of the West Bengal state (India): Asansol and Kolkata and its suburbs. The control strain used in the study was *Escherichia coli* ATCC 25922, while *E. coli* V517 strain was used as the molecular marker for plasmid.

The isolates, which were stored and maintained in cystine tryptone agar stabs with or without antibiotics, were tested for susceptibility to ampicillin (Am), chloramphenicol (Chl), co-trimoxazole (Cot), tetracycline (Tet), nalidixic acid (Nx), ciprofloxacin (Cp) and ceftriaxone (Ctx) by disc diffusion method using the guidelines provided by the National Committee for Clinical Laboratory Standards (NCCLS).[7] The antibiotic contents ($\mu$g/disc) of the discs.
(Hi-Media, Mumbai, India) were Am (25), Chl (30), Cot (25), Tet (10), Nx (30), Cp (5) andCtx(30) and the media used were Mueller-Hinton agar and Mueller-Hinton broth (Hi-Media, Mumbai, India).

For all the *S. paratyphi* A isolates, minimum inhibitory concentration values (MICs) of the antibiotics: Am, Chl, Nx and Ctx (Sigma Chemicals, St. Louis, USA) and Tet, Cp and Cot (sulphamethoxazole + trimethoprim) from Hi-Media Laboratory Limited, Mumbai, India, were determined by agar dilution method according to the NCCLS criteria,[8] using Mueller-Hinton agar.

Transferability of antibiotic resistance was determined by *in vitro* conjugation experiments performed according to the protocol of Jevanand et al.,[9] with slight modification,[10] using drug resistant *S. paratyphi* A isolates as donors and *Escherichia coli* C 600 (Nx, F) as recipient.

MDR *S. paratyphi* A, their transconjugants and the *S. paratyphi* A strains showing susceptibility to antibiotics (Am, Chl, Cot and Tet) were selected for plasmid DNA isolation by alkaline lysis method of Birnboim and Doly,[11] with some modifications. Briefly, from 10 ml of bacterial culture (in nutrient broth, Hi-Media, India) plasmid DNAs were isolated using 0.48 ml, 1 ml and 0.8 ml of solution I, II and III, respectively. After the addition of solution III, the lysate was kept in ice for 30 minutes and centrifuged for 15 minutes. Phenol-chloroform (1:1) was added with the clear supernatant, plasmid DNA was precipitated with 0.8 volume of chilled isopropyl alcohol and DNA pellet was dissolved in 100 µl of TE buffer. Agarose gel electrophoresis of the isolated plasmid DNAs was carried out in tris-borate buffer system,[12] using 0.8% agarose, for 4 h at 50v. The gel was stained with ethidium bromide and results were documented in gel-doc system. Electrophoretic separation of plasmid by molecular weight and subsequent size estimations were accomplished using reference strain of *E. coli* V517.

The association between Nx-resistance and decreased Cp susceptibility was determined using the t test wherein difference between mean MIC values of Cp and Nx for Nx-resistant *S. paratyphi* A isolates was compared. The 95% confidence interval of the mean MICs of Nx and Cp for Nx-resistant isolates was calculated.

**Results**

The antibiotic resistance patterns of *S. paratyphi* A isolates are shown in Figure 1. In 1991, two types of antibiotic resistance patterns were noticed: “AmChlCotTet” in 9 (69.23%) and “Cot” in 4 (30.77%) isolates. All the isolates of 1992-1994 and 9 (75%) and one (11.11%), respectively of the year 1995 and 1996 were susceptible to antibiotics studied. The rest of the isolates of 1995 (n=3, 25%) and 1996 (n=8, 88.89%) and all isolates of the year 1997 (n=10) were Nx-resistant. Between the years 1998 and 2001, including Nx-resistance, different patterns of resistance were found among the isolates of *S. paratyphi* A: AmChlCotTetNx in 1998 (n=3, 30%), in 2000 (n=2, 25%) and in 2001 (n=1, 16.67%); ChlCotTetNx in 2000 (n=2, 25%); and CotNx in 1998 (n=1, 10%), in 1999 (n=1, 33.33%), in 2000 (n=1, 12.5%) and in 2001 (n=1, 33.33%). Among 11 *S. paratyphi* A isolates, which were obtained during 2002-2005, two (18.2%) showed “NxCot” resistance pattern, while the remaining 9 (81.8%) were resistant to Nx, but susceptible to four antibiotics namely Am, Chl, Cot and Tet. The MDR isolates showed high level of MIC to Am (500-6000 µg/ml), Chl (500-3000 µg/ml), Cot (125-600 µg/ml) and Tet (75-600 µg/ml).

The MIC values of Cp for 95 *S. paratyphi* A isolates (1991-2005) are shown in Figure 2. All the *S. paratyphi* A isolates (n=26) obtained during 1991-1994, 9 (75%) isolates in 1995 and one (11.11%) in 1996 showed Cp MICs 0.005-0.05 µg/ml. Remaining 3 (25%) and 8 (88.89%) isolates obtained during 1995 and 1996, respectively and all 48 (100%) isolates during 1991-2005 had Cp MICs 0.5-1.25 µg/ml.

![Figure 1: Antibiotic resistance pattern of *S. paratyphi* A isolates (n=95); 1991-2005. Resistance patterns are indicated within the small box of the graphics. Am: ampicillin; Chl: chloramphenicol; Cot: cotrimoxazole; Tet: tetracycline; Nx: nalidixic acid.](image1)

![Figure 2: Scattergram showing minimum inhibitory concentration (MIC) values of ciprofloxacin (Cp) for *S. paratyphi* A isolates (1991-2005), n=95. The numbers within the graphic indicate the number of *S. paratyphi* A isolates.](image2)
The correlation between the MIC values of Cp and that of Nx is shown in Figure 3. Among 95 S. paratyphi A isolates, 36 (37.9%) isolates for which Cp MICs were ≤ 0.05 μg/ml were susceptible to Nx (MICs ≤ 16 μg/ml) and remaining 59 (62.1%) isolates for which Cp MICs were 0.1-1.25 μg/ml were Nx-resistant (MICs 32-256 μg/ml). Table 1 represents the association between Nx-resistance and reduced susceptibility to Cp among 59 S. paratyphi A isolates (P values 0.001). With 95% confidence, the mean Nx MIC ranged from 86.14 to 130.80 μg/ml and the mean Cp MIC from 0.58 to 0.8 μg/ml. All the isolates showed susceptibility to Ctx by both disc diffusion and agar dilution methods. The MICs of the agent was in between 0.005-0.25 μg/ml.

The MDR isolates having resistance patterns of AmChlCotTetNx and AmChlCotTet transferred the resistance property for Am, Chl, Cot and Tet to E. coli C600 recipients; Nx-resistance was not transferred. MDR S. paratyphi A strains and their corresponding transconjugants contained a plasmid of the same molecular size (approximately 55 Kb) conferring resistance to Am, Chl, Cot and Tet. The isolates, which were susceptible to these four agents, were plasmidless.

**Table 1: Mean MICs (μg/ml) of Nx and Cp for Nx-resistant and Nx-susceptible S. paratyphi A isolates**

<table>
<thead>
<tr>
<th>Agents</th>
<th>S. paratyphi A isolates</th>
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<tr>
<td></td>
<td>Nx-resistant</td>
<td>Nx-sensitive</td>
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<tr>
<td>Nalidixic acid (Nx)</td>
<td>108.474 ± 9.88* (32-256)</td>
<td>6.435 ± 1.026</td>
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<td>(0.125-16)</td>
<td>(0.005-0.05)</td>
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<tr>
<td>Ciprofloxacin (Cp)</td>
<td>0.694 ± 0.048* (0.1-1.25)</td>
<td>0.0161 ± 0.0023</td>
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<tr>
<td></td>
<td>(0.005-0.05)</td>
<td>(0.005-0.05)</td>
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* P< 0.001 compared to Nx-sensitive S. paratyphi A isolates. Values are mean ± S.E. Figures in the parentheses are the MIC ranges Nx: Nalidixic acid

Emergence of MDRS. paratyphi A isolates causing enteric fever has been reported from different parts of the world including India.[5,13,14] In the present study, both susceptible and resistant isolates were obtained and the resistant isolates showed flip-flop patterns of antibiotic resistance with high MICs of Am, Chl, Cot and Tet. All the isolates involved in the enteric fever outbreak during 1991 were drug resistant. Enteric fever outbreak caused by drug resistant S. paratyphi A has been reported earlier from India.[15] Emergence of such drug resistant S. paratyphi A isolates of high MIC values might be due to indiscriminate use of antibiotics in enteric fever.

Drug resistance in S. paratyphi A isolates was reported to be plasmid mediated. Rangnekar et al.[4] reported R-plasmid encoding resistance for Am, Chl, sulfonamides (Su) and Tet in 3 strains of S. paratyphi A. The present investigation also provides the evidence of involvement of a transferable R-plasmid encoding AmChlCotTet-resistance, in S. paratyphi A isolates. Results of *in vitro* transfer studies, presence of plasmid of same molecular size in the donor S. paratyphi A strains and in their corresponding transconjugants and absence of plasmid in the antibiotic sensitive S. paratyphi A isolates supports this view. Thus, vigilance for R-plasmids in *Salmonella* isolates, including other *Enterobacteriaceae* is essential.

Since 1990, Cp has formed the mainstay of anti-microbial therapy for patients with MDR enteric fever in India. This practice relieved the selection pressure on antibiotics such as Am, Chl and Cot and led to the re-emergence of S. paratyphi A isolates susceptible to these antibiotics. This is evident in our study from the fact that all S. paratyphi A isolates obtained during 1992-1994 were susceptible. However, with the rampant use of Cp, the price was paid in the form of emergence of S. paratyphi A isolates with high MICs of Cp (≥ 0.1 μg/ml), as recorded in our study. The present study showed a gradual increase in MICs of Cp, from 0.005 to 1.25 μg/ml, among the isolates pointing towards the emergence of Cp-resistant isolates in recent years.[10] But, such resistance of S. paratyphi A isolates to Cp was not detected by the NCCLS guidelines, which use the MICs of ≤ 1 μg/ml and ≥ 4 μg/ml as respective breakpoints for Cp susceptibility and resistance.[8] In the present study, association of Nx resistance (≥ 32 μg/ml) with decreased Cp susceptibility (≥ 0.1 μg/ml) was noticed among the isolates. Therefore, Nx resistance can be used as a tool to detect Cp resistance of *Salmonella* isolates.[15] Emergence of S. paratyphi A causing treatment failure due to high Cp MICs has been reported earlier.[11] In this situation, there seems to be room for considering using the conventional antibiotics (Am, Chl and Cot) for the treatment of enteric fever. The fact that most of the S. paratyphi A isolates of 2000 and 2001 to 2005 in the present investigation were susceptible to these agents supports such a strategy. However, for MDR S. paratyphi A infection, Ctx therapy was found effective.

Considering the small number of samples studied, the
resistance pattern obtained in our study may not represent the true picture of S. paratyphi A resistance in the community. The fact that most of the patients suspected to have enteric fever in our country do not undergo blood culture and antibiogram studies, may be responsible for the small number of isolates obtained. Nevertheless, the study underlines the importance of testing anti-microbial susceptibility of S. paratyphi A isolates from different parts of the West Bengal state (India), in order to prepare effective treatment regimens to combat antibiotic resistance in different regions of the state.

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References


Announcement

JPGM gets indexed with Science Citation Index

Journal of Postgraduate Medicine (www.jpgmonline.com) has been included by ISI into its prestigious databases – Current Contents/Clinical Medicine, Science Citation Index, Web of Science and Journal Citation Report. The journal will get its first official Impact Factor in 2009. As of now, it’s unofficial Impact Factor for 2005 places it at number 1 position amongst the biomedical journals from India.

This is a moment of pride for all involved in editing and publishing of the journal. The journal also attracts over 1 lakh unique visitors a month and gets more than 1 million article downloads per year. The handles over 750 articles per year with a turnaround time of just about 30 days. Over 35% of the articles are submitted from outside India; UK and USA contributing the maximum number of overseas submissions. The journal, thus, deservedly got indexed with SCI and hopes to further improve its performance over the years.