

# Antimicrobial Sensitivity Pattern of *Salmonella* spp. Clinical Isolates in Blood Culture: A Tertiary Center Study

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**Conflict of Interest:** None

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### Abstract:

**Objective:** Bangladesh is an endemic area for enteric fever. Adequate and timely antimicrobial treatment invariably cures this disease. But resistant strains of *Salmonella* spp. have made it difficult to treat nowadays. This study will help clinicians in understanding the local resistance pattern of enteric fever and identifying recent changes in the trends of the sensitivity pattern of commonly used antibiotics in Bangladesh.

**Materials and Methods:** This cross-sectional study was conducted during the period from January 2016 to June 2019 in Sheikh Hasina Medical College, Tangail, to determine the antimicrobial sensitivity patterns of *Salmonella typhi* and *paratyphi* isolated by blood culture from clinically suspected typhoid fever patients. The blood sample was processed on BACTEC 9050 and isolates obtained from subculture were serotyped. Then antibiotic susceptibility testing was carried out using the disk diffusion method.

**Results and Discussion:** A total of 137 cases were recorded in four years. All cephalosporins were found sensitive in more than 90% of cases. We did not find any resistance to Ceftriaxone in our patients' group. Moxifloxacin and Levofloxacin showed higher sensitivity than Ciprofloxacin (100% and 96% Vs 90%). Nalidixic acid demonstrated lower sensitivity than previously reported (less than 5%), while Gentamicin showed the highest sensitivity around 100%.

**Conclusion:** Multidrug-resistant (MDR) *Salmonella typhi* and *paratyphi* are emerging rapidly. To overcome this global issue, rational use of antibiotics has to be ensured. Physicians should also be aware of the local resistance pattern so that they can treat their patients effectively.

### Key Words:

*Salmonella typhi*, Antibiotic, Resistance, Sensitivity

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### Introduction

Typhoid and paratyphoid, collectively known as enteric fever, are life-threatening infectious diseases caused by *Salmonella enterica* serovars *typhi* and *paratyphi* (A, B and C). Although the mortality rate remains low (1%) worldwide, approximately 17 million people are infected

every year, and 136,000 deaths occur annually by these pathogens<sup>1</sup>. These are the most common bacterial causes of morbidity worldwide, and are endemic in tropical countries, especially in Africa, South and Southeast Asia including Bangladesh<sup>2</sup>. Multidrug-resistant bacterial strains happen to be one of the major health problems in Bangladesh<sup>3</sup>. Specially for the children, it is an epidemic. Typhoid fever colonizes only humans and is transmitted through the fecal-oral route<sup>4</sup>. Ample, well timed antimicrobial treatment dependably cures typhoid fever. However, the treatment options are severely getting limited day by day owing to the sharp rise in the antimicrobial resistance of *S. typhi* and *paratyphi*. Specific antimicrobial treatment of patients with enteric fever depends on an understanding of local resistance patterns to antimicrobials. This resistance pattern is known from the results of antimicrobial susceptibility testing. Drug-resistant variants are evolving. So, clinicians need to be

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aware of the patients who are getting infected with resistant *Salmonella* variants and who will not respond adequately. In this study, we tried to observe the recent changes in the trends of the sensitivity pattern of the commonly used antibiotics in Bangladesh. The results of this study will help physicians decide which antibiotics are to be chosen to treat this disease. Moreover, identifying the changing resistance patterns will help us in formulating future directives to prevent further dissemination of antimicrobial resistance.

### Materials and Methods

This cross-sectional study was carried out from January 2016 to June 2019 in Sheikh Hasina Medical College, Tangail, Bangladesh. Patients of different age and sex provisionally diagnosed as having typhoid (enteric) fever were asked to undergo blood culture test. Only the culture-positive cases for *Salmonella spp.* were included in this study.

### Isolation and Identification of *Salmonella spp.*:

With all aseptic precautions, 5 ml of peripheral blood from adults and 2 ml from pediatric patients were collected. Blood samples were collected in the FAN (Fast Antibiotic Neutralizer) bottles according to WHO guidelines. After collection, the bottles were put in the Bactec machine, where it was incubated at 37 °C and agitated continuously. In the case of unloading a positive bottle when there was any growth, both the machine and the computer would indicate the growth by alarm message on the computer screen. All alarm-positive samples were inoculated on MacConkey agar and Blood agar media and incubated overnight at 37 °C. Then the isolates were identified by colony characteristics, Gram stain reaction, and biochemical reaction such as catalase, oxidase, Motility Indole Urease (MIU) test, Triple sugar Iron (TSI) test, Simmons Citrate utilization test, methyl red test, Voges Proskauer test. The isolates of *Salmonella* were further confirmed by serotyping through agglutination with polyvalent O antiserum.

### Determination of antibiotic susceptibility of *Salmonella* isolates:

Susceptibility to antimicrobial agents of all isolates was done by Kirby-Bauer modified disk diffusion technique<sup>5</sup>. The isolated colony from various media was inoculated on Mueller- Hinton Agar media by spreading technique. The zone of inhibition produced by each drug was considered into two susceptibility categories, namely sensitive (S) and resistant (R), using the National Committee for Clinical Laboratory Standards (NCCLS) 2006. Each plate was examined after 24 hours of incubation at 37°C. The antimicrobial discs were used according to the standard antibiotic panel for specific

samples and isolated organisms. Antibiotic discs were obtained from commercial sources (Oxoid, UK). The isolated organisms were tested against ampicillin (10 µg), tetracycline (30 µg), cotrimoxazole (25 µg), nalidixic acid (30 µg), ceftriaxone (30 µg), erythromycin (15 µg), ciprofloxacin (5 µg), Gentamycin (30 µg), Cefixime (5 µg), Chloramphenicol (30 µg), Moxifloxacin (5 µg), Levofloxacin (5 µg), Cefipime (30 µg).

Only culture-positive enteric fever patients were validated and included in the study.

### Ethical clearance:

After the Ethical Review Committee's approval, the study was performed. Informed consent was taken before the collection of blood from the patients.

### Results

A total of 137 cases were recorded in four years. Cases having positive blood cultures for *Salmonella typhi* and *paratyphi* were covered in the study. A maximum of 52 cases was included in 2018, while the lowest number of documented cases was 12 in 2019 (Figure 1).

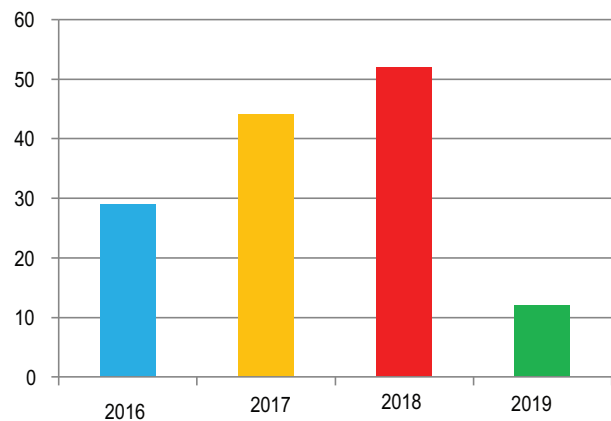


Fig.-1: Case recorded in different years

Out of 137 patients, 101 patients were male, and 36 were female (73% vs 27%) (Figure 2).

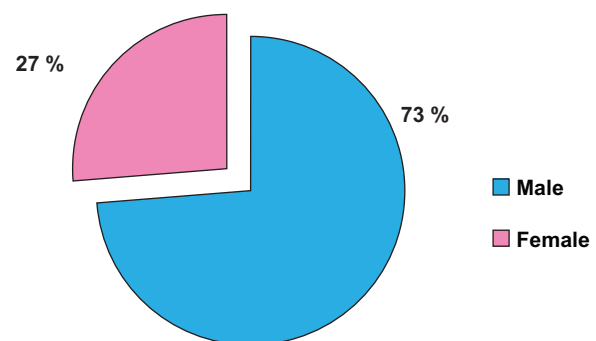


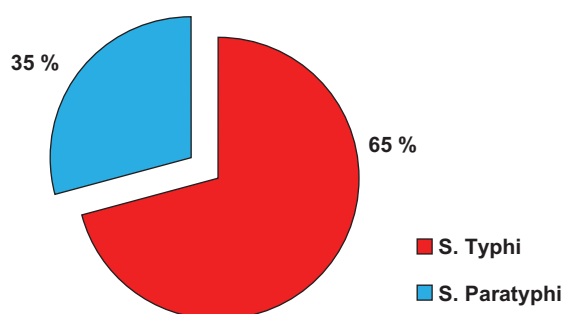
Fig.-2: Sex distribution of patients

Eighty-eight patients were aged between 10-30 years (Table 1). Mean age of the patients was 21.6 years with a standard deviation of 12.29.

**Table 1**

<i>Age group of the affected patients</i>	
Age Group	Affected Patients
<9	21
10-19	44
20-29	44
30-39	17
40-49	7
>50	4
Total	137

97 patients were infected by *S. Typhi*, while 40 patients were infected by *S. Paratyphi* (65% vs 35%) (Figure 3).

**Fig.-3: Distribution of Infecting Organisms**

The sensitivity of fourteen commonly prescribed and available antibiotics was assessed. In 2016, the Cephalosporin group had shown a higher sensitivity pattern than other groups of antibiotics. All Cephalosporines were sensitive in more than 90% of the cases, except Amoxicillin (about 80% sensitive). The Macrolide group exhibited a variable sensitive pattern, e.g. Erythromycin vs Azithromycin (less than 10% vs 70%). Among Quinolones, Moxifloxacin and Levofloxacin showed higher sensitivity than Ciprofloxacin (100% and 96% vs 90%). And Cotrimoxazole and Chloramphenicol showed moderate sensitivity of about 70%. Nalidixic acid was found with the lowest sensitivity (less than 5%), while Gentamicin showed highest sensitivity around 100%. (Table II).

**Table-II**

Antibiotics	<i>Sensitivity pattern of Antibiotic in different years</i>			
	2016	2017	2018	2019
Amoxicillin	82	80	83	91
Cephadrine	100	97	95	70
Ceftriaxone	100	100	100	100
Cefixime	100	97	96	83
Cefepime	96	100	93	100
Cefuroxime	100	97	98	80
Cotrimoxazole	75	88	92	91
Nalidixic Acid	10	0	2	9
Azithromycin	74	59	52	83
Moxifloxacin	96	97	90	100
Ciprofloxacin	96	97	90	100
Levofloxacin	100	95	93	100
Chloramphenicol	72	84	94	100
Gentamicin	100	100	100	100

Sensitivity pattern of Cephalosporin group was almost similar in 2016, 2017, and 2018. But it was different in 2019. The sensitivity of Cephadrine and Cefixime also declined in 2019. In 2019, they were less than 80% sensitive compared to more than 90% sensitivity before 2019. Azithromycin sensitivity was slightly higher in 2019 compared to 2017 and 2018 (> 80% vs <60%). The sensitivities of Nalidixic acid, Cotrimoxazole, and Quinolones were almost similar in all four years. Chloramphenicol sensitivity increased in between 2016 and 2019 (around 70% vs 80% vs 90% vs 100%). (Table II).

In 2016 and 2018, cases were distributed throughout the months. In 2017, more cases were clustered in later months of the year (September to December).

### Discussion

137 patients were included in the study from January 2016 to June 2019. Most were young adult and children aged between 10 to 30 years (n=88). All Patients were culture-positive for Enteric fever and most of the cases were *S. Typhi* positive (n=97). The sensitivities of different commonly prescribed antibiotics were assessed. Cephalosporin group was found to have the highest sensitivity, while Macrolides (Azithromycin) were found less sensitive than they were thought to be. Moreover, Nalidixic acid was found highly resistant. In a 2007 study from Bangladesh, Nalidixic acid resistance was observed

in 81.6% of the isolates<sup>6</sup>, but it became almost 100% in our study. This finding indicates that more resistant organisms are emerging now.

Azithromycin was formerly chosen to treat MDR enteric fever. But our study shows that its sensitivity is on the decline. It was sensitive in 74%, 59%, and 52% of the cases in 2016, 2017, and 2018 respectively. On the other hand, Ciprofloxacin, which was considered resistant in our clinical setting, was found a better drug to treat enteric fever in this trial. Ciprofloxacin was found sensitive in 96%, 97%, and 90% of the cases in 2016, 2017, and 2018 respectively. These findings are not consistent with the previous report where Ciprofloxacin resistance was observed in 39.5% isolates from Bangladesh<sup>6</sup>. Drugs like Levofloxacin and Moxifloxacin, which we did not clinically recognize as first line treatment in case of Enteric fever, also showed a higher sensitivity pattern. Levofloxacin was sensitive in 100%, 95%, and 93% of the cases, while Moxifloxacin was sensitive in 96%, 97%, and 90% of the cases in 2016, 2017, and 2018 respectively. In this trial, typhoidal *Salmonella* showed no resistance to Aminoglycoside group (Gentamicin). We think it might be a good choice in treating future resistant pathogens.

When we compared sensitivity patterns from different years, we found that sensitivities of Cephadrine, Cefixime, and Cefuroxime decreased gradually from 2016 to 2018, although by a negligible percentage. There have been two reports of Ceftriaxone-resistant *S. Typhi* from Bangladesh to date<sup>7,8</sup>. But we did not find any resistance to Ceftriaxone in our patients' group. Sensitivities of Macrolides and Quinolones were also lower in 2018 compared to that of 2016. On the other hand, sensitivities of Cotrimoxazole and Chloramphenicol gradually increased from 2016 to 2018.

### Conclusion

Antimicrobials are the mainstays of therapy for typhoid patients. However, the intensive use of first-line antimicrobials has led to the emergence and global spread of MDR *S. Typhi* strains. This study reveals that

Levofloxacin and Moxifloxacin have become more sensitive against *Salmonella* spp. than before, and Cephalosporines and Gentamicin are the most effective drugs. The burden of typhoid fever can be substantially overcome by appropriate diagnosis and rational antibiotic use.

### Limitations

The total number of patients is too small to draw a conclusion about the sensitivity pattern. Moreover, it is not a multicenter trial. A multicenter trial with a large number of patients may overcome these limitations.

### Conflict of Interest

The author has declared no competing interest.

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