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Research Article

Antibiotic Resistance among *Salmonella* Isolates - A Five-year Trend

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A B S T R A C T

Introduction: In India, enteric fever is an endemic febrile illness, caused by *Salmonella enterica* serovar Typhi/ Paratyphi A, B, and C. The incidence of *Salmonella enterica* serovar Typhi/ Paratyphi isolates with decreased susceptibility/ resistance to fluoroquinolones is very high, though these isolates have been reported to be sporadically resistant to third-generation cephalosporins. This retrospective study was planned to analyse the trends of drug resistance along with the emergence of ceftriaxone resistance, if any, among *Salmonella enterica* isolates.

Materials and Methods: This retrospective study in a tertiary care hospital over a span of five years (2018–2022). All the blood specimens were inoculated in blood culture bottles and were processed using automated systems as per standard protocols. All the isolates obtained were identified and their antimicrobial susceptibility pattern was determined using VITEK 2.

Results: A total of 1083 *S. enterica* were isolated, of which, 779 (71%) were *Salmonella* Typhi, and 304 (29%) were *Salmonella* Paratyphi A. The positivity of blood culture for *Salmonella enterica* varied from 0.7% to 1.3%. The distribution of S. Typhi (70%–80%) and S. Paratyphi (20%–30%) remained almost uniform during all the years. A significant increase in ciprofloxacin resistance was observed in S. Typhi isolates in all the years. 0.7%–3.2% and 1.1%–1.4% of S. Typhi and S. Paratyphi A isolates, respectively, were found resistant to ceftriaxone over the years.

Conclusion: A high resistance to fluoroquinolone among S. Typhi and S. Paratyphi A isolates was observed with the emergence of ceftriaxone resistance and re-emergence of susceptibility to first-line medications.

Keywords: Antimicrobial Resistance, Enteric Fever, Emerging Ceftriaxone Resistance, *Salmonella*

Introduction

Enteric fever/ typhoid is an endemic disease mainly caused by *Salmonella enterica* serotype Typhi and, to a lesser extent, by *S. enterica* serotypes Paratyphi A, B, and C, and may involve multiple organ systems with high morbidity (1000–20,000 per million population). The disease is transmitted by ingestion of contaminated food and water, hence it is an important public health problem in developing countries including India. Antibiotics are the mainstay for the treatment of typhoid, which can successfully reduce mortality.[1–3] However, the emergence of multidrug resistance among S. Typhi and Paratyphi isolates, against first-line antibiotics like chloramphenicol, ampicillin and cotrimoxazole further complicated the situation.[2,4–7] This resulted in the use of ciprofloxacin as the treatment of choice against drug-resistant *Salmonella* enterica,[8] leading to the emergence of ciprofloxacin-resistant strains.[9,10] Based on the current revised MIC breakpoints of ciprofloxacin by CLSI, it is necessary to have clarity regarding the incidence/ prevalence of resistance, especially to ciprofloxacin. With the emergence of ciprofloxacin resistance, third-generation cephalosporins such as ceftriaxone are extensively used empirically for the treatment of enteric fever.[2] However, sporadic cases of reduced susceptibility or resistance to ceftriaxone against S. Typhi and Paratyphi have also been reported recently.[11,12] Hence, this retrospective study was planned to analyse the trends of drug resistance along with the emergence of ceftriaxone resistance, if any, among *Salmonella enterica* isolates over a period of 5 years.

Material and Methods

This retrospective study was conducted in the Microbiology Department of a tertiary care hospital in Ludhiana, North India during 2018–2022 after obtaining approval from the Institutional Ethics Committee (IEC number 2023-852). Blood for culture was collected under strict aseptic conditions. All the blood specimens received (N = 102,600) in the microbiology laboratory for culture and sensitivity were inoculated for enrichment in plus aerobic/ F culture vials and were processed using automated systems (BACTEC 9240/ BacT-Alert 3D, Biomerieux) as per standard protocols. Immediately after the bottle was marked positive in the system, Gram staining was performed on the smear made from the bottle content and a subculture from the bottle was made using blood agar and MacConkey agar plates. The organisms were presumptively identified on the basis of colony morphology and Gram stain, from the culture plates after overnight incubation. Gram-negative bacilli were further identified using automated VITEK 2 GNB ID cards and were confirmed by serotyping with the help of commercially available antisera obtained from the Central Research Institute, Kasauli, India. Antimicrobial susceptibility for ampicillin, ciprofloxacin, cotrimoxazole and ceftriaxone was performed using VITEK 2 AST-N 235 cards, while chloramphenicol (30 µg) and azithromycin (15 µg) were tested by Kirby–Bauer disc diffusion method[13] as per the CLSI guidelines[14] (Table 1).

Table 1.Minimum Inhibitory Concentration (MIC) Breakpoints

Antibiotics	Minimum inhibitory Concentration (MIC)/µg/ml		
	Sensitive	Inter-mediate	Resistant
Ampicillin	≤ 8	16	≥ 32
Cotrimoxazole	≤ 40	--	> 80
Chloramphenicol	≤ 8	16	≥ 32
Azithromycin	≤ 16	--	≥ 32
Ciprofloxacin	≤ 0.06	0.12-0.5	≥ 1
Ceftriaxone	< 1	2	≥ 4

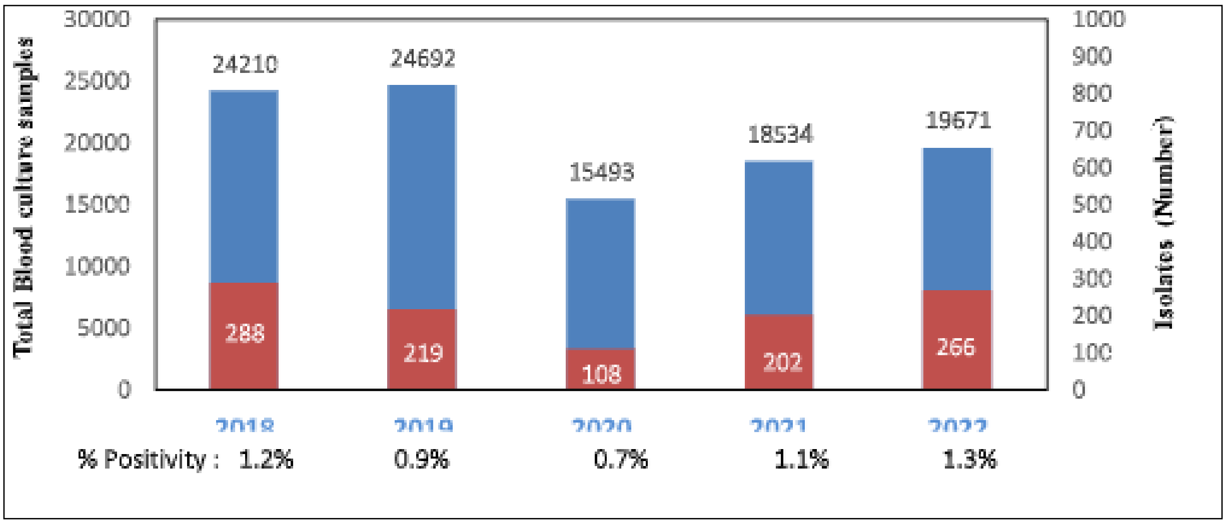

Figure 1.Distribution of *Salmonella* spp. Isolates Over a Period of Five Years (2018–2022)

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Results

A total of 1083 *Salmonella enterica* isolates were obtained from 102600 blood samples; 779 isolates (71%) were identified as *Salmonella enterica* serovar Typhi, and 304 (29%) were identified as *Salmonella enterica* serovar Paratyphi A. Males were predominantly infected (64%) as compared to females (36%). During the study period, the positivity of blood culture for *Salmonella enterica* in 2018, 2019, 2020, 2021, and 2022 were 1.2%, 0.9%, 0.7%, 1.1%, and 1.3% respectively. The year-wise distribution of blood culture samples and isolates obtained is shown in Figure 1. The distribution of *Salmonella enterica* isolates, during the years of study, remained almost uniform, i.e. approximately 70%–80% and 20%–30% of the isolates being S. Typhi and S. Paratyphi, respectively. However, the year 2019 was an outlier with S. Typhi isolation (57%) (Figure 2).

All S. Typhi and S. Paratyphi A isolates were susceptible to azithromycin and cotrimoxazole, except in 2018 when S. Paratyphi A isolates (1.5%) showed resistance to cotrimoxazole. S. Typhi isolates showed resistance to chloramphenicol (0.9%–2.1%), whereas all S. Paratyphi A isolates were sensitive to chloramphenicol. The majority of isolates were susceptible to ampicillin except in 2018 when 30% of S. Paratyphi A isolates showed resistance. A significant increase in ciprofloxacin resistance was observed in S. Typhi isolates in all the years (56.8% to 87.3%), whereas S. Paratyphi A showed 99%–100% resistance. Ceftriaxone resistance was observed in the year 2018 in the case of S. Paratyphi A (1.4%), in 2019 in the case of both S. Typhi (3.2%) and S. Paratyphi A (1.1%), and in 2021 in the case of S. Typhi (0.7%) (Table 2).

Out of *Salmonella enterica* isolates, seven isolates were ceftriaxone resistant (five S. Typhi and two S. Paratyphi A) and three isolates showed intermediate susceptibility (one isolate of S. Typhi in 2021 and two isolates of S. Paratyphi in 2019). All ceftriaxone-resistant isolates were sensitive to azithromycin, chloramphenicol, and cotrimoxazole and 71% were resistant to ampicillin. Out of five S. Typhi isolates, 4 had a MIC of ≥ 64 µg/ml while 1 had a MIC of 16 µg/ml. Out of two S. Paratyphi, 1 had a MIC of ≥ 64 µg/ml and the other had a MIC of ≥ 32 µg/ml (Table 3).

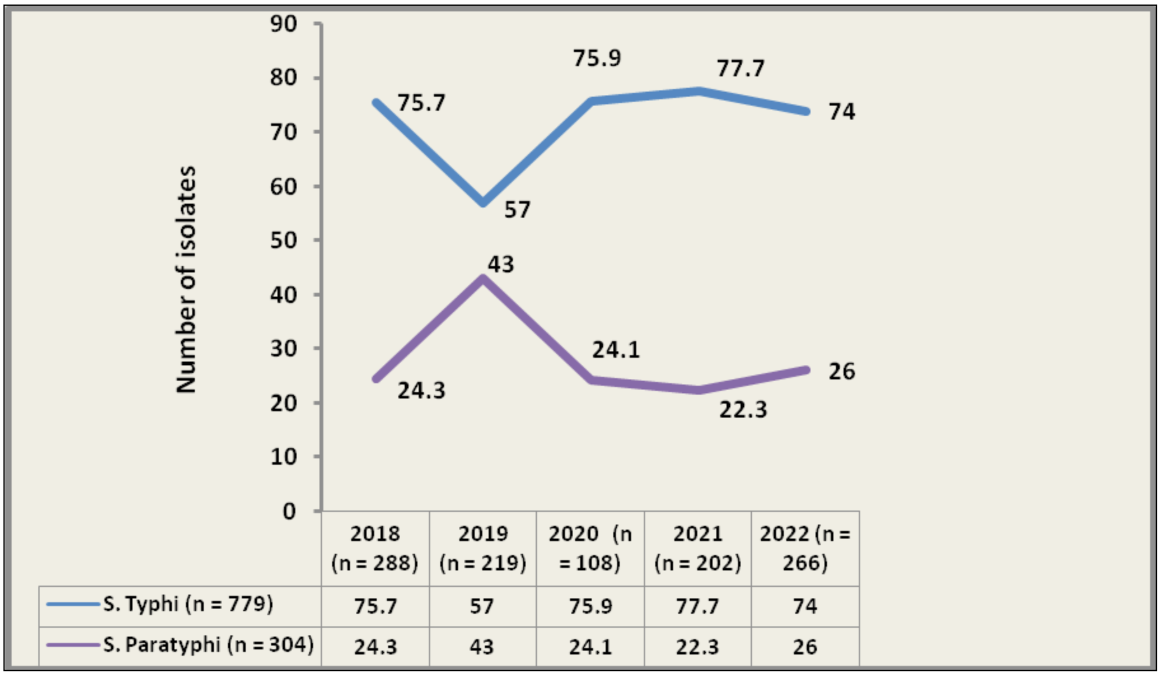

Figure 2.Percentage Distribution of *Salmonella* Typhi and Paratyphi Over a Period of 5 Years

Table 2.Year-wise Antibiotic Resistance Pattern of Salmonella spp

P	S. Typhi					S. Paratyphi				
	2018 (218) n (%)	2019 (125) n (%)	2020 (82) n (%)	2021 (157) n (%)	2022 (197) n (%)	2018 (70) n (%)	2019 (94) n (%)	2020 (26) n (%)	2021 (45) n (%)	2022 (69) n (%)
Ampicillin	6 (2.8)	8 (6.2)	0 (0.0)	5 (3.1)	1 (0.7)	21 (30.0)	3 (3.4)	1 (3.9)	1 (3.1)	0 (0.0)

Cotrimoxazole	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Chloramphenicol	4 (1.9)	1 (0.8)	1 (1.7)	3 (2.1)	1 (0.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Azithromycin	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Ciprofloxacin	124 (56.8)	85 (68.0)	68 (82.9)	116 (73.8)	172 (87.3)	70 (100.0)	93 (98.9)	26 (100)	45 (100.0)	69 (100.0)
Ceftriaxone	0 (0.0)	4 (3.2)	0 (0.0)	1 (0.7)	0 (0.0)	1 (1.4)	1 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)

Table 3.Minimum Inhibitory Concentration of *Salmonella* spp. to Ceftriaxone

Isolates	MIC Breakpoint (µg/ml)						
	1	2	4	8	16	32	64
2018 S. Typhi (n = 218) S. Paratyphi (n = 70)	218 (100.0) 69 (98.5)	0 (0.0) 0 (0.0)	0 (0.0) 0 (0.0)	0 (0.0) 0 (0.0)	0 (0.0) 0 (0.0)	0 (0.0) 0 (0.0)	0 (0.0) 1 (1.4)
2019 S. Typhi (n = 125) S. Paratyphi (n = 94)	121 (96.8) 91 (96.8)	0 (0.0) 2 (2.1)	0 (0.0) 0 (0.0)	0 (0.0) 0 (0.0)	1 (0.8) 0 (0.0)	0 (0.0) 1 (1.7)	3 (2.4) 0 (0.0)
2020 S. Typhi (n = 82) S. Paratyphi (n = 26)	82 (100.0) 26 (26.0)	0 (0.0) 0 (0.0)	0 (0.0) 0 (0.0)	0 (0.0) 0 (0.0)	0 (0.0) 0 (0.0)	0 (0.0) 0 (0.0)	0 (0.0) 0 (0.0)
2021 S. Typhi (n = 157) S. Paratyphi (n = 45)	155 (98.7) 45 (100.0)	1 (0.6) 0 (0.0)	0 (0.0) 0 (0.0)	0 (0.0) 0 (0.0)	0 (0.0) 0 (0.0)	0 (0.0) 0 (0.0)	1 (0.6) 0 (0.0)
2022 S. Typhi (n = 197) S. Paratyphi (n = 69)	197 (100.0) 69 (69.0)	0 (0.0) 0 (0.0)	0 (0.0) 0 (0.0)	0 (0.0) 0 (0.0)	0 (0.0) 0 (0.0)	0 (0.0) 0 (0.0)	0 (0.0) 0 (0.0)

Discussion

Enteric fever is a public health issue, especially for underdeveloped countries where the availability of clean potable water to a large part of the population is a matter of concern. The problem is further increased by the growing antimicrobial resistance and diminishing antimicrobial armamentarium amongst *Salmonella enterica* serovar Typhi/ Paratyphi. As a result, the management of the disease has become problematic. In our study, the positivity of blood culture for *Salmonella enterica* varied from 0.7% to 1.3% while in a study conducted by Biswas et al., the positivity varied from 0.4% to 1.7% (2017–2022).[15] In concordance with other studies, S. Typhi was found to be the leading isolate in comparison to S. Paratyphi A.[16,17] In our study, males (64%) were predominantly infected as compared to females (36%), which is corroborated by a study conducted by Biswas et al., in which 66.6% of the enteric fever patients were males,[15] whereas in contrast, only 47.7% males were involved in a study conducted in South India[18].

In the recent past, the drugs of choice for the treatment of enteric fever were fluoroquinolones and third-generation cephalosporins due to the global emergence of resistance to ampicillin, cotrimoxazole, and chloramphenicol, over a period of two decades. However, the effectiveness of ciprofloxacin as an empirical option for the treatment of enteric fever has been questioned because of multiple reports from various parts of the world, reporting *S. enterica* serovar Typhi/ Paratyphi isolates with decreased ciprofloxacin susceptibility leading to treatment failure.[4,5,19] In the present study, resistance to ciprofloxacin during 2018–2022 varied from 56.8% to 87.3% and from 99% to

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100% in S. Typhi and S. Paratyphi A, respectively. Various authors reported resistance to ciprofloxacin in S. Typhi isolates (13.6%,[20] 67.3%,[11] 68.5%,[21] 80%,[15] and 100%[22]) and S. Paratyphi A (11%,[22] 82.6%,[21] 90%,[15] and 97.6%[11]). A report from the United Kingdom has also shown an increase in the incidence of *S. enterica* serovar Typhi isolates with decreased ciprofloxacin susceptibility (from 0.9% to 33%) in a span of just 8 years.[23] Further, several failures of clinical treatment of typhoid patients with ciprofloxacin and other fluoroquinolones were also a matter of concern.[23–25] Non-judicious prescription and usage of fluoroquinolones, not only for the treatment of enteric fever but other infections as well, may be directly correlated with the resurgence of resistance to these drugs. In contrast, the removal of adaptive pressure ascribed to the limited use of chloramphenicol has been linked to the re-emergence of its susceptibility.[26,27] Decreased resistance to chloramphenicol and cotrimoxazole in the present study may be attributable to the above-said fact.

In contrast to the reports from Gupta et al.[20] and Malini et al.,[22] the current study showed a variable resistance to ceftriaxone against S. Typhi and S. Paratyphi A, from 0.7% to 3.2% and 1.1% to 1.4%, respectively. A few studies reported none of the isolates to be resistant to ceftriaxone.[20,22] However, the emergence of ceftriaxone resistance, though a very small proportion (0.08%) has been reported in one of the studies done in Karachi during the year 2010–11.[28] Other studies conducted by Taneja et al.[21] and Kaira[29] have reported ceftriaxone resistance to S. Typhi (12.3% and 20%, respectively). On the other hand, they reported 34.7% and 22.0% ceftriaxone resistance against S. Paratyphi in their studies, respectively. The highest MIC of ceftriaxone was found to be 64 μg/ml for both S. Typhi and Paratyphi A, similar to those reported in the literature.[29]

Azithromycin, belonging to the macrolide class, is another drug which is highly effective for the treatment of enteric fever in all age groups and can be conveniently administered by oral route. In our study, 100% of the isolates were susceptible to azithromycin, similar to a few other reports from different parts of the country.[20,22] However, this is in contradiction to the reports published by Taneja et al.[21] They reported 28% of S. Typhi and 21.7% of S. Paratyphi A isolates to be resistant to azithromycin. Nonetheless, Dutta et al. from Kolkata also reported 28.1% and 21.8% of S. Typhi and S. Paratyphi A isolates, respectively, to be resistant to azithromycin.[30]

In our study, S. Typhi isolates showed resistance to ampicillin (0%–6.2%), cotrimoxazole (0.0%) and chloramphenicol (0.8%–2.1%). Various authors reported resistance to ampicillin (34.1%–100%,[20] 0.0%,[15,22] and 23.1%[21]), cotrimoxazole (0%–9.7%,[20] 0.0%,[15,22] 24.1%[21]) and chloramphenicol (0%,[15] 2.4%,[20] 11.0%,[22] and 25.6%[21]).

In our study, S. Paratyphi isolates showed resistance to ampicillin (0%–30%), cotrimoxazole (1.5%) and chloramphenicol (0.0%). Resistance to ampicillin, cotrimoxazole, and chloramphenicol was reported by Gupta et al.[20] (36.4%–62.5%, 0%–11.1%, and 5.5%, respectively), Malini et al.[22] (9.0%, 11.0%, and 3.0%, respectively) and Taneja et al.[21] (21.4%, 4.3%, and 17.3%, respectively), whereas 100% susceptibility was observed by Biswas et al.[15]

Conclusion

The incidence of fluoroquinolone resistance among S. Typhi and S. Paratyphi A isolates is very high in the present study with a risk for the emergence of ceftriaxone resistance. However, there is a re-emergence of increased susceptibility to first-line medications. Therefore, it is empirical to continuously monitor and analyse the resistance profile of *Salmonella* species for the rationalisation of treatment protocols.

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