



Antimicrobial Susceptibility Pattern of Typhoidal *Salmonella* species in Chittagong Medical College Hospital

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Abstract

Background: Typhoid and paratyphoid fevers result from infections from *Salmonella enterica* serovar Typhi and Paratyphi, and the increasing prevalence of antibiotic resistance to these pathogens has raised concerns about incurable infections. It is a serious public health issue in underdeveloped nations. The widespread development of antibiotic resistance is complicating the treatment of enteric fever. **Objectives:** The primary objective of this study was to determine the antimicrobial susceptibility patterns of *Salmonella enterica* serovar Typhi and Paratyphi in clinically suspected enteric fever patients at Chittagong Medical College Hospital. **Methodology:** This cross-sectional study was conducted from July 2023 to June 2024 at the Microbiology laboratory, Chittagong Medical College. A total of 220 clinically suspected enteric fever patients from both the inpatient and outpatient departments of Paediatric and Medicine were enrolled in this study. Blood culture was carried out by the automated method. The isolates of *Salmonella enterica* serovar Typhi and Paratyphi were obtained from blood by culture, Gram staining, relevant biochemical tests and using specific antisera. The antimicrobial susceptibility test was done by the Modified Kirby-Bauer disc diffusion method as per the CLSI guideline of 2024. Levofloxacin sensitivity was detected by MIC as per the CLSI guideline. **Results:** The isolation rate of *Salmonella spp.* from blood was 14.5 %, *Salmonella enterica* serovar Typhi was 14.05%, and Paratyphi A was 0.45%. The isolates showed 49% resistance to azithromycin, 46% to ciprofloxacin, 35% to ampicillin, 25% to chloramphenicol, 22% to cotrimoxazole and 3% to tetracycline and 2% resistant to levofloxacin. No resistance was detected in ceftriaxone and meropenem. MDR strain was detected in 12.5% isolates. The data were analysed by SPSS 29 and the results were summarized by using graphs and tables. **Conclusion:** In vitro susceptibility pattern of *Salmonella spp.* showed the highest sensitivity against ceftriaxone and meropenem, followed by levofloxacin, tetracycline, chloramphenicol and cotrimoxazole, whereas azithromycin resistance was highest.

Keywords: Antimicrobial resistance; Blood culture; Enteric fever; *Salmonella enterica* serovar Typhi; Typhoid fever.

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Introduction

Enteric fever, also known as typhoid and paratyphoid fever, is caused by *Salmonella enterica* serovar Typhi

and Paratyphi A, B, and C¹. This is a common bacterial infection that results in significant death and illness in low to middle-income nations across Asia, Africa and South America. It is directly associated with sanitation, sewage and water treatment systems². South Asia accounted for 69.6% of all deaths worldwide in 2017, with the highest death rates and total number of deaths worldwide due to enteric fever³. WHO estimates up to 9 million cases of enteric fever globally with a mortality rate of 1,10,000 deaths each year worldwide⁴.

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Bangladesh is an endemic country for enteric fever, with an incidence of 252 per 100000 person⁵.

Antibiotics serve as the mainstay for the treatment of enteric fever. When the appropriate antibiotics are used, the death rate is reduced from 25.0% to 1.0% cases⁶. Drug-resistant enteric fever poses an increasing threat to public health. The escalating incidence of antibiotic-resistant strains of enteric fever has instigated concerns regarding the possibility of untreatable infections, thereby restricting available treatment alternatives. Irrational antibiotic prescribing, over-the-counter drugs and indiscriminate antibiotic use in farming are also responsible for antimicrobial resistance in enteric fever, which obstructs the efficacious treatment, prolonging the fever duration and placing those who could have additional health issues^{7,8}. Failure to treat an infection properly leads to prolonged illness, thus increasing the chance of developing a carrier state in which persons are contagious and able to spread the resistant strain to others⁹. Over the past 30 years, antibiotic susceptibility profiles of *Salmonella* species have changed significantly in Asia due to point mutations and the horizontal acquisition of resistance genes¹⁰. Multidrug-resistant (MDR) enteric fever isolates, defined as combined resistance to chloramphenicol, ampicillin, and co-trimoxazole, were a common occurrence in the 1990s and necessitated the use of fluoroquinolones, subsequently cephalosporins and azithromycin¹¹. Concerningly, cases of extensively drug-resistant (XDR) enteric fever cases (resistant to ampicillin, chloramphenicol, co-trimoxazole, fluoroquinolones and third-generation cephalosporine) has been reported from many corners of the world¹². Resistance to ceftriaxone poses significant local and global health risks because of the limited treatment alternatives. In 2016, an outbreak of typhoidal *Salmonella* resistant to ceftriaxone was documented in Sindh, Pakistan. In addition, Bangladesh, India and Sri Lanka have reported similar cases¹³.

The present study was undertaken to assess the current antimicrobial sensitivity pattern and reevaluate the trends of antibiogram of typhoidal *Salmonella* isolates, so that the therapeutic value of common drugs and the empirical treatment of enteric fever can be rationalized.

Methodology

Study Setting and Design: This cross-sectional study was conducted in the Department of Microbiology of Chittagong Medical College from July 2023 to June

2024. Prior to commencement, the study protocol was approved by the Institutional Review Board and the concerned authority. Blood samples were collected aseptically from 220 suspected enteric fever patients irrespective of age and sex, who attended the laboratory of the Department of Microbiology from inpatient and outpatient departments of Paediatric and Medicine of Chittagong Medical College Hospital.

Identification of Typhoidal *Salmonella* species: Blood culture was carried out by automated method (BD BACTEC™ FX 40, Becton, Dickinson and Company, Sparks, MD 21152, USA), which was followed by subculture on Blood agar and MacConkey agar media (procured from Oxoid Ltd. UK). The identification was made by relevant biochemical tests such as oxidase test, followed by inoculation into KIA media, MIU media and citrate utilization test. Later confirmed by *Salmonella*-specific antisera (Deben Diagnostic, UK).

Antimicrobial Susceptibility testing: Antimicrobial Susceptibility was assessed by the modified Kirby-Bauer disc diffusion method, and the discs were procured from commercial sources (Oxoid Ltd, UK). The zone of inhibition was interpreted according to the Clinical and Laboratory Standards Institute. Antimicrobial agents were selected according to CLSI guideline, 2024. Ampicillin (10µg), cotrimoxazole (25µg), Tetracycline (30µg), Chloramphenicol (30µg), Ciprofloxacin (5µg), Azithromycin (15µg), Ceftriaxone (30µg), Meropenem (10µg). (Azithromycin is indicated for *Salmonella enterica* Serovar Typhi only). *Escherichia coli* ATCC 25922 was used as control strain to assess the performance of the method. Within 30 minutes of the placement of antibiotic discs, inoculated plates were incubated aerobically 37° C for overnight.

Detection of Multidrug-Resistant *Salmonella*: *Salmonella* strains that were resistant to all three first-line anti-typhoidal drugs, namely ampicillin, chloramphenicol, and cotrimoxazole, were detected as MDR organisms.

Determination of MIC of Levofloxacin: The MIC of levofloxacin was determined using the agar dilution method, where Mueller-Hinton agar plates were prepared with varying concentrations of levofloxacin (0.008 µg/ml to 4 µg/ml). After preparing the agar, it was stored at 2-8°C. Bacterial colonies from blood agar were standardized to a 0.5 McFarland standard and diluted to achieve a final concentration of approximately 10⁴ CFU per spot on the agar plates, which were then inoculated and incubated overnight at

37°C.14 The MIC was assessed based on the lowest concentration that inhibited growth, with sensitive defined as MIC ≤ 0.125 $\mu\text{g/ml}$, intermediate sensitive as 0.25-1 $\mu\text{g/ml}$, and resistant as ≥ 2 $\mu\text{g/ml}$, as per CLSI guidelines for 2024.

Quality Control: *Salmonella* ATCC 700931 was used for different biochemical tests. Reference strains of *Escherichia coli* (ATCC 25922) was used as a control reference strain for identification and antimicrobial susceptibility testing.

Statistical Analysis: Statistical analysis was performed following standard procedures utilizing SPSS (version 29.0) and Microsoft Excel.

Ethical Consideration: Ethical approval for the study was obtained from the Institutional Review Board. As this was a prospective study, written informed consent was obtained from all study participants. All methods were performed in accordance with the relevant guidelines and regulations.

Results

A total 220 blood samples were collected from clinically suspected enteric fever cases in the study period of one year of which, 44 (20%) samples yielded positive growth and the remaining 176 (80%) showed no growth. (Figure I)

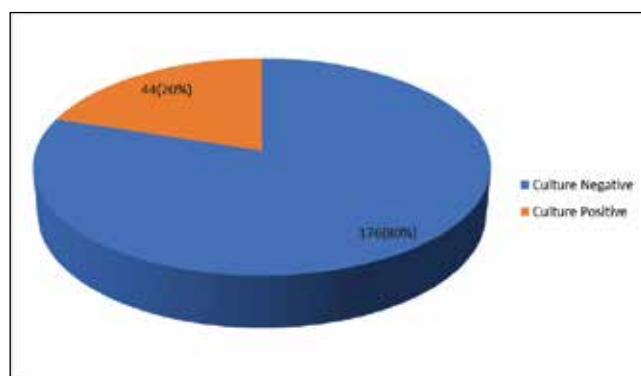


Figure I: Culture Positive Specimens among the Study Population (N=220)

Among cultures positive isolates that included 31(14.09%) *Salmonella* Typhi, 1(0.45%) *Salmonella* Paratyphi A and 12 (5.4%) growth of other organisms. (Figure II)

Among the 44 isolated bacteria, 31(70.5%) were *Salmonella* Typhi, 1 (2.3%) was *Salmonella* Paratyphi A followed by *Staphylococcus aureus* 6 (13.6%), *Acinetobacter* spp. 4 (9.0%) and *Klebsiella* species 2

(4.54%). (Figure III)

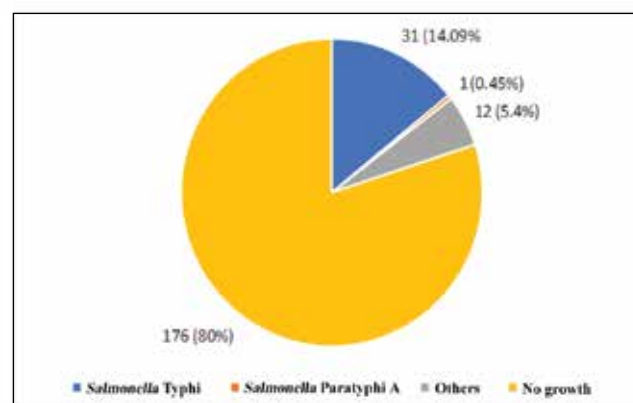


Figure II: Distribution of *Salmonella* species and Other Organisms among the Study Population (N=220)

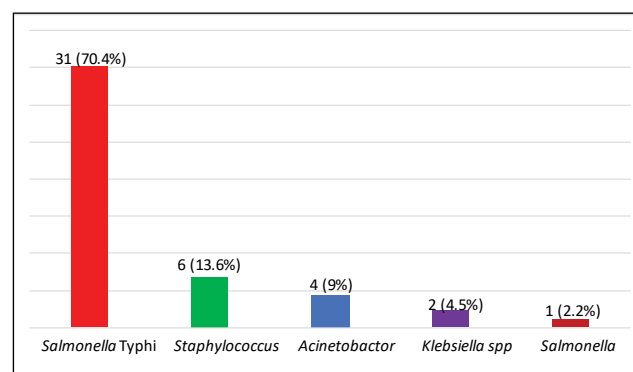


Figure III: Distribution of Isolated Bacteria among Culture-Positive Specimens (n=44)

Among 32 *Salmonella* Typhi and Paratyphi isolates, highest 100% sensitivity found to meropenem and ceftriaxone followed by tetracycline 91%, levofloxacin 91%, chloramphenicol 75%, cotrimoxazole 72%, ampicillin 62% and azithromycin 51%. ciprofloxacin was 54% intermediate sensitive and 46% resistant.

Table-I: Antimicrobial susceptibility pattern of isolated *S. Typhi* and Paratyphi (n=32)

Antibiotics	Sensitive	Intermediate	Resistant
Ampicillin	20 (62.0%)	1 (3.0%)	11 (35.0%)
Chloramphenicol	24 (75.0%)	-	8 (25.0%)
Cotrimoxazole	23 (72.0%)	2 (6.0%)	7 (22.0%)
Ciprofloxacin	0 (0.0%)	17(54.0%)	15 (46.0%)
Levofloxacin	29 (91.0%)	1 (3.0%)	2 (6.0%)
Ceftriaxone	32 (100.0%)	0 (0.0%)	0 (0.0%)
Azithromycin	16 (51.0%)	-	15 (49.0%)
Tetracycline	29 (91.0%)	2 (6.0%)	1 (3.0%)
Meropenem	32 (100.0%)	0 (0.0%)	0 (0.0%)

Sensitivity to azithromycin was detected in *Salmonella enterica* serovar Typhi only as per CLSI guideline, 2024. Table-II shows the distribution of MDR strains among isolated *Salmonella* spp. Among 32 isolated *Salmonella* strains 4 (12.5%) were MDR and 28 (87.5%) were non-MDR (Table 1).

Table 2: Distribution of MDR strains among isolated Typhoidal *Salmonella* (n=32)

Resistance	S. Typhi (n=31)	S. Paratyphi A (n=1)	Total (n=32)
MDR	4 (12.9%)	0 (0.0%)	4 (12.5%)
Non-MDR	27 (87.1%)	1 (100.0%)	28 (87.5%)
Total	31 (100.0%)	1 (100.0%)	32 (100%)

Azithromycin is indicated in *Salmonella enterica* serovar Typhi only as per CLSI guideline, 2024. Levofloxacin sensitivity was detected by MIC (Minimum inhibitory concentration).

Discussion

Widespread abuse of antibiotic in enteric fever is rampant in Bangladesh. The emergence of non-susceptibility among the commonly used antibiotics for enteric fever has been associated with a worse clinical response, higher rate of complications and death, as well as prolonged fecal shedding, which sustains transmission and propagates secondary cases within the community. So, drug-resistant enteric fever is becoming a cause of concern in enteric fever¹⁵⁻¹⁷.

In this study, out of 220 samples, 44 samples (20%) were found to be culture positive, similar to the findings of the studies conducted by Dola et al¹⁸ which is 25.6% in Bangladesh. Out of 44 culture-positive cases, 31 (70.4%) were *Salmonella* Typhi and 1 (2.2%) was *Salmonella* Paratyphi A. Dola et al¹⁸ also reported a study in which *Salmonella* Typhi were 80.0% and Paratyphi A were 20.0%. Begum et al¹⁹ reported similar findings in which 73.0% *Salmonella* Typhi and 27% *Salmonella* Paratyphi A were isolated in Uttara Adhunik Medical College. The differences may be related to a variety of factors, including location, differences in climate as well as environmental and ecological factors²⁰.

In the present study, nine different antibiotics representing different categories were tested on isolated *Salmonella* spp. to perceive the antimicrobial susceptibility pattern. Among 32 *Salmonella* isolates, the highest 100% sensitivity were seen in meropenem and ceftriaxone, followed by tetracycline 91% and

levofloxacin 91%. Haque et al²¹ also reported 100% meropenem sensitivity and Chowdhury et al²² stated 96.5% tetracycline sensitivity. Khanam et al²³ (2022) also reported 80% tetracycline sensitivity, which is slightly lower than the present study. Other studies showed 100% sensitivity of ceftriaxone by Dola et al¹⁸ and Akhtar et al²⁴ in Dhaka. A study by Begum et al²⁵ showed 2% ceftriaxone resistance in United Hospital, Dhaka, which is a matter of concern regarding treatment of enteric fever. On the other hand, 32% ceftriaxone resistance was seen in Pakistan, which is much higher than the present study. Ceftriaxone remains the preferred treatment for enteric fever; however, it is important to use it with caution due to the emergence of resistance in various regions globally²⁶.

A comparatively higher sensitivity rate was exhibited by chloramphenicol 75%, cotrimoxazole 72% and ampicillin 62%, which is almost similar to the study conducted by Rahman et al²⁷. A study from Iraq reported 61.5% resistance to ampicillin and 51.2% to chloramphenicol²⁸. Mutai et al²⁹ from Kenya reported that 72% were resistant to both ampicillin and chloramphenicol with 70% resistant to cotrimoxazole. This variation of resistance may be due to different geographical areas and samples.

In this study, 54% of *Salmonella* strains were intermediate sensitive to ciprofloxacin and 46% were resistant; no sensitive strain was found which is correlated with the study of Rahman et al²⁷. Akhtar et al²⁵ showed only 4.4% sensitivity against ciprofloxacin, which is also similar to the present study. But Hasan et al³⁰ and Jha et al³¹ showed 81% and 86% sensitivity rates of ciprofloxacin in Bangladesh and India, respectively. The resistance patterns of typhoidal *Salmonella* have shown variability over time and across different geographical regions. This pattern is also probably due to the indiscriminate and over-the-counter use of this drug in countries with large populations in the treatment of typhoid and other unrelated infections³².

An overall resistance of 49% for azithromycin was observed in the present study. Dola et al¹⁸ found 30% azithromycin resistance at Dhaka, which was similar to the present study. 81.5% and 95% azithromycin resistance reported by Sazzad et al³³ and Ahsan and Rahman³⁴ in Bangladesh, which were much higher than the findings of the present study. Narasanna et al³⁵ showed 0% resistance in India, which was lower than the present study. In Bangladesh, the widespread

resistance to azithromycin can be linked to factors such as improper prescribing practices and the irrational use of the drug by healthcare professionals. Moreover, availability and incomplete treatment due to many reasons may also be the factors contributing to the development of resistance to this drug^{36,37}.

MDR strain was found in 12.5% *Salmonella* isolates. Similar reductions in MDR rates have been documented in enteric fever surveillance studies, including India, Nepal, Indonesia, Vietnam, Laos³⁸. Whereas in Iraq reported 43.5% MDR *Salmonella* strains which is much higher than this study²⁸.

In the present study, levofloxacin showed 91% sensitivity, whereas 1 (3.1%) was intermediate sensitive and 2 (6.2%) were resistant. In India, study found 21 (53%) intermediate sensitive, 15 (38%) resistant and 3 (7.6%) sensitive which is dissimilar to the present study¹⁴.

Conclusion

This study highlights the growing public health concern of drug-resistant enteric fever in Chittagong. Increasing azithromycin resistance has limited oral treatment options, and rising resistance to both ciprofloxacin and azithromycin may force a shift toward injectable therapies, adding pressure on already strained healthcare systems. Third-generation cephalosporins remain effective, but their judicious use is crucial to prevent further resistance. Notably, sensitivity to first-line antibiotics has rebounded to over 70%, suggesting a possible return of their effectiveness. To address these challenges, region-specific antibiotic guidelines, improved public awareness, and development of new therapeutics are needed. Ongoing antimicrobial resistance surveillance is essential to sustain effective treatment strategies and reduce the public health impact of enteric fever.

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Conflict of Interest

The authors declare no conflict of interest.

Financial Disclosure

This study was not funded.

Authors' contributions

Masuma Jannat: conceptualized and designed the study, analyzed the data, interpreted the results, and wrote up the draft manuscript. Pompy Dey, Md Gias Uddin and Ayesha Ahmed Khan contributed to the data

analysis, interpretation of the results, and critical review of the manuscript. Abu Hena Md. Saiful Karim Chowdhury, Ripon Barua contributed to the analysis of the data. All authors read and approved of the final manuscript.

Data Availability

Any inquiries regarding supporting data availability of this study should be directed to the corresponding author and are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

Ethical approval for the study was obtained from the Institutional Review Board. All methods were performed in accordance with the relevant guidelines and regulations.

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