Defining the concurrent pattern of antimicrobial sensitivity of *Typhoidal Salmonella*

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Abstract

Objective: Salmonella-associated typhoidal fever presents a significant public health challenge in Bangladesh. Factors such as limited access to safe water, inadequate sanitation, low awareness of infection control measures among the general population, and inappropriate use of antibiotics contribute to the high incidence of *Salmonella typhi* infections. Moreover, the emergence of resistance to multiple advanced antibiotics complicates treatment options. Methods: monitor the prevalence and drug resistance patterns of *Salmonella typhi* among patients across northern and southern Dhaka. Blood samples were collected aseptically from febrile patients, identifying *Salmonella typhi* as the causative agent. These isolates were then tested against twelve commonly prescribed antibiotics across eight different classes to assess their resistance profiles. Results: Among the 200 blood samples tested from suspected typhoid patients, 28% tested positive for *Salmonella typhi*. Distribution was skewed, with 43% of cases from northern Dhaka and 57% from southern Dhaka. Antibiotic resistance analysis revealed high resistance rates to first-line antibiotics and fluoroquinolones. However, susceptibility was retained against third-generation cephalosporins and carbapenems. Azithromycin showed moderate sensitivity, but the rapid emergence of resistance is concerning, limiting treatment options primarily to injectable carbapenems. Conclusion: The findings underscore the urgent need for tailored antibiotic prescribing guidelines and the development of novel therapeutic agents to address these healthcare challenges in tropical developing countries like Bangladesh. Effective strategies are essential to combat rising antibiotic resistance and ensure effective treatment outcomes for typhoid fever.

Keywords: Typhoid fever, *Salmonella typhi*, Antibiotic resistance, Bangladesh, Public health

Significance | High prevalence of multidrug-resistant Salmonella Typhi in Dhaka underscores urgent need for tailored antibiotic guidelines and novel treatment strategies.

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Introduction

Typhoid fever, a severe and potentially fatal systemic infection caused by Salmonella enterica serovars Typhi and Paratyphi, continues to be a significant public health challenge, particularly in low- and middle-income countries like Bangladesh (World Health Organization, 2018). The disease is primarily transmitted through the ingestion of contaminated food or water, making it a major concern in regions where access to clean water and adequate sanitation is limited (Mannan et al., 2014). According to the World Health Organization (WHO), typhoid fever accounts for approximately 30 million cases globally each year, resulting in an estimated 60,000 deaths annually. In Bangladesh, the burden of typhoid fever is particularly high, with an incidence rate exceeding 1 infection per 1,000 individuals per year (Ghurnee et al., 2021).

The prevalence of typhoid fever in Bangladesh is exacerbated by several socio-economic and environmental factors (Saha et al., 2020). Poor water quality, inadequate sanitation, and a lack of public awareness about infection control contribute significantly to the spread of the disease. Despite efforts to combat typhoid fever

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through the use of antibiotics, the situation is further complicated by the rapid emergence of multidrug-resistant (MDR) strains of *Salmonella Typhi* (Saha et al., 2020). The inappropriate use of antibiotics, driven by the demand for quick symptomatic relief and a general lack of understanding about antibiotic resistance, has led to a significant challenge in the treatment of typhoid fever in Bangladesh (Holt et al., 2011).

Historically, first-line antibiotics such as ampicillin, chloramphenicol, and cotrimoxazole were effective in treating typhoid fever (Akter et al., 2016). *Salmonella Typhi* in Bangladesh has developed resistance to these drugs, necessitating the use of alternative antibiotics like ciprofloxacin and other fluoroquinolones (Kidgell et al., 2002). Unfortunately, resistance to ciprofloxacin has also been on the rise, creating an urgent need for effective alternatives. The increasing resistance to these commonly used antibiotics mirrors a global trend, where *Salmonella Typhi* is rapidly adapting and diminishing the effectiveness of previously reliable treatments (Mbatchou et al., 2011).

In response to the growing challenge of antibiotic resistance, third-generation cephalosporins (such as cefixime, ceftriaxone, and ceftazidime) and carbapenems have become the drugs of choice for treating typhoid fever in Bangladesh (Bauer, 1966). These antibiotics are currently considered effective against *Salmonella Typhi*; however, the emergence of resistance to these drugs has also been reported, raising concerns about the future efficacy of these treatments (Rowe et al., 1997). Azithromycin, another antibiotic that has been used in cases of ciprofloxacin resistance, has also shown signs of decreasing effectiveness, further limiting treatment options (Islam et al., 2019).

Given the critical nature of this issue, it is essential to continuously monitor and evaluate the antibiotic sensitivity patterns of *Salmonella Typhi* to inform treatment protocols and public health strategies (Klemm et al., 2018). This study focuses on the Dhaka metropolitan area, where significant differences in sewage treatment and water quality exist between the northern and southern regions (White et al., 1996). By analyzing data from hospitals across these areas, the study aims to identify regional variations in antibiotic resistance patterns and contribute to the development of more effective treatment guidelines for typhoid fever in Bangladesh (Saha et al., 1999; Djeghout et al., 2018). The findings underscore the urgent need for a multifaceted approach that includes improved public health infrastructure, enhanced awareness of antibiotic resistance, and the development of novel therapeutic agents to address the growing threat of multidrug-resistant *Salmonella Typhi*.

Materials and Methods

Sample Collection

A total of 200 patients presenting with septicemia symptoms and febrile illness were selected for this study. The patients were recruited from various hospitals located in both the northern and southern regions of Dhaka, Bangladesh (Islam et al., 2007; Gautam et al., 2002; Khan & Hoque, 1992). To ensure the accuracy and reliability of the results, blood samples were drawn aseptically from each patient, using sterile techniques to prevent any contamination. These samples were collected in specially designed sterile bottles, which were immediately sealed and labeled for identification (Afroz et al., 2013; Akter et al., 2012).

Isolation and Identification of Salmonella Typhi

Once collected, the blood samples were introduced into the BACTEC system, a fully automated machine designed to detect microbial growth in blood cultures. The samples were incubated at 37°C and continuously agitated to promote bacterial growth (Rahman et al., 2005; Afroj et al., 2011). Monitoring for growth was conducted over a period of 72 to 120 hours. Samples that showed positive growth within this period were subjected to further processing (Shahriar & Kabir, 2010; Dimitrov et al., 2005).

For the isolation of *Salmonella Typhi*, the positive blood cultures were sub-cultured onto sterile Xylose Lysine Deoxycholate (XLD) agar and MacConkey agar plates. These plates were then incubated overnight at 37°C. The colonies that grew on these media were identified based on their colony morphology and appearance. On XLD agar, Salmonella typically produces red colonies with black centers, while on MacConkey agar, it forms non-lactose fermenting, whitish colonies (CLSI, 2006; Kumar et al., 2006).

To confirm the identification of *Salmonella Typhi*, standard microbiological tests were performed on the isolated colonies. These tests included Gram staining to determine the gram-negative nature of the bacteria, as well as a series of biochemical tests such as oxidase and catalase tests, motility test, citrate utilization, methyl red test, Voges-Proskauer test, triple sugar iron (TSI) agar test, and urease test (Alam et al., 2010; Benschop et al., 2008). Each of these tests provided specific information that confirmed the presence of *Salmonella Typhi* in the blood samples.

Determination of Antibiotic Resistance Using the Kirby-Bauer Disk Diffusion Method

Antimicrobial susceptibility testing of the isolated *Salmonella Typhi* strains was conducted using the Kirby-Bauer disk diffusion method. This method was performed following the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2006). The Kirby-Bauer method involves placing antibiotic-impregnated paper discs on agar plates that have been inoculated with a standardized bacterial suspension (Boni-Cissé et al., 2012).

In this study, twelve commonly prescribed antibiotics across eight different classes were selected for testing. These included Ampicillin, Chloramphenicol, Co-trimoxazole, Azithromycin, Ciprofloxacin, Cefixime, Nalidixic acid, Ceftriaxone, Imipenem,

Meropenem, Gentamicin, and Amikacin. The bacterial inoculum was adjusted to 0.5 McFarland standard, ensuring a uniform bacterial density on the Mueller-Hinton agar plates. The antibiotic discs were then placed on the surface of the inoculated agar, and the plates were incubated at 37°C for 24 hours (Pourakbari et al., 2012). After the incubation period, the zones of inhibition around each antibiotic disc were measured in millimeters (mm). The sizes of these zones were compared to CLSI guidelines to determine whether the bacterial isolates were susceptible, intermediate, or resistant to the antibiotics tested. The isolates were classified as multidrug-resistant (MDR) if they showed resistance to at least two different classes of antibiotics (Akinyemi et al., 2012; Aggarwal et al., 2007).

Results and Discussion

In this study, we examined 200 blood samples from patients presenting with febrile illness across various hospitals in northern and southern Dhaka, Bangladesh. Of these, 56 samples (28%) were confirmed positive for *Salmonella Typhi* through microbiological and biochemical identification methods. The prevalence of *S. Typhi* infection was distributed unevenly, with 43% (24) of positive cases originating from northern Dhaka and 57% (32) from southern Dhaka. This regional disparity might reflect differences in sanitation practices, access to clean water, and healthcare infrastructure between the two areas (World Health Organization, 2018).

Colony Morphology and Biochemical Identification

The *S. Typhi* isolates displayed characteristic colony morphology when sub-cultured on XLD and MacConkey agar. On XLD agar, the colonies appeared as red with black centers, indicative of hydrogen sulfide production, while on MacConkey agar, the colonies were non-lactose fermenting and exhibited a whitish appearance. These findings are consistent with typical Salmonella morphology, reinforcing the reliability of our isolation techniques (Bauer, 1966).

Biochemical tests further confirmed the identity of *S. Typhi*. The isolates were Gram-negative, oxidase-negative, and catalase-positive, with motility observed under the microscope. The biochemical reactions aligned with standard profiles for *S. Typhi*, including positive results for citrate utilization, methyl red test, and negative results for the Voges-Proskauer and urease tests. The combination of selective media and biochemical assays provided a robust framework for accurately identifying *S. Typhi* in the clinical samples (Rowe, Ward, & Threlfall, 1997).

Antibiotic Sensitivity and Resistance Patterns

The antibiotic susceptibility of the *S. Typhi* isolates was assessed using the Kirby-Bauer disk diffusion method against twelve commonly prescribed antibiotics from eight different classes. The results revealed a concerning pattern of resistance, highlighting the ongoing challenges in treating typhoid fever in Bangladesh (Islam, Rahman, Rouf, Islam, Khaleque, Siddika, et al., 2007).

First-Line Antibiotics Resistance: All isolates showed significant resistance to the first-line antibiotics ampicillin, chloramphenicol, and co-trimoxazole, with resistance rates ranging from 70% to 80%. This resistance is indicative of the widespread use and subsequent ineffectiveness of these drugs in treating typhoid fever, a trend that has been observed in several regions across Bangladesh and the broader Southeast Asian context (Mannan, Shohel, Rajia, Mahmud, Kabir, & Hasan, 2014). Although some previous studies suggested residual sensitivity to chloramphenicol in certain Salmonella isolates from Dhaka, the current study underscores a pervasive resistance, highlighting the limited utility of these once-standard treatments (Ghurnee et al., 2021).

Fluoroquinolone Resistance: Resistance to fluoroquinolones, specifically ciprofloxacin and nalidixic acid, was also alarmingly high. Almost all isolates demonstrated no sensitivity to these antibiotics, which is particularly concerning given that ciprofloxacin has been a primary treatment option following the decline in effectiveness of first-line drugs. The resistance observed in this study aligns with global reports of declining ciprofloxacin efficacy, which poses a significant challenge for public health (Saha, Sajib, Garrett, & Qamar, 2020).

Third-Generation Cephalosporins and Carbapenems: In contrast to the high resistance rates observed with first-line and fluoroquinolone antibiotics, third-generation cephalosporins (cefixime and ceftriaxone) and carbapenems (imipenem and meropenem) exhibited high sensitivity across all isolates, with sensitivity rates between 96% and 98%. These findings confirm that these antibiotics remain effective against *S. Typhi* in Dhaka, making them crucial for current treatment regimens (Klemm et al., 2018). However, there is growing concern over the potential emergence of resistance to cephalosporins, which has already been reported in other studies and could lead to future treatment challenges (Holt et al., 2011).

Azithromycin Sensitivity: Azithromycin, often used as an alternative treatment in cases of fluoroquinolone resistance, showed moderate sensitivity among the isolates. Although this antibiotic is currently effective, the observed emergence of resistance patterns is worrisome. As azithromycin is increasingly used, its efficacy may diminish, as has been the case with other antibiotics (Afroj, Ilias, Islam, & Saha, 2011).

Aminoglycosides Resistance: The aminoglycosides, represented by amikacin and gentamicin, showed moderate resistance in the *S. Typhi* isolates. While these antibiotics are not the first choice for treating typhoid fever, their reduced effectiveness further limits the available treatment options. The moderate resistance observed aligns with other reports of aminoglycoside resistance in Salmonella

Table 1. List of antibiotics used for determining susceptibility towards *Salmonella typhi*

Antibiotic	**Short form**	**Antibiotic Classes**
Ampicillin	AMP	B-lactam
Chloramphenicol	CRO	Chloramphenicol
Cotrimoxazole	SXT	Sulfonamide
Azithromycin	AZI	Macrolides
Ciprofloxacin	CIP	Fluoroquinolones
Nalidixic acid	NAL	
Cefixime	CEFI	3rd generation Cephalosporin
Ceftriaxone	CFT	
Imipenem	IPM	Carbapenem
Meropenem	MEM	
Amikacin	AMK	Aminoglycosides
Gentamycin	GEN	

Table 2. Morphological and Cultural Characteristics of *Salmonella*

	Morphological characteristics	**Cultural characteristics**	
	Gram staining	**XLD Agar**	**MacConkey Agar**
Characteristics of *Salmonella* Positive isolates	Gram negative, Bacillus	Circular, Small, Red colored colonies with blackish center	Circular, Medium, Translucent, Non-lactose fermenting colonies
	Biochemical characteristics		

Catalase	oxidase	TSI	Motility	MR	VP	Indole	Citrate	Urease
+	-	Acid butt Alk slant H_2S + Gas -	+	+	-	-	-	-

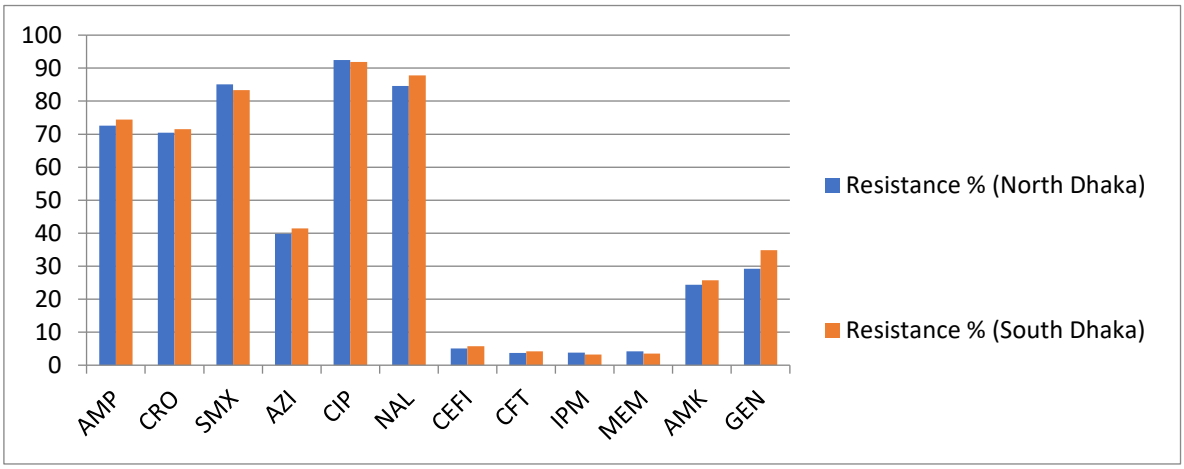

Figure 1. Determination of resistance pattern of test isolates

strains, emphasizing the necessity for careful antibiotic selection (Akter, Hossain, Khan, Al Sanjee, Fatema, & Datta, 2016).

Comparison with Previous Studies

The results of this study are consistent with earlier research conducted in Dhaka and other regions of Bangladesh, which have reported similar patterns of multidrug-resistant (MDR) *S. Typhi*. The high prevalence of resistance to first-line antibiotics and fluoroquinolones mirrors findings from other studies, reinforcing the notion that *S. Typhi* in this region has developed significant resistance due to overuse and misuse of antibiotics (Shahriar & Kabir, 2010). The retention of sensitivity to third-generation cephalosporins and carbapenems is reassuring, though the potential for resistance development requires continuous monitoring (Boni-Cissé et al., 2012).

Previous studies have documented sporadic cases of cephalosporin resistance, particularly in areas with high antibiotic use, suggesting that even these more advanced antibiotics could eventually become less effective (Djeghout et al., 2018). The emergence of resistance to azithromycin, although moderate at present, highlights the dynamic nature of antibiotic resistance and the need for ongoing vigilance in tracking these trends (Afroz et al., 2013).

Implications for Public Health and Treatment Strategies

The findings from this study have significant implications for public health in Bangladesh. The high rates of multidrug resistance observed in *S. Typhi* isolates underscore the urgent need for revised antibiotic prescribing practices and more stringent guidelines to prevent further resistance development. Public health initiatives should focus on educating healthcare providers and the general public about the dangers of antibiotic misuse, as well as promoting the use of effective treatment options based on current resistance patterns (Aggarwal, Vij, & Oberoi, 2007; Mohammad et al., 2020).

There is a clear need for continued research into alternative treatment strategies, including the development of new antibiotics and the exploration of combination therapies that might mitigate the risk of resistance. The high sensitivity observed with third-generation cephalosporins and carbapenems suggests that these drugs should be prioritized in treatment protocols, but their use must be carefully managed to preserve their effectiveness (Pourakbari et al., 2012).

Conclusion

This study highlights the significant public health challenge posed by multidrug-resistant *Salmonella Typhi* in Dhaka, Bangladesh. With a 28% prevalence rate among febrile patients, and high resistance to first-line antibiotics and fluoroquinolones, the findings underscore an urgent need for revised treatment strategies. Third-generation cephalosporins and carbapenems remain effective, but their potential for resistance emergence necessitates careful use. The moderate sensitivity to azithromycin further complicates treatment options. Addressing this issue requires tailored antibiotic guidelines, public awareness campaigns, and the development of novel therapeutic agents to ensure effective management of typhoid fever in this region. Continuous surveillance of antibiotic resistance patterns is essential to ensure that treatment strategies remain effective and that the public health burden of typhoid fever is minimized.

Author contributions

S.D., conceptualized and developed the methodology, D.R.B. and B.J., prepared the original draft and collected, M.B.Z. and K.R.M., reviewed and edited the writing.

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Competing financial interests

The authors have no conflict of interest.

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