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Culture Positive Cases of Enteric Fever and Their Antibiotic Susceptibility Patterns in a Tertiary Care Hospital in Dhaka, Bangladesh

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Abstract

Background: Enteric fever is a systemic bacterial infection caused by *Salmonella enterica* subspecies *enterica* serotype Typhi (*S.* Typhi) or Paratyphi A, B, or C (Paratyphi A, B, or C). It is a significant public health concern in developing countries. Periodic monitoring of sensitivity and resistance patterns is necessary to support therapeutic care at both national and local levels, given the increasing antibiotic resistance observed in their management. Additionally, this will enable the planning of antibiotic recycling whenever possible.

Objective: This study aimed to identify *Salmonella*, the most prevalent pathogen in bloodstream infections, as well as to determine their demographics, seasonal variations, and antibiotic susceptibility to create a complete picture of how their antibiotic susceptibility has changed over time.

Methods: This retrospective study was conducted in the Department of Microbiology and Immunology, Bangladesh Medical University (BMU), Dhaka, between July 2023 and June 2024. Ten thousand, fifty-three blood samples were collected in adult and pediatric Brain Heart Infusion broth (BHI) bottles for culture, and the bacterial profile was retrieved. The collected samples were cultured using a standard technique in a medical microbiology laboratory. The isolated bacteria were identified by colony morphology, Gram staining, and biochemical reaction. Antibiotic susceptibility was tested by Kirby-Bauer disc diffusion methods per the National Committee for Clinical Laboratory Standards guidelines.

Results: A total of 10053 blood samples were collected, of which 513(5.10%) were culture positive. Among 513 culture growth, the prevailing isolate was *Salmonella* Typhi 216 (42.10%), followed by *Staphylococcus aureus* 81(15.80%), *Salmonella* Paratyphi A 60 (11.70%). Following that, *Acinetobacter* spp. (10.90%), *Klebsiella* spp. (9.90%), *Pseudomonas* spp. (4.30%), *E. coli* (3.30%), *Serratia* spp. (0.97%), *Burkholderia cepacia* (0.60%), *Enterobacter* spp., and *Stenotrophomonas maltophilia* (0.39%). The majority of blood culture-positive cases (*S.* Typhi or *S.* Paratyphi A) were below 10 years old, with male predominance. There were two surges of enteric fever, the highest prevalence in October through January and March through May. It was found that *S.* Typhi (13%–19.4 %) and *S.* Paratyphi A (10-11.7%) had decreasing resistance to the first-line medications amoxicillin, chloramphenicol, and cotrimoxazole. In cases of enteric fever, the majority of isolates exhibited fluoroquinolone resistance, but none of them exhibited ceftriaxone resistance. There is an increase in azithromycin resistance (85.2%).

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Conclusion: The findings of this study highlight the declining resistance to first-line antimicrobials, and it could be reintroduced as an empirical treatment option for enteric fever. Antibiotic use without prescription should be minimized, and prescribing practice should be modified. These results underscore the necessity for judicious use of antimicrobials and the implementation of an antimicrobial stewardship program in tertiary care hospitals across Bangladesh.

Keywords: Enteric fever, antibiotic susceptibility, BHI

Introduction

Karl Joseph Eberth initially described the bacillus thought to cause typhoid in 1980, and four years later, pathologist Georg Graffky confirmed these findings. As a consequence, the bacillus was given the name Eberthella typhi, which is now more commonly referred to as Salmonella enterica [1]. A major public health concern in developing countries is enteric fever [2], which is brought on by *Salmonella enterica* subspecies *enterica* serotype Typhi (*S.* Typhi) or Paratyphi A, B, or C [3]. According to the World Health Organization (WHO), there are between 11 and 20 million typhoid infections worldwide each year, with a mortality rate of 128,000 to 161,000 deaths [4]. Bangladesh is one of the nations that are badly afflicted, with an annual incidence rate of 252 per 100,000 affected [5]. The most cases occurred among children aged 5–9 years; 56% were in individuals under <15 years [3]. Under five-year-olds, who are particularly immunocompromised [6], are significantly more susceptible than older people [7]. Lacking clean water, food, and inadequate sanitation in poor communities are also prone [4,6]. According to South Asia's overall sex discrepancies in enteric fever reporting, men made up the majority of cases (59%) [8]. In many Asian and African nations, enteric fever frequently recurs around the same time of year and exhibits a seasonal pattern [9]. The seasonal pattern of enteric fever in North America and Europe indicated that August to September was the peak time. The peak seasons in Asia, Africa, and the Middle East lasted for several months. Africa and the Middle East from July to November, while Asia is from May to October. Due to the Southern Hemisphere's distinct seasonal scheduling, South America's peak season lasted from January to May [10], suggesting that their transmission may be influenced by meteorological conditions [11]. In Bangladesh, Nepal, and Cambodia (South and Southeast Asia), incidence peaks around May to October [9], whereas it manifests in a variety of seasonal rhythms throughout Africa. A clear seasonal cycle with a peak in March–June after the rainy season was noted in Blantyre, Malawi [12]; however, Nairobi, Kenya, revealed more intricate dynamics [13]. The disease has been reported to occur more frequently in Cameroon and the Democratic Republic of the Congo [14].

Geographical and temporal variations affect the distribution of *Salmonella* species and the pattern of antibiotic resistance [15,16]. South Asia is particularly vulnerable in terms of the emergence and spread of antimicrobial resistance [17]. This harms clinical outcomes [18]. Public health is seriously threatened by antimicrobial resistance (AMR), which was expected to have contributed to 1.27 million deaths in 2019 [19]. Deaths from AMR infections could increase tenfold by 2050 if left unchecked because of the extensive use of antibiotics and a paucity of new antimicrobials [20, 21]. The burden of morbidity from infectious diseases in poor countries is primarily caused by enteric fevers. Due to the growing antibiotic resistance seen in their management, Salmonella and its many species need to be regularly assessed for sensitivity and resistance patterns in order to guide treatment at the local level [22]. Our study aimed to ascertain demographic information and seasonal fluctuation and monitor antibiotic sensitivity patterns that are therapeutically significant because studies from different countries have shown a fluctuating pattern of susceptibility to conventional medications. This will allow for the recycling of antibiotics wherever possible and provide appropriate treatment guidance.

Materials and Methods

Study design

A retrospective review of the laboratory data of blood samples obtained from the inpatient and outpatient departments of Bangladesh Medical University (BMU), Dhaka, between July 2023 and June 2024 was carried out by the Department of Microbiology and Immunology.

Study population

A total of 10053 conventional blood samples were collected during that period. Reports of Salmonella infection from blood cultures were retrieved and analyzed. A comprehensive dataset regarding demographic data, seasonal variation, laboratory results of bacterial isolation, and susceptibility patterns was collected from the Laboratory specimen logbooks using the standard data collection form.

Laboratory procedures

Sample collection

All the samples were aseptically collected in adult and pediatric Brain Heart Infusion (BHI) broth bottles. About 5 -10 ml of blood/bottle for adults and 2-5 ml of blood/bottle for pediatric patients was collected in the blood culture bottle, labeled properly, and transported to the Microbiology laboratory without delay for the bacteriological examination.

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Organism isolation and antimicrobial susceptibility

Incubation of the blood in BHI broth lasted 18–24 hours at 37°C, followed by a 7-day observation period. Following 18–24 hours of incubation, blind subculture onto Blood agar and MacConkey agar, and then every other day until day 7. Daily check the bottle of BHI broth for any obvious growth indicators, such as turbidity, gas generation, pellicle development, clotting, hemolysis, etc. The inoculated cultures were incubated overnight for 24 to 48 hours at 37 degrees Celsius. Organisms were identified based on morphology, culture characteristics, and biochemical reactions according to standard microbiological techniques. All the isolates were tested for antimicrobial susceptibility on Muller Hinton Agar (HI Media, India) by the Kirby Bauer disc diffusion method, according to the Clinical Laboratory Standard Institute (CLSI) guidelines [23]. The following antibiotics were used for *Salmonella* spp.: amoxicillin (10µg), ciprofloxacin (5µg), nalidixic acid (30µg), ceftriaxone (30µg), trimethoprim-sulfamethoxazole (1.25/23.75µg). And additional azithromycin (30µg) for *Salmonella* Typhi. All the antibiotic disks were commercially purchased from Biomaxima, Poland. *Salmonella* Typhimurium ATCC 14028 was included as a quality control strain for antimicrobial susceptibility testing.

Data analysis

Data were cleaned manually, entered, and analyzed by using SPSS version 24 software. The statistical analysis used in the study was descriptive and involved categorical data analysis. Frequency and percentage were examined for categorical independent variables. Results were presented through graphs and tables.

Results

A total of 10053 conventional blood samples were collected, of which 513(5.10%) yielded bacterial growth (Table 1).

In the current observation, among 513 culture positive cases 216 (42.10%) were caused by *Salmonella* Typhi, with *Staphylococcus aureus* 81 (15.80%) and *Salmonella* Paratyphi A 60 (11.70%) following closely behind *Acinetobacter* spp., *Klebsiella* spp., and *Pseudomonas* spp. were then found in the following proportions: 56 (10.90%), 51 (9.90%), and 22 (4.30%). Lastly, *E. coli* 17 (3.30%), *Serratia* spp. 5 (0.97%), and strains of *Burkholderia cepacia* 3 (0.60%) were identified. *Stenotrophomonas maltophilia* and *Enterobacter* spp. were the other two species detected (0.39%) (Figure 1).

Table 1: Frequency of Bacterial isolates in blood sample (n=10053)

Culture	Frequency	Percentage (%)
Growth	513	5.1
No Growth	9540	94.89
Total	10053	100

Males (134,62%) were more affected than females (82,38%) in the current study's culture-positive *Salmonella* Typhi cases. Males made up (34,56.7%) of *Salmonella* Paratyphi A, while females made up (26.43.3%), showing male preponderance, and male to female ratios were 1.6:1 and 1.3:1, respectively (Figure 2).

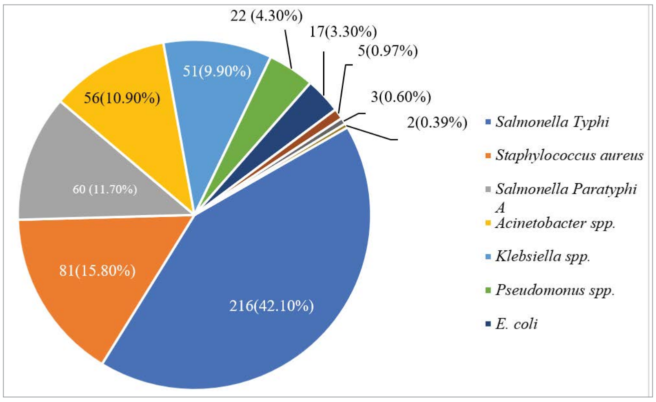

Figure 1: Distribution of Culture-Positive cases

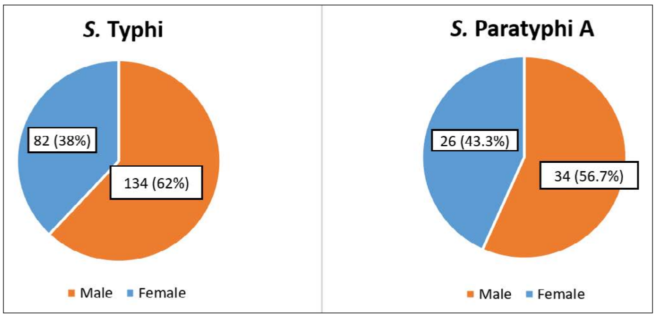

Figure 2: Sex wise distribution of culture-positive cases of *Salmonella* Typhi (n=216) and *Salmonella* Paratyphi A (n=60)

With a positive culture for *Salmonella* Typhi, the current study found that nearly half (89, 41.2%) of the 216 patients were under 10 years old, followed by those who were 11 to 20 years old (57, 26.4%), 21 to 30 years old (55, 25.5%), 31 to 40 years old (6, 2.8%), 41 to 50 years old (6, 2.8%), and only (3, 1.4%) older than 50. Nearly half of the 60 *Salmonella* Paratyphi A patients (26, 43.3%) were under ten years old. Only one person was older than 50 (1, 1.7%), followed by those between the ages of 11 and 20 (12, 20%), 21 and 30 (17, 28.3%), 31 and 40 (2, 3.3%), and 41 and 50 (2, 3.3%) (Figure 3).

Both cases exhibit a nearly identical seasonal pattern, with the highest number of cases occurring from late monsoon to mid-winter (October to January), followed by the summer season (March to May). Figure 4 provides an illustration of this.

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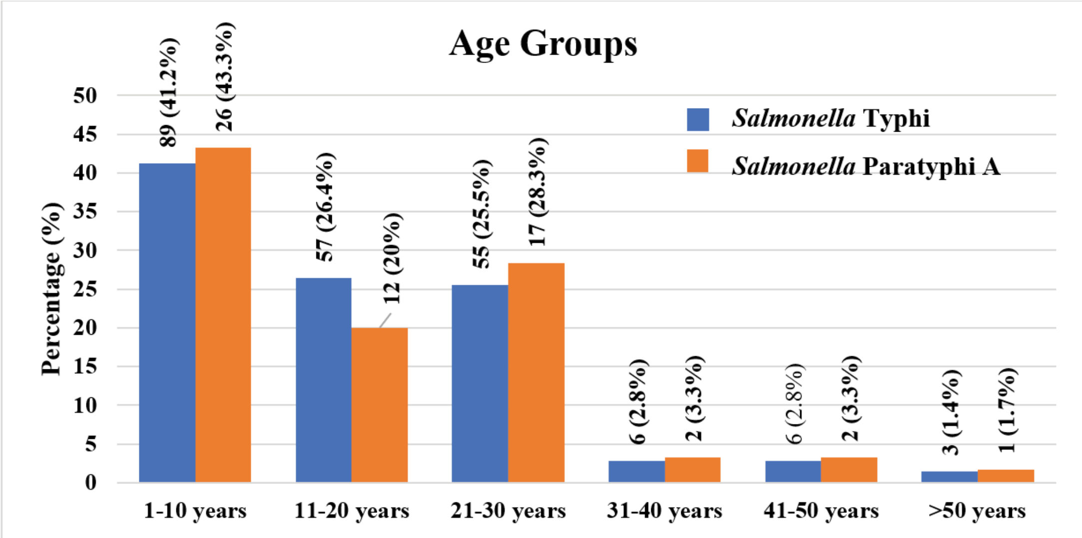

Figure 3: Age-wise distribution of culture-positive cases of *Salmonella Typhi* (n=216) and *Salmonella* Paratyphi A (n=60)

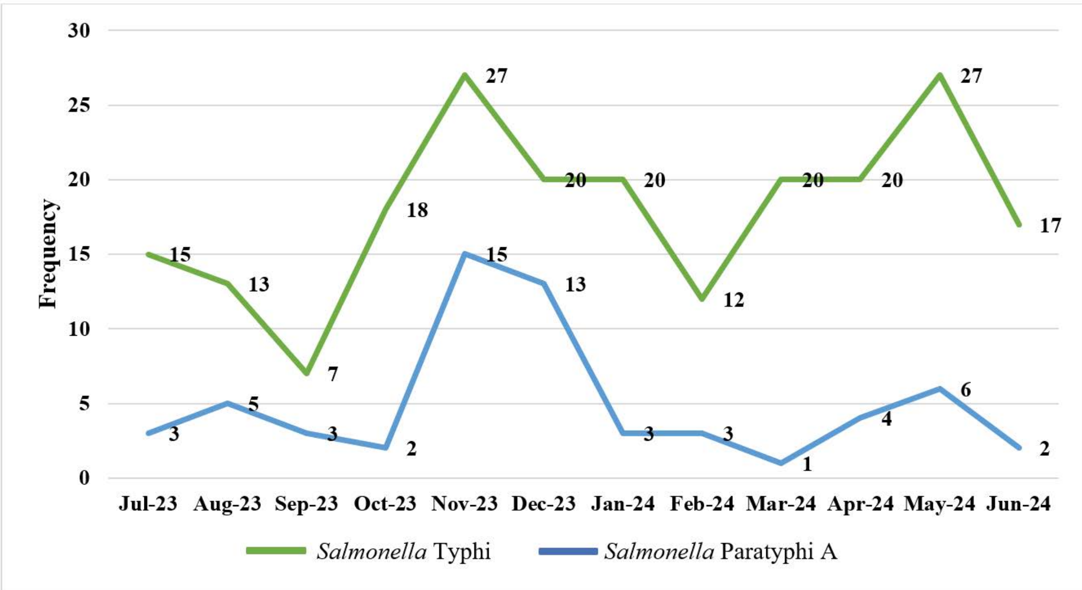

Figure 4: Month-wise distribution of cases

Table -2. Antibiotic resistance pattern of *Salmonella* Typhi isolated from blood culture.

Resistance to the first-line antimicrobials-amoxicillin, chloramphenicol, cotrimoxazole

We observed the lowest resistance among *S.* Typhi isolates to irst-line antimicrobials amoxicillin (33, 15.3%), chloramphenicol (28,13.0%), trimethoprim-sulfamethoxazole (cotrimoxazole) (42, 19.4%).

Resistance to fluoroquinolone (nalidixic acid, cipro loxacin) and macrolide (azithromycin)

Non-susceptibility to nalidixic acid (210, 97.2%) and cipro loxacin (160, 74.1%) in *S.* Typhi was notably high. And azithromycin was (184, 85.2%) resistant.

Resistance to third-generation cephalosporin - ceftriaxone

None of the *S.* Typhi isolates were resistant to ceftriaxone.

Table 3: Antibiotic resistance pattern of *Salmonella* Paratyphi A isolated from blood culture.

Resistance to the first-line antimicrobials-amoxicillin, chloramphenicol, cotrimoxazole

Declining resistance was displayed among *S.* Paratyphi A isolates to irst-line antimicrobials amoxicillin (7,11.7%), chloramphenicol (6,10%), trimethoprim-sulfamethoxazole (cotrimoxazole) (6, 10%).

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Table 2: Antibiotic resistance pattern of *Salmonella* Typhi to different antibiotics (n=216)

Antibiotic Class	Antibiotics	Sensitive	Resistance	Percentage of Resistance (%)
Penicillin	Amoxicillin	183	33	15.3 (%)
Amphenicol	Chloramphenicol	182	28	13.0 (%)
Sulfonamides	Trimethoprim-Sulfamethoxazole	174	42	19.4 (%)
Cephalosporin	Ceftriaxone	216	0	0 (%)
Fluoroquinolones	Nalidixic acid	6	210	97.2 (%)
	Ciprofloxacin	50	160	74.1 (%)
Macrolide	Azithromycin	32	184	85.20%

Table 3: Antibiotic resistance pattern of *Salmonella* Paratyphi A to different antibiotics (n=60)

Antibiotic Class	Antibiotics	Sensitive	Resistance	Percentage of Resistance (%)
Penicillin	Amoxicillin	53	7	11.7 (%)
Amphenicol	Chloramphenicol	54	6	10 (%)
Sulfonamides	Trimethoprim-Sulfamethoxazole	54	6	10 (%)
Cephalosporin	Ceftriaxone	60	0	0 (%)
Fluoroquinolones	Nalidixic acid	10	50	83.3 (%)
	Ciprofloxacin	8	52	86.7 (%)

Resistance to fluoroquinolone (nalidixic acid, ciprofloxacin) and ceftriaxone

Non-susceptibility to nalidixic acid (50, 83.3%) and ciprofloxacin (52, 86.7%) in *S.* Paratyphi A was also alarming. While ceftriaxone showed 100% sensitivity.

Discussion

Bangladesh is one of the developing nations where enteric fever is still a major health concern. *S.* Typhi bacteremia accounts for the majority of these instances, while *S.* Paratyphi A bacteremia accounts for the remaining occurrences. The bacterial isolation rate in this study was 5.10%, comparable to the 3% in Bangladesh and 0.53%, 4.1%, and 2.2% in India, Nepal, and Pakistan, respectively, reported in Barkume et al.'s (2018) multiphase surveillance study for the Surveillance for Enteric Fever in Asia Pacific (SEAP) [8]. However, Nasin et al. (2021) reported a 13% isolation rate [24]. Additionally, 10-year retrospective research conducted in Bangladesh found that the isolation rate ranged from 10.7 to 17.3% [25]. These differences in the bacterial isolation rate could be due to the different institutions' employment of varied sample collection techniques, culture techniques, and infection control protocols. *Salmonella* Typhi (42.10%) was the most common isolate in the present study, followed by *Staphylococcus aureus* (15.80%) and *Salmonella* Paratyphi A (11.70%). *Salmonella* Paratyphi B was not found in any of the isolates during our analysis. Numerous studies carried out in Bangladesh have found that *Salmonella* Typhi is a frequent cause of bloodstream infections in this region [7,26–27]. According to Nasrin et al. (51%) were *S.* Typhi, and (15%) were *S.* Paratyphi A, which is almost consistent with our study, and 6 percent of isolates were *Staphylococcus aureus* [24]. Ahmed et al. reported *S.* Typhi (36.9%) and *S.* Paratyphi A, B (8.9%) [25]. However, Singh et al. demonstrated that *Staphylococcus aureus* (33.10%) was their most common isolate [22], nearly twice as high as our study. The culture, technique, and infection control policies of each unique institution are the cause of this discrepancy.

However, according to Date et al. (2016), the first thorough analysis of National Typhoid and Paratyphoid Fever Surveillance System (NTPFS) data over a five-year period, 80% of cases in the United States were typhoid, and 20% were paratyphoid A [28]. This discrepancy results from the fact that an increasing proportion of cases have been linked to travelers returning from Southern Asia [2]. In addition, *Acinetobacter* species, *Klebsiella* species, and *Pseudomonas* species were isolated in this study; their respective percentages were (10.90%), (9.90%), and (4.30%). Finally, it was found that *E. coli* (3.30%), *Serratia* spp. (0.97%), and *Burkholderia cepacia* (0.6%) were present. Two more organisms, *Stenotrophomonas maltophilia* and *Enterobacter* spp., were found (0.39%). This is nearly in line with the Singh et al. study, which found that *Acinetobacter* species accounted for 10.61%, *Klebsiella* species for 7.69%, and *Pseudomonas* species for 6.42% [22]. However, E. coli levels in other studies were slightly higher than ours (12.07%

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- 15%) [22, 24]. Ahmed et al found *Pseudomonas* species (12.5%) and *Acinetobacter* species (5.1%); besides this, *E. coli*, *Enterobacter* spp., and *Serratia* species had a steady isolation rate over the ten years, while *Klebsiella* species showed an increasing trend in their isolation rate from 22.6 to 50.9% [25]. The rise in BSI from different organisms may have resulted from patients' greater access to medical services at that time in their study period [25]. The reason for this difference from our study is due to the organisms' shifting patterns.

In this study, we found that males were predominantly suffering from enteric fever than females, which is in line with the typical sex differences in enteric fever reporting in South Asia [8]. These results show a significant degree of sex-specific variance in illness, even though Zabeen et al. and Mina et al. reported a female predominance [29,30]. Whether any factors contribute to the sex-specific vulnerability of enteric fever patients can be ascertained by additional research. In any microbiological infection, the patient's age is always a significant factor. Enteric fever can infect people of any age [30]. The majority of the *S*. Typhi and *S*. Paratyphi A culture-positive patients in our study were from the age group below 10 years. This finding was consistent with a different study that found children aged 5 to 9 years and under 10 years had the highest incidence [3,13]. Those aged 11-30 years were similarly affected by *S*. Typhi, whereas the age group of 11–20 years old is less affected than the group of 21–30 years old in *S*. Paratyphi A culture-positive patients in our study. The previous study represented that the patients infected by *S*. Typhi were observed under the age of 20 years, though the maximum of them were under the age of 5 [7,8, 30,31]. While Barkume et al. reported, in India and Nepal, a high proportion of participants aged 15-25 years, and in a previous study in Bangladesh and Pakistan were aged ≤ 5 years [8]. Because young children typically suffer from malnutrition, changes in the gut flora or other host defenses may increase susceptibility to reinfection [32]. Evidence from past studies that shows environmental variation in the risk of infection between children and adults is emphasized by Akullian et al. [13]. Also, this infection is brought on by school-age children's unsanitary behavior, poor hygiene practices, or developing a habit of consuming local street foods, as it is more affordable and convenient in a developing nation like Bangladesh. Adults are particularly at risk since they make up the majority of the workforce and engage in more outside activities in poor nations, which allows them to consume cool water and consume undercooked or street cuisine on hot days.

The two positive spikes of enteric fever in the current study occurred in the fall and winter (late monsoon to cool dry winter) months of October through January, and in the summer (pre-monsoon hot season) months of March through May. It is consistent with the study by Jayaprasad et al., who reported the highest case positivity in January 2017[33]. In contrast, several previous studies in which the South Asian case burden was described as highest during periods of peak rainfall or just after, the months of May to October [27,29,10,33]. This shifting pattern of wintertime enteric fever may be brought on by past travel or improper storage of food consumption. Additionally, cold air impairs immunity, making it more difficult to fend off infections. And in summer dry season peak in our study correlate with other study that represent increase temperature would promote the growth and reproduction of Salmonellae, leads to water source pollution, water scarcity, using contaminated water sources, engage in more outdoor recreational activities and encouraging the purchase of prepared foods or barbecues during warmer weather leads to infection [34, 35] In a range of geographical contexts, numerous studies have shown a positive correlation between temperature and foodborne disease like enteric fever [36-38]. In our study, the antibiotic resistance pattern was almost the same for *S*. Typhi and *S*. Paratyphi A. Resistance to the first-line antibiotics amoxicillin, chloramphenicol, and cotrimoxazole decreased in *S*. Typhi (13%–19.4 %) and *S*. Paratyphi A (10–11.7%), respectively. This result is similar to that of a 24-year retrospective observational study by Tanmoy et al. (2024), in which resistance peaked declined for *S*. Typhi from 80% to less than 20% for all three first-line antimicrobials, and Ahmed et al. (2017) resistance declines from 61.7 to 23.7% [38,25,8]. Alongside a decline in resistance for *S*. Paratyphi A was (5-7%) to these first-line antimicrobials [24]. Enteric fever surveillance studies across various countries, including Bangladesh, India, Nepal, Pakistan, Vietnam, Laos, Indonesia, have shown that this fall was linked to a decrease in multidrug resistance (MDR) [38–45]. This decrease in resistance may be the consequence of fewer doctors prescribing first-line antibiotics and a drop in usage, especially of cotrimoxazole. In our present observation, we did not find any MDR Salmonella cases. Whereas Khan et al (2024) in Pakistan showed high resistance rates to ampicillin (81.40%) and chloramphenicol (90.2%), respectively, and did not mention cotrimoxazole, they also mention MDR cases [46]. This difference is due to geographical and environmental variance and a decline in the consumption of these first-line antimicrobials in our country.

Our study found that (85.2%) of *Salmonella* Typhi were resistant to azithromycin, which is quite concerning. This is in line with the research report, Bangladesh suggests a rising effective population size of azithromycin-resistant isolates [47]. High consumption of the drug may exert selective pressure, fostering resistance determinants in the gut [48]. However, another study highlights the potential of azithromycin as a viable treatment option with a notable (90.40% -93%) sensitivity [46,49,50]. This concurs with the WHO's recognition of azithromycin as an alternative treatment for MDR typhoid fever, particularly in regions facing high resistance to conventional drugs [51]. We did not test for *Salmonella* Paratyphi A sensitivity against azithromycin. In the present study, we found remarkable resistance against the fluoroquinolones class of antibiotics, nalidixic acid, and ciprofloxacin in both *Salmonella* Typhi and *Salmonella* Paratyphi A. This is consistent with several studies where the majority of isolates in all countries were resistant to fluoroquinolones [8,49,38,46]. It suggests that these medications are being abused and used irrationally to treat many other nonspecific illnesses. In the current study, no isolates showed resistance to ceftriaxone in enteric fever cases; similarly, multiple studies showed 100% sensitivity to ceftriaxone [49]. However, Hooda et al. discovered 47 cases of ceftriaxone-resistant *Salmonella* Typhi in Bangladesh between April and September 2024, in addition to a few solitary cases that had already been reported in Bangladesh and India [52–54]. Since ceftriaxone empirical treatment is so common in South Asia, public health officials need to keep an eye out for the emergence and spread of ceftriaxone-resistant *Salmonella* Typhi [55]. Antimicrobial resistance in enteric fever is mostly caused by the inappropriate, excessive, and misused use of antimicrobial drugs. Hence, the result may not reflect the national scenario of this endemic infection; physicians should consider this before prescribing antimicrobials.

Conclusion

According to the current study, children under the age of ten are primarily afflicted by enteric fever, which is endemic throughout the year, with two significant incidence spikes from October to January and another peak from March to May. Third-generation cephalosporins need to be closely monitored, and reintroducing first-line antibiotics to treat this infection is one possible antibiotic stewardship technique that we suggest in order to observe the AMR pattern in enteric fever cases. These results indicate that treatment practices need to change, and immediate action is required to curb antibiotic abuse. It is essential to routinely evaluate the microbiological profile in order to guarantee efficient management of enteric fever cases, especially in light of the expanding problem of medication resistance.

Limitation

Because the study is retrospective, we are unable to distinguish between samples from patients outside of our hospitals and those from our hospitals. Apart from age and sex, we were also unable to gather information about the clinical manifestation of the patient or any other patient features. Clinical connection is therefore not possible to some degree.

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Conflict of interest

The authors declare no conflicts of interest regarding the publication of this paper.

Authors Contributions

All authors contributed equally to this work.

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