

Antimicrobial susceptibility profile, treatment outcome and serotype distribution of clinical isolates of *Salmonella enterica* subspecies *enterica*: a 2-year study from Kerala, South India

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Background/purpose: Typhoid and paratyphoid fever continue to be important causes of illness and death in parts of Asia, being associated with poor sanitation and consumption of unsafe food and water. Antimicrobial resistance has emerged to traditional first-line drugs, namely, the fluoroquinolones, as well as to third-generation cephalosporins, posing challenges to treatment. Azithromycin has proven to be an effective alternative for treatment of uncomplicated typhoid fever. The purpose of this study was to determine the antimicrobial susceptibility, clinical outcome and serotype distribution pattern of clinical isolates belonging to *Salmonella enterica* subspecies *enterica*.

Methodology: All clinical isolates of *S. enterica* obtained from blood, sterile body fluids, as well as stool and urine samples at Amrita Institute of Medical Sciences and Research Centre, Kerala, India, between August 2011 and July 2013 were included in the study and processed based on standard microbiology protocols.

Results: A total of 118 isolates of *Salmonella* were obtained during the study period. Out of these, 79 were of *S. Typhi* (66.95%), followed by isolates of *S. Paratyphi A* (22; 18.64%) and *S. Typhimurium* 12 (10.17%). Five isolates could not be identified further. There was 100% susceptibility to ceftriaxone in all *S. enterica* subspecies. Ciprofloxacin susceptibility was 32.91% for *S. Typhi* and 40.90% for *S. Paratyphi A* as determined by the disk diffusion method. The susceptibility profile of *S. Typhi* isolates to different antimicrobials was as follows: chloramphenicol (94.93%), ampicillin (77.21%), cotrimoxazole (75.94%) and azithromycin (78.48%). For *S. Typhi*, the minimum inhibitory concentration (MIC) of ciprofloxacin required to inhibit the growth of 50% of organisms was 0.5 µg/mL (intermediate) and MIC required to inhibit the growth of 90% of organisms was 1 µg/mL (resistant). *S. Typhimurium* was 100% susceptible to cotrimoxazole, ampicillin, ceftriaxone, chloramphenicol, ofloxacin and azithromycin. Susceptibility to ciprofloxacin was 66.66%. Patients from whom *S. Typhimurium* was isolated had comorbidities with documented risk. Of the 118 patients, 3 expired. Two had typhoid fever and were in sepsis at admission. One had *S. Typhimurium* and was suffering from multiple myeloma.

Conclusion: *S. Typhi* was the predominant isolate. All isolates were susceptible to ceftriaxone. Chloramphenicol susceptibility was >90%. No multidrug-resistant *Salmonella* strains were isolated. Susceptibility to ciprofloxacin for *S. Typhi* was 33%. Recovery rate was 97%.

Keywords: *Salmonella enterica*, susceptibility profile, clinical isolates

Introduction

Typhoid and paratyphoid fever continue to be important causes of illness and death, particularly among children and adolescents in South-Central and Southeast Asia. Enteric fever is associated with poor sanitation and unsafe food and water. Antimicrobial resistance has sequentially emerged to traditional first-line drugs, namely, the fluoroquinolones, as well as to third-generation cephalosporins, posing challenges to treatment. Azithromycin has proven to be an effective alternative for treatment of uncomplicated typhoid fever. The availability of full-genome sequences for *S. enterica* serovar Typhi and *S. enterica* serovar Paratyphi A confirms their place as monomorphic, human-adapted pathogens vulnerable to control measures if international efforts can be redoubled.¹ Not all serovars of *S. enterica* subspecies *enterica* cause disease in humans, and only a small subset causes debilitating/life-threatening disease, the most well-known being serovars Typhi, Paratyphi A–C, Enteritidis, Typhimurium and Choleraesuis.² The purpose of this work was to study the predominant *Salmonella* serotypes isolated at Amrita Institute of Medical Sciences, a tertiary care hospital in Kochi, Kerala, South India, over a 2-year period. With ciprofloxacin being the traditional drug of choice for enteric fever, difference in susceptibility results of ciprofloxacin and nalidixic acid was often noted while reporting *Salmonella* serotypes in our clinical laboratory. The knowledge that isolates that test sensitive to ciprofloxacin and resistant to nalidixic acid on routine disk diffusion tests may have decreased ciprofloxacin susceptibility, which may not be detected unless minimum inhibitory concentration (MIC) testing is performed, propelled us to check the MIC of ciprofloxacin. Literature search did not reveal much information on isolation of *S. Typhimurium* from patients; this prompted us to include *S. Typhimurium* isolates in our study.

Methodology

All clinical isolates of *S. enterica* obtained from blood, sterile body fluids as well as stool and urine samples from patients treated at the Amrita Institute of Medical Sciences and Research Centre, Kochi, Kerala, India, a tertiary care center, between August 2011 and July 2013 were included in the study. The study was approved by the Thesis Protocol Review Committee (Scientific, Ethical and Financial). Samples were collected after getting written informed consent from the subjects. Briefly, motile Gram-negative bacilli that were oxidase negative and catalase positive were subjected to preliminary identification in a VITEK 2 Compact (bioMérieux, SA, France) microbial identification system and manual biochemical reactions and subsequently confirmed using the slide agglutination test with *Salmonella* polyvalent O antiserum groups A–E and with

type-specific *S. Typhi* O9 and H:d, *S. Paratyphi A* O2 and H:a, and *S. Typhimurium* O4 and H:i antisera sourced from the Central Research Institute, Kasauli, Himachal Pradesh, India. Our repertoire of antisera only allowed for the identification of three serovars and isolates that were positive for *Salmonella* polyvalent O group A–E agglutination but gave no agglutination with type-specific antisera were reported as *Salmonella* species. The typing scheme followed was Grimont and Weill's *Antigenic Formulae of the Salmonella Serovars, 9th Edition*.³ Antibiotic susceptibility testing was performed by Kirby–Bauer disk diffusion method using commercial antibiotic disks (Hi Media Laboratories, Mumbai, Maharashtra, India). Susceptibility to cotrimoxazole, ampicillin, ciprofloxacin, nalidixic acid, ceftriaxone, chloramphenicol, ofloxacin and azithromycin was determined.^{4,5} Zone diameter interpretation was based on the 2013 guidelines of Clinical and Laboratory Standards Institute. Interpretive criteria for the disk diffusion test for ciprofloxacin were as follows: susceptible ≥ 31 mm, intermediate 21–30 mm and resistant ≤ 20 mm.⁵ The MICs (micrograms per milliliter) for ciprofloxacin (Sigma-Aldrich, Bangalore, Karnataka, India) were determined using the microbroth dilution technique. The MIC interpretive criteria used were as follows: MIC ≥ 1 $\mu\text{g/mL}$ as resistant, MIC 0.12–0.5 $\mu\text{g/mL}$ as intermediate and MIC ≤ 0.06 $\mu\text{g/mL}$ as susceptible.^{5,6} Treatment outcomes of these patients were also recorded.

Results

During the study period, 118 isolates of *Salmonella* were obtained. Of these, 79 were of *S. Typhi* (66.95%), followed by those of *S. Paratyphi A* 22 (18.64%) and *S. Typhimurium* 12 (10.17%); 5 isolates could not be identified further (Table 1). *S. enterica* serotype distribution in clinical samples is depicted in Table 2. The total number of *Salmonella* samples isolated was 118. The majority (112, 94.91%) of isolates were from the blood, 4 (3.38%) from the stool, 1 (0.84%) from the urine and 1 (0.84%) from the synovial fluid. Moreover, 78 (98.7%) *S. Typhi* isolates were obtained from blood. All isolates of *S. Paratyphi A* (22, 100%) were also from blood. Of the 12 isolates of *S. Typhimurium*, 8 (66%) were also from blood culture. Of the *Salmonella* that could not be further identified, 4 (80%) were from blood and 1 (20%) was from synovial fluid.

Percentage susceptibility results of the *S. enterica* isolates are presented in Table 3. The salient findings were as follows:

Table 1 Isolation of *Salmonella enterica* subspecies *enterica* serotypes

Organisms	Number (N=118)	%
<i>Salmonella Typhi</i>	79	66.95
<i>Salmonella Paratyphi A</i>	22	18.64
<i>Salmonella Typhimurium</i>	12	10.17
<i>Salmonella species</i>	5	4.23

Table 2 *Salmonella enterica* serotype distribution in clinical samples (N=118)

Clinical sample	Number of isolates	<i>Salmonella</i> Typhi	<i>Salmonella</i> Paratyphi A	<i>Salmonella</i> Typhimurium	<i>Salmonella</i> spp.
Blood	112	78	22	8	4
Stool	4	0	0	4	0
Urine	1	1	0	0	0
Synovial fluid	1	0	0	0	1

Table 3 Percentage susceptibility of *Salmonella enterica* isolates (August 2011–July 2013)

Isolates	<i>Salmonella</i> Typhi		<i>Salmonella</i> Paratyphi A		<i>Salmonella</i> spp.		<i>Salmonella</i> Typhimurium		
	N=79		N=22		N=5		N=12		
	Number of isolates	n	%	n	%	n	%	n	%
Cotrimoxazole 25 µg	60		75.94	21	95.45	5	100	12	100
Ampicillin 10 µg	61		77.21	22	100	5	100	12	100
Ciprofloxacin 5 µg	26		32.91	9	40.90	5	100	8	66.66
Nalidixic acid 30 µg	10		12.65	0	0	5	100	7	58.33
Ceftriaxone 30 µg	79		100	22	100	5	100	12	100
Chloramphenicol 30 µg	75		94.93	20	90.90	5	100	12	100
Ofloxacin 5 µg	52		65.82	18	81.81	5	100	12	100
Azithromycin 15 µg	62		78.48	21	95.45	4	80	12	100

there was 100% susceptibility to ceftriaxone in all *S. enterica* species. Ciprofloxacin susceptibility was 32.91% for *S. Typhi* and 40.90% for *S. Paratyphi A* as determined by the disk diffusion method. The susceptibility profile of *S. Typhi* isolates to antimicrobials was as follows: chloramphenicol 94.93%, ampicillin 77.21%, cotrimoxazole 75.94% and azithromycin 78.48%. Susceptibility pattern of the 22 isolates of *S. Paratyphi A* was as follows: 100% to ampicillin, 95.45% to cotrimoxazole, 90.90% for chloramphenicol, 81.81% to ofloxacin and 95.45% to azithromycin. *S. Typhimurium* was 100% susceptible to cotrimoxazole, ampicillin, ceftriaxone, chloramphenicol, ofloxacin and azithromycin. Susceptibility was 66.66% to ciprofloxacin. The MICs of all *Salmonella* isolates when considered together were as follows: MIC required to inhibit the growth of 50% of organisms (MIC₅₀)=0.25 µg/mL and MIC₉₀=1 µg/mL. For *S. Typhi* alone, the values were MIC₅₀=0.5 µg/mL and MIC₉₀=1 µg/mL.

Treatment outcome

Data were available for 93 patients. All of them excluding three patients recovered. Ceftriaxone was the most common monotherapeutic agent used in 52/93 (55.91%) patients. Quinolones were used as monotherapy based on susceptibility results in 16/93 (17.2%) patients. One patient with typhoid fever was treated with azithromycin. Other agents used as monotherapy were cotrimoxazole in 1/93, cefoperazone–sulbactam in 1/93, piperacillin–tazobactam in 1/93 and amoxicillin–clavulanate in 1/93. Ten of the 12 patients in whom *S. Typhimurium* was detected had comorbidities with

documented risk. Of the 118 patients, 3 expired. Among these, two patients had typhoid fever and were in sepsis at admission. One patient grew *S. Typhimurium* and was suffering from multiple myeloma.

Discussion

S. Typhi was the predominant isolate at 67%, 18% of isolates were of *S. Paratyphi A* and 10% were of *S. Typhimurium*. Isolation of *S. Typhi* as the most common serotype is similar to the results from other Indian studies from Chennai, Shimla, Chandigarh and New Delhi.^{7–11} Susceptibility of *S. Typhi* and *S. Paratyphi A* was 100% for ceftriaxone; similar susceptibility has been reported from Chennai.⁷ A study from Bengaluru has reported 97.50% susceptibility to ceftriaxone in *S. Typhi* and 100% in *S. Paratyphi A*.¹² In our study, only 26/79 (32.9%) of *S. Typhi* were sensitive to ciprofloxacin. The percentage of nalidixic acid-resistant *S. Typhi* isolated was 87.43. The percentage of nalidixic acid-resistant *S. Paratyphi A* isolated was 100. A study from Chandigarh has also observed very high resistance to nalidixic acid (~86% in 2005 and 100% in 2012) and decreasing susceptibility to ciprofloxacin in *S. Typhi*.¹¹

There have been many reports of multidrug-resistant (MDR) *Salmonella* resistant to ampicillin, trimethoprim–sulfamethoxazole and chloramphenicol in the Indian subcontinent, but we did not isolate any MDR during our study.¹ A notable decline in MDR strains has been reported by Singhal et al¹¹ in a 12-year study from Chandigarh in North India. Our observation of reemergence of susceptibility to chloramphenicol is comparable with studies by other Indian workers.^{7,11,13,14}

S. Typhimurium was 100% susceptible to all antimicrobials tested; susceptibility to ciprofloxacin was 66.66%. Of the 12 *S. Typhimurium* strains isolated during the study period, 4 were from stool and 8 from blood stream; moreover, 9 patients had 3 or more comorbidities. Several comorbidities have been reported in patients with nontyphoidal salmonellosis, including malignancy, diabetes and treatment with immunotherapy agents.^{15,16}

The lone urinary carrier of *S. Typhi* was a male with no history of enteric fever. He had bilateral renal calculi, obstructive uropathy, peripheral neuropathy and end-stage kidney disease. *S. Typhi* bacteriuria has been documented in patients with renal calculi and also in those with urinary tract abnormalities.¹⁷ The two patients who expired due to typhoid fever were septicemic; their blood cultures were positive for *S. Typhi*. One died a few hours after admission, and the other – despite intensive management and treatment with ceftriaxone – passed away within 48 hours of admission. On administration of appropriate antibiotic therapy in a prompt manner, typhoid fever is a short-term febrile illness with about 6 days of hospitalization. The risk of mortality is 0.2%.¹⁸ The third patient who expired grew *S. Typhimurium*. He was immunocompromised, suffering from multiple myeloma with renal failure and was on chemotherapy. The severity of nontyphoidal salmonellosis depends on host factors and the strain of *Salmonella*.¹⁸

Five isolates belonging to the genus *Salmonella* could not be identified further. The lack of antisera for other *Salmonella* serotypes and nonavailability of molecular methods for confirmatory identification were limitations of the study.

Conclusion

S. Typhi was the predominant isolate. This was followed by *S. Paratyphi A*. Ceftriaxone was the common monotherapeutic agent used in our hospital. All isolates of *Salmonella* were susceptible to ceftriaxone. Chloramphenicol susceptibility was >90%. No MDR *Salmonella* strains were isolated. Susceptibility of *S. Typhi* to ciprofloxacin was 33%. Furthermore, 97% of patients recovered. A urinary carrier of *S. Typhi* was detected during the study period. There were 17 cases of nontyphoidal salmonellosis.

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Disclosure

The authors report no conflicts of interest in this work.

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