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Original Article

Trends in antibiotic susceptibility of enteric fever isolates from South India, 2002–2013

Sangeeta Joshi ^{a,*}, Ranjeeta Adhikary ^b, Hosdurg Bhaskar Beena ^b,
Malavalli Venkatesh Bhavana ^b, Air Vice Mshl Rajvir Bhalwar, (Retd) ^c

^a SRCC Childrens Hospital-Narayana Health, 1-1A, Haji Ali Park, K. Khayde Marg, Mahalaxmi, Mumbai 400034, India

^b Microbiologist (Laboratory Medicine), Manipal Hospital, 98 Rustom Bagh, Old Airport Road, Bangalore 560017, India

^c Former Commandant, Command Hospital Air Force, Bangalore, India

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ABSTRACT

Background: Enteric fever is endemic in India. Trends in antibiotic resistance in *Salmonella enterica* subspecies *enterica* serovars Typhi and Paratyphi A isolates over the past 12 years were studied.

Methods: A retrospective analysis of consecutive blood culture isolates of *Salmonella* Typhi and *Salmonella* Paratyphi A was performed from 2002 to 2013. Antibiotic susceptibility testing was carried out for ampicillin, chloramphenicol, cotrimoxazole, nalidixic acid (NA), ciprofloxacin and ceftriaxone by disc diffusion. The minimum inhibitory concentration of ciprofloxacin and azithromycin was determined using E-test strips. Mantel-Haenszel extended chi-square test was used for analysis of trends across years.

Results: Three thousand two hundred ninety-six *Salmonella* spp. were isolated; of which, 1905 were identified as *Salmonella* Typhi (58%) and 1393 as *Salmonella* Paratyphi A (42%). Multidrug resistance (chloramphenicol, ampicillin and cotrimoxazole) was relatively stable throughout the study period. NA resistance increased from 18% in 2007 to 100% in 2013 among *Salmonella* Paratyphi A isolates and from 67% to 82% among *Salmonella* Typhi isolates. Complete susceptibility to ceftriaxone and azithromycin was observed in this study. **Conclusions:** Knowledge of the local patterns of resistance would help in appropriate therapy for enteric fever. With increasing rates of fluoroquinolone resistance in our hospital setting, it is probably prudent to revert back to the first-line agents for treatment and save azithromycin and third-generation cephalosporins for difficult and non-responsive cases.

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Introduction

Salmonella serovars Typhi and Paratyphi A are the 2 leading causes of enteric fever in India. A systematic review to

estimate global morbidity and mortality of typhoid and paratyphoid fever in 2010 suggested that there were 13.5 million episodes of typhoid fever globally.¹ In another study of disease burden in low- and middle-income countries, a high incidence estimate for South Asia was noted. The estimated number of

* Corresponding author.

E-mail address: sangeetajo@yahoo.com (S. Joshi).

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typhoid fever in low- and middle-income countries in 2010 was 11.9 million cases.²

The incidence of *Salmonella* Paratyphi A has been gradually increasing over the past decade both in South East Asia and in India.^{3,4} A 2-decade trend analysis of enteric fever in Nepal revealed the emergence of *Salmonella* Paratyphi A as a leading agent.⁵

The pattern of antibiotic resistance has also seen changes over the last decade. By the late 1980s, multidrug-resistant (MDR) *Salmonella* Typhi was reported from multiple countries.⁶ With the increasing use of fluoroquinolones, resistance to ciprofloxacin has increased, while resistance to ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole has decreased.⁷ Third-generation cephalosporins and azithromycin are increasingly being used for the treatment of enteric fever. This record-based study was undertaken to evaluate the trends and antibiotic resistance patterns of enteric fever isolates from 2002 to 2013.

Material and methods

This 12-year record-based study was performed in a 600 bedded tertiary care hospital in Bangalore, South India, for the period January 2002 to December 2013 from medical and laboratory records. All consecutive blood culture isolates of *Salmonella* Typhi and *Salmonella* Paratyphi A were included in the analysis. Blood cultures were obtained by using the BacTALERT system (Biomérieux, France). The isolates were identified manually by standard biochemical tests⁸ and confirmed serologically using polyvalent O antiserum and serovar-specific antisera (Denka Seiken, Japan).

Antibiotic susceptibility testing was carried out using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar using discs from HiMedia, India, for the following antimicrobials: ampicillin (A, –5 µg), chloramphenicol (C, –30 µg), trimethoprim-sulfamethoxazole (Co, –1.25/23.75 µg), nalidixic

acid (NA, 30 µg), ceftriaxone (30 µg), ciprofloxacin (30 µg) and azithromycin (15 µg). Disc diffusion for NA, azithromycin, and ceftriaxone was started in 2007.

From 2012, the minimum inhibitory concentration (MIC) levels were determined for ciprofloxacin and azithromycin using Epsilometer (E)-test strips (BioMérieux, France). The results of antimicrobial susceptibility testing were analysed using the Clinical and Laboratory Standards Institute (CLSI, USA) breakpoints.

From July 2012, the revised CLSI breakpoints for ciprofloxacin for *Salmonella* were used.⁹ For azithromycin, isolates with MIC <16 µg/ml were considered susceptible.¹⁰ A standard strain of *Escherichia coli* ATCC 25922 was included as the quality control. Isolates resistant to ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole were defined as MDR isolates.

Results

Of the total 3298 *Salmonella enterica* spp. isolated over the 12-year study period, 1905 isolates were identified as *Salmonella* Typhi (58%) and 1393 as *Salmonella* Paratyphi A (42%). The increased isolation of *Salmonella* Typhi from 2005 to 2012 and *Salmonella* Paratyphi A from 2005 to 2009 (Fig. 1) was found to be statistically significant with a p value <0.001. Mantel-Haenszel extended chi-square test was used for analysis of trends across years.

The rate of recovery of MDR *Salmonella* Typhi rate fell from 6.3% in 2004 to 0.7% in 2013, whereas NA-resistant (NAR) *Salmonella* Typhi recovery increased from 67.4 to 82.4 during the same period (Table 1). The MICs for ciprofloxacin was determined for 340 *Salmonella* Typhi (Fig. 2) in 2012 and 2013, and the MICs ranged from 0.06 to more than 1 µg/ml.

There was no MDR *Salmonella* Paratyphi A detected in our study. Nalidixic acid resistant (NAR) of *Salmonella* Paratyphi A increased during the study period (Table 2); the MICs for

Fig. 1 – Trend of *Salmonella*, 2002–2013.

Table 1 – *Salmonella* Typhi resistance (2002–2013).

Year	<i>Salmonella</i> Typhi (number)	MDR (%)	NAR (%)	Cipro % resistant/intermediate
2002	64	3.1	ND	0
2003	47	4.3	ND	0
2004	79	6.3	ND	0
2005	138	2.9	ND	0
2006	169	1.2	ND	0
2007	184	5.4	67.4	0
2008	255	5.1	86.3	0
2009	197	4.1	84.8	4.5
2010	164	4.9	89	9.7
2011	224	2.7	95.4	8.0
2012 ^a	253	1.9	96.8	98.4
2013 ^a	131	0.7	82.4	95.4

Cipro, ciprofloxacin; MDR, multidrug resistant; ND, not done; NAR, nalidixic acid resistant.

^a The 2012 CLSI guidelines were followed.

Table 2 – *Salmonella* Paratyphi A resistance (2002–2013).

Year	<i>Salmonella</i> Paratyphi A (number)	NAR (%)	Cipro % resistant/intermediate
2002	46	ND	0
2003	48	ND	0
2004	156	ND	0
2005	135	ND	0
2006	231	ND	0
2007	132	18.2	0
2008	204	89.7	0
2009	106	99.1	3.7
2010	60	98.3	0
2011	73	97.3	10.9
2012 ^a	91	100	79.1
2013 ^a	111	100	100

Cipro, ciprofloxacin; ND, not done; NAR, nalidixic acid resistant.

^a The 2012 CLSI guidelines were followed.

Fig. 2 – Ciprofloxacin MICs for *Salmonella* Typhi and *Salmonella* Paratyphi A as per the 2012 CLSI guidelines. Intermediate susceptible range: 0.12–0.5 µg/ml. Resistant: ≥1 µg/ml.

ciprofloxacin was determined for 180 *Salmonella* Paratyphi A (Fig. 2) in 2012 and 2013, and the MICs ranged from 0.06 to more than 1 µg/ml.

The MICs for azithromycin was determined for 300 *Salmonella* Typhi and 169 *Salmonella* Paratyphi A isolates, and the MICs ranged from less than 0.19 to 12 µg/ml (Fig. 3). No resistance to third-generation cephalosporins (ceftriaxone) was detected.

A subset of paediatric patients (532 patients) was studied during the period from November 2008 to December 2012. All were culture positive; 85.2% had *Salmonella* Typhi and 24.8% had *Salmonella* Paratyphi A isolated from the blood culture. Four hundred seventy-six isolates were NAR. The children presented with fever of more than 102 °C in 80% of cases. In almost 80% of cases, the presentation was fever with more than 5 days of duration; 19.1% had splenomegaly and 18.7% had hepatomegaly. All the children recovered after treatment with ceftriaxone/azithromycin.

Discussion

Enteric fever continues to be a problem in developing countries. In a study from Gujarat, India, there was a rising trend of

enteric fever reported from 2005 to 2011 with a case rate of 26–71 per 100,000 population per year.¹¹ Enteric fever is also an important cause of traveller's disease. A geosentinel survey from 2000 to 2010 showed a steady increase of enteric fever in travellers returning from South-Central Asia, South-East Asia and sub-Saharan Africa.¹² However, in Singapore, there is a declining trend of enteric fever from 4.3 per 100,000 population in 1990 to 0.26 per 100,000 population in 2009. This was attributed to vast improvements in environmental sanitation, potable water supplies and sewage disposal facilities. More than 90% of the reported cases were imported, mainly from India and Indonesia.¹³ The incidence of enteric fever in Australian residents increased from 4 to 5 before 2003 to 7 cases per million person-years after 2003.¹⁴

In the present study, which is from South India, there was a gradual increase in cases of *Salmonella* Paratyphi A over the years till 2006 followed by a decline. In another study from South India,¹⁵ *Salmonella* Paratyphi A cases ranged from 52.5% in 2006 to 30.6% in 2010; there was a dramatic rise of *Salmonella* Paratyphi A isolates from 2006 to 2009 in this study. On the other hand, in a 12-year study from North India, *Salmonella* Typhi was the predominant serotype (83.8%) followed by *Salmonella* Paratyphi A (16.2%).¹⁶ In another 3-year study from North India, *Salmonella* Paratyphi A isolations were 14.9%, whereas *Salmonella* Typhi isolations were 85.1%.¹⁷ Thus, we see that in the same country, we have regional differences in the prevalence of *Salmonella* Paratyphi A and *Salmonella* Typhi.

MDR salmonellae have shown a gradual decline after the peak in the 1990s. A re-emergence of susceptibility to first-line antibiotics and a notable decline in MDR strains was noted in a 12-year study from North India, and 84.5% of their strains had a decreased susceptibility to ciprofloxacin.¹⁶

NA has been reported to be a surrogate marker for predicting reduced ciprofloxacin susceptibility. However, NA may not detect all mechanisms of fluoroquinolone resistance.⁹ Increasing recovery of NAR *Salmonella* Typhi has been associated with intermediate susceptibility of these isolates to ciprofloxacin. NA Resistance may indeed be taken as a clue to increased MIC of ciprofloxacin for *Salmonella* Typhi. In such instances, the clinicians need to be alerted, and dosage of ciprofloxacin will have to be increased for adequate response.

Fig. 3 – Azithromycin MICs for *Salmonella Typhi* and *Salmonella Paratyphi A* (Ref.¹⁰). Susceptible MIC: ≤ 16 µg/ml.

Increasing resistance and intermediate susceptibility to ciprofloxacin was noted in our study. No resistance to third-generation cephalosporins was observed. NAR was 100% and ciprofloxacin resistance was 13.6% in another study from North India.¹⁷ In a 5-year study from South India that was conducted from 2005 to 2009, 22% of *Salmonella Typhi* were found to be MDR and 78% were NAR. A high rate of resistance (8%) was found for ciprofloxacin.¹⁸ In a multicentric study from India for the period 2008 to 2010, NAR was seen in 83% of *Salmonella Typhi* and 93% of *Salmonella Paratyphi A* strains.¹⁹

NAR and MDR strains have also been reported globally. During the period from 1985 to 2010, multidrug resistance in Australia was between 4% and 11%.¹⁴ In a meta-analysis from Nepal, there was an increasing trend of NAR in *Salmonella Typhi* and *Salmonella Paratyphi A*. The MDR rate had decreased in both these organisms over the past 2 decades.⁵ In another study from Nepal, 90% NAR in *Salmonella Typhi* and *Salmonella Paratyphi A* and no MDR strains were reported.²⁰ In a study from Middle-East and Central Asia, the MDR prevalence was found to be 13–83%; 22% had reduced susceptibility to ciprofloxacin and 22% were NAR.²¹

All isolates in our study were susceptible to azithromycin. A high MIC 90 to azithromycin in *Salmonella* isolates was reported in a study from North India.²² A study from South India found a 33% of resistance to azithromycin.²³ On the other hand, in London, a stable antibiotic resistance to ceftriaxone and azithromycin was noted during the period 2005–2012.²⁴ A recent study from Mumbai found all isolates of *Salmonella Typhi* and *Salmonella Paratyphi A* to be susceptible to azithromycin.²⁵

While comparing the 2012 CLSI guidelines with the previous 2011 guidelines, it was found that as per the 2012 CLSI guidelines for ciprofloxacin susceptibility, only 3% of their strains were susceptible, and 88% were moderately susceptible, as compared with the 2011 CLSI breakpoints in which 95% were susceptible. Thus, reinterpreting MIC results with 2012 guidelines indicates the end of the road for ciprofloxacin as the drug of choice for *Salmonella Typhi*.²⁶ In a study using the CLSI 2012 guidelines, it was found that NAR was seen in 94% of *Salmonella Typhi* and in 96% of *Salmonella Paratyphi A*; the MIC of ciprofloxacin for 76.8% of *Salmonella Typhi* and 71.4% of *Salmonella Paratyphi A* was in the intermediate sensitive range

(MIC 0.12–0.5 µg/ml) and 11.1% of *Salmonella Typhi* and 2.19% of *Salmonella Paratyphi A* were resistant (MIC ≥ 1 µg/ml) as per the 2012 CLSI guidelines.²⁷

NAR strains of *Salmonella* may be associated with clinical failure or delayed response in fluoroquinolone-treated patients.⁹ For enteric fever isolates with ciprofloxacin MICs in the intermediate range, it is recommended that maximal doses of oral parental ciprofloxacin be given. Although ciprofloxacin is an economical drug for the treatment of enteric fever, with increasing rates of fluoroquinolone resistance, it is probably prudent to revert back to the first-line agents for treatment and save azithromycin and third-generation cephalosporins for difficult and non-responsive cases.²⁸ Azithromycin is a preferred alternative agent in cases of uncomplicated enteric fever.²⁹

Conclusions

Salmonella Typhi and *Salmonella Paratyphi A* are almost equally isolated in our region, and the majority of the isolates are NAR. The MDR rate for *Salmonella Typhi* is very low, and we have not encountered any MDR resistance in *Salmonella Paratyphi A*. Although a vaccine for *Salmonella Typhi* exists, the increased isolation of *Salmonella Paratyphi A* warrants a vaccine too. Susceptibility to the first-line agents and third-generation cephalosporins remains high, and they continue to be the drugs of choice for the treatment of enteric fever.

Conflicts of interest

The authors have none to declare.

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