

## Antimicrobial Susceptibility Pattern of *Salmonella enterica* Species in Blood Culture Isolates

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### Abstract

**Introduction:** Enteric fever continues to be a major health problem in under developed countries including south Asian nations. In this study, we evaluate the prevalence and susceptibility pattern of *Salmonella enterica* (Serotype typhi, Paratyphi A and Paratyphi B).

**Methods:** Blood samples were obtained from 3210 patients, suspected with enteric fever. The sample was processed on BACTEC 9050 and isolates obtained from subculture were serotyped and antibiotic susceptibility testing was carried out using disk diffusion (Kirby–Bauer).

**Result:** Out of 3120 samples 370 isolates of *S. enterica* were isolated. The prevalence of *Salmonella enterica* was 11.8 % where 78.4% of these isolates were *S. enterica* serotype Typhi, 20.8% were *S. enterica* serotype Paratyphi A and 0.8% were *S. enterica* serotype Paratyphi B. The isolates demonstrated poor susceptibility to oral antibiotics including Nalidixic acid, Ciprofloxacin, Ofloxacin, Azithromycin, Amoxicillin, Tetracycline, Ceftriaxone and Trimethoprim–sulfamethoxazole whereas all of the isolates of *S. enterica* demonstrated 100% susceptibility to Chloramphenicol.

**Conclusion:** There was greater prevalence of *Salmonella enterica* serotype typhi isolates resistant to fluoroquinolones (Nalidixic acid, Ofloxacin and Ciprofloxacin). However Chloramphenicol was sensitive to all isolates. This study suggests Chloramphenicol as a drug of choice for enteric fever and further monitoring of efficacy of older and newer antibiotics are desirable.

**Keywords:** *Salmonella enterica*, Blood culture, Multi Drug resistant, Antibiotics Susceptibility

### Introduction

Enteric fever continues to be a major health problem in under developed countries including South Asian nations. It afflicts local inhabitants as well as travelers to endemic areas. Increasing multidrug resistance in *Salmonella enterica* serotype Typhi has been reported from various parts of the world [1-5]. Enteric fever is endemic in Nepal. *S. enterica* serotype Typhi and *S. enterica* serotype Paratyphi A have been reported as the most common culture isolates from patients with febrile illnesses needing hospital admission [6,7]. Over the past decade, increasing antibiotic resistance in *S. enterica* has led to a shift in the antibiotics used against this organism from chloramphenicol and ampicillin to trimethoprim–sulfamethoxazole, fluoroquinolones (ofloxacin, ciprofloxacin), and ceftriaxone. Even with the use of these antibiotics, the positive response to treatment has been only seen in the range of 16-40% in Nepal [7].

Over the last decade, fluoroquinolones have emerged as the mainstay of therapy for enteric fever. At the same time, increasing incidence of infection with *Salmonellae* resistant to nalidixic acid, which usually display decreased susceptibility to fluoroquinolones, has raised considerable global concern [8]. The vast majority of nalidixic acid resistant isolates remains within the current susceptibility range for ciprofloxacin (1 µg/ml) as recommended by the National Committee for Clinical Laboratory Standards (NCCLS). However, the probability of clinical response to fluoroquinolone therapy in patients with invasive *Salmonella* infection is lower in those with Nalidixic acid resistant than with susceptible isolates [9]. In 1993, 23% of *Salmonella enterica* serotype Typhi isolates from patients in the United Kingdom exhibited decreased susceptibility to ciprofloxacin more than half of these were also resistant to Chloramphenicol, Ampicillin and Trimethoprim. Increasing numbers of treatment failures were noted. Most infections were noted in patients with a recent history of travel to India and Pakistan, Nepal, Sri Lanka, Bangladesh, and Thailand [10].

### Materials and Methods

The study was conducted from February to August 2013, at Civil Service Hospital at Kathmandu, Nepal. Blood samples were obtained from patients suspected with enteric fever. The samples to be tested were inoculated in Bactec® culture bottle vial with soybean- casein digested broth which was inserted into the BACTEC 9050® for incubation and periodic reading. Each culture vial contains a chemical sensor which can detect CO<sub>2</sub> produced by the growth of microorganism. The sensor was monitored by the instrument every 10 minutes for an increase in its fluorescence, which was proportional to the amount of present. A positive reading indicated the presumptive presence of viable microorganism in the vial [11]. From the positive vial subculture was done on Blood agar and MacConkey agar, Identification of bacteria was done using standard microbiological techniques [12-14]. Serotyping of *Salmonella enterica* was done by using polyvalent O-antisera (Denka Seiken, Japan), *Salmonella* 9-O, *Salmonella* Vi (Remel Europe, UK). Antimicrobial susceptibility for *Salmonella enterica* was performed by Kirby- Bauer disk diffusion method on Mueller Hinton Agar following National Committee for Clinical Laboratory Standards (NCCLS) recommendations [12-15]. The antibiotics tested were: Amoxicillin (10 µg) and Azithromycin (15 µg) with zone of inhibition

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(ZOI)≥18 mm, Ceftriaxone (30 µg) and Ciprofloxacin (5 µg) ZOI≥21 mm, Chloramphenicol (30 µg) ZOI≥18 mm, Co-trimoxazole (25 µg) ZOI≥mm, Nalidixic acid (30 µg) ZOI≥19 mm and Tetracycline (30 µg) ZOI ≥15 mm (Hi Media Laboratory Ltd., Mumbai, India) [15]. The disk strength and zone-size interpretation were in accordance with the National Committee for Clinical Laboratory Standards (NCCLS) [15]. MDR was categorized if they were resistant to at least two classes of first-line agents including Amoxicillin, Chloramphenicol, Trimethoprim-sulfamethoxazole, Fluoroquinolones (Ciprofloxacin and Ofloxacin), and Cephalosporins (Ceftriaxone).

Statistical comparisons of prevalence rates between the two serotypes and differences in resistance rates against the antibiotics were done by Fisher's exact tests and Chi-square tests and Fisher's exact test (where the sample size was less than 5) using SPSS software version 22.0 (SPSS Inc., Chicago, USA).

## Result

Out of 3120 samples 370 isolates of *S. enterica* were isolated between

February to August 2013, at Civil Service Hospital Kathmandu. Among the blood cultures obtained from 3120 patients, 11.8 % were positive for bacterial growth of *S. enterica*. Serotyping showed that 290 (78.4%) of these isolates were *S. enteric* serotype Typhi, 77 isolates (20.8%) were *S. enteric* serotype Paratyphi A and 3 isolates (0.8%) were *S. enteric* serotype Paratyphi B. (p=0.000)

Out of 370 isolates, (Table 1) 143 (38.6%) isolates of *Salmonella enterica* serotype Typhi were sensitive to all antibiotics, 44 (11.8%) isolates of *Salmonella enterica* serotype Paratyphi A and 2 (0.54%) isolates *Salmonella enterica* serotype paratyphi B were sensitive to all antibiotics. Total 181 (48.91%) isolates were resistant to two or more drugs.

Further, 35.5% of MDR *Salmonella enterica* serotype typhi were combinely resistant to fluoroquinolones (Nalidixic acid, Ofloxacin and Ciprofloxacin) furthermore 18.7% isolates were resistant to the combination of Nalidixic acid and Ciprofloxacin whereas only 2.2% isolates were resistant to the combination of Nalidixic acid and Ofloxacin (Table 2).

Antibiotics	Serotype typhi			Serotype Paratyphi A			Serotype Paratyphi B			P value
	R	I	S	R	I	S	R	I	S	
Amoxicillin	16	8	266	11	1	65	1	0	2	0.041
Azithromycin	28	32	230	29	30	45	0	0	3	0
<b>Ceftriaxone</b>	<b>1</b>	<b>2</b>	<b>287</b>	3	0	74	0	0	3	0.102
<b>Ciprofloxacin</b>	122	49	119	3	20	54	0	1	2	<b>0</b>
Trimethoprim-sulfamethoxazole	3	1	286	2	0	75	0	0	3	0.84
Chloramphenicol	0	0	290	0	0	77	0	0	3	-
Ofloxacin	87	33	170	2	0	75	0	0	3	0
Nalidixic_acid	240	0	50	60	0	17	3	0	0	0.443
Tetracycline	0	4	286	0	0	77	0	0	3	0.572

S, susceptible; I, intermediately susceptible; R, resistant

**Table 1:** Antibiotic susceptibilities of *Salmonella enteric* serotype by Kirby-Bauer method.

S.N.	MultiDrug Resistant	Typhi	Paratyphi A	Paratyphi B	Total
1	Non	143	44	2	189
2	<b>Azithromycin+Nalidixic acid</b>	<b>13</b>	<b>22</b>	<b>0</b>	<b>35</b>
3	Ciprofloxacin+Ofloxacin+Nalidixic acid	68	0	0	68
4	Azithromycin+Ciprofloxacin+Ofloxacin+Nalidixic acid	6	0	0	6
5	Amoxicillin+Nalidixic acid	3	6	1	10
6	Amoxicillin+Azithromycin+Ceftriaxone+Nalidixic acid	1	0	0	1
7	Amoxicillin+Azithromycin+Nalidixic acid	0	1	0	1
8	Ofloxacin+Nalidixic acid	4	0	0	4
9	Amoxicillin+Azithromycin+Ceftriaxone+Ciprofloxacin+Ofloxacin+Nalidixic acid	0	1	0	1
10	Ciprofloxacin+Nalidixic acid	34	0	0	34
11	Amoxicillin+Ceftriaxone+Ciprofloxacin+Trimethoprim+Nalidixic acid	0	1	0	1
12	Amoxicillin+Ceftriaxone+Trimethoprim	0	1	0	1
13	Trimethoprim+Ofloxacin+Nalidixic acid	1	0	0	1
14	Azithromycin+Ciprofloxacin+Nalidixic acid	1	0	0	1
15	Azithromycin+Ofloxacin+Nalidixic acid	1	0	0	1
16	Amoxicillin+Ciprofloxacin+Nalidixic acid	4	0	0	4
17	Amoxicillin+Ciprofloxacin+Ofloxacin+Nalidixic acid	4	1	0	5
18	Azithromycin+Ciprofloxacin	1	0	0	1
19	Ciprofloxacin+Ofloxacin	3	0	0	3
20	Amoxicillin+Ofloxacin+Nalidixic acid	1	0	0	1
21	Amoxicillin+Ciprofloxacin+Trimethoprim+Ofloxacin+Nalidixic acid	1	0	0	1
22	Trimethoprim+Nalidixic acid	1	0	0	1
	Total	290	77	3	370

Pearson Chi-Square=109.564, P value =0.000

**Table 2:** Susceptibilities of MDR *Salmonella enteric* serotype.

In the other hand 7.18% of *Salmonella enterica* serotype typhi isolates were resistant to Azithromycin and Nalidixic acid whereas in case of *Salmonella enterica* serotype paratyphi A, the resistant pattern has increased up to 11.7%.

## Discussion

The result of this study showed that a prevalence rate of 11.8% for the *S. enteric* in the study area, a similar trend has been reported in a recent study done in Nepal [15]. Chloramphenicol, Ampicillin, and Trimethoprim-sulfamethoxazole were the antibiotics widely used as the primary for the treatment of treatment of enteric fever. In our study 48% of isolates were resistant to two or more drugs where 81.2% were *S. enteric* serotypes Typhi and 18.2% were Paratyphi A and only 0.5% were Paratyphi B (Table 3). All of these multidrug resistant isolates showed reduced susceptibility to fluoroquinolones (Table 2). A similar results show a high sensitivity of both *Salmonella enterica* serovar typhi (96%) and *Salmonella enterica* serovar paratyphi A (100%) to chloramphenicol. Sensitivity to ciprofloxacin and amikacin was 88% and 84% respectively (Table 1). Sensitivity of *Salmonella enterica* serovar paratyphi A was 100% to Chloramphenicol, Ciprofloxacin, Ofloxacin, Nalidixic acid and Ceftriaxone, 95% to Amikacin and 30% to ampicillin [16]. Our result (Table 1 and 4) showed different comparable view (i.e. Chloramphenicol 100% susceptibility) than that of others studies reporting Chloramphenicol resistant in *S. enterica* [17-19]. In 1970s the isolates resistant to Chloramphenicol were reported in various countries like UK, India, Mexico, Greece, Israel, and Pakistan [2,4]. While In the 1990s, the patten showing resistance of Chloramphenicol suggested to the use of fluoroquinolones (Ciprofloxacin and Ofloxacin) [20], which were found to be highly resistant in this study which accounts as 40%. Similarly a different view was reported on Pakistan reporting *Salmonella enteric* serotype paratyphi-A were sensitive to Ciprofloxacin, Ofloxacin and Nalidixic acid and 62.7% isolates were resistant to first-line antibiotics (Ampicillin, Chloramphenicol and Co-Trimoxazole) on disc diffusion testing [21]. According to Thonget *et al.* [5] The MDR *S. typhi* isolates were resistant to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole. Analysis by PFGE showed that 50 MDR isolates of *S. typhi* had a single, homogenous PFGE profile, which was distinctly different from that of 50 antibiotic-sensitive isolates obtained in the same time frame from the same area [22].

Organism	N	Percent	Chi-Square	P Value
<i>Salmonella enterica</i> serotype Typhi	290	78.37	360.038	0
<i>Salmonella enterica</i> serotype Paratyphi A	77	20.81		
<i>Salmonella enterica</i> serotype Paratyphi B	3	0.81		
Total	370	100		

Table 3: Frequency of *Salmonella enteric* serotypes.

Antibiotic	Resistant	Intermidate	Sensitive
Amoxycillin	28 (7.6%)	9 (2.4%)	333 (90%)
Azithromycin	57 (15.4%)	35 (9.5%)	278 (75.1%)
Ceftriaxone	4 (1.1%)	2 (0.5%)	364 (98.4%)
Ciprofloxacin	125 (33.8%)	70 (18.9%)	175 (47.3%)
Trimethoprim-sulfamethoxazole	5 (1.4%)	1 (0.3%)	364 (98.4%)
Chloramphenicol	0	0	370 (100%)
Ofloxacin	89 (24.1%)	33 (8.9%)	248 (67%)
Nalidixic acid	303 (81.9%)	0	67 (18.1%)
Tetracycline	0	4 (1.1%)	366 (98.9%)

Table 4: Antibiotic susceptibility.

## Conclusion

Our study has further accentuated concern about the sensitivity pattern and the status of multi drug resistant *Salmonella enterica* spp. This study suggests Chloramphenicol as a drug of choice for enteric fever and further monitoring of efficacy of older and newer antibiotics are desirable.

## Authors' Contributions

**Sunil Poudel:** Sample collection, Identification and Antibiotic Susceptibility Testing, Monitoring and Evaluation, Literature Review, Data analysis, Manuscript preparation and Submission.

**Saroj Kumar Shrestha:** Identification and Antibiotic Susceptibility Testing, Monitoring, Evaluation, Literature and Review

**Ashish Pradhan:** Sample collection, Identification and Antibiotic Susceptibility Testing, Monitoring and Evaluation, Literature Review and Manuscript preparation

**Dr. Binaya Sapkota:** Literature Review, Data analysis and Manuscript preparation

**Manoj Mahato:** Sample collection, Identification and Literature review

All authors read and approved the final manuscript.

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