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Frequency of Antimicrobial-Resistant *Salmonella Typhi* Among Blood Culture–Positive Patients Across Age and Sex Groups in Mohakhali, Dhaka, Bangladesh

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Abstract

Antimicrobial-resistant Salmonella Typhi has emerged as a significant clinical and public health concern in developing countries, including Bangladesh. This study was conducted to determine the frequency of antimicrobial-resistant S. Typhi isolated from blood samples of patients in Dhaka city and to evaluate their antimicrobial susceptibility patterns using conventional cultural, biochemical, serological, and antibiogram methods. Blood culture–confirmed S. Typhi isolates were identified following standard laboratory procedures, and antimicrobial susceptibility testing was performed using the disc diffusion method. Resistance among the isolates varied across the tested antibiotics, with complete susceptibility observed to meropenem and the highest resistance noted against sulfamethoxazole. Gentamicin demonstrated high effectiveness, while a considerable proportion of isolates exhibited multidrug resistance, defined as resistance to five or more commonly used antibiotics. Despite widespread clinical use, most isolates remained susceptible to ciprofloxacin. The findings indicate a high burden of multidrug-resistant S. Typhi in this setting, emphasizing the need for continuous antimicrobial resistance surveillance, rational antibiotic use, and evidence-based treatment strategies to ensure effective management of typhoid fever.

Keywords: Typhoid fever; Salmonella Typhi; Antimicrobial resistance; Multidrug resistance; Blood culture

Introduction

Typhoid fever remains a major public health concern in low- and middle-income countries, particularly in South Asia, where inadequate sanitation, unsafe water supplies, and overcrowded living conditions facilitate sustained transmission [1]. The disease continues to impose a substantial burden in terms of morbidity, mortality, healthcare expenditure, and socioeconomic loss. Bangladesh is recognized as a highly endemic setting, with typhoid fever affecting individuals across all age groups, although children and young adults are disproportionately impacted [2,3]. The etiological agent, Salmonella enterica serovar Typhi, is a Gram-negative, motile, non-spore-forming bacillus belonging to the family Enterobacteriaceae. It is a strictly human-adapted pathogen transmitted via the fecal–oral route, primarily through contaminated food and water [4]. Following intestinal invasion, the organism can enter the bloodstream, leading to systemic infection characterized by prolonged fever and potentially severe complications if untreated. Virulence factors such as endotoxin, capsular Vi antigen, and intracellular survival

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mechanisms contribute to its pathogenicity and clinical severity [5,6]. Appropriate antimicrobial therapy has historically been the cornerstone of typhoid fever management and has significantly reduced case fatality rates. However, the progressive emergence and spread of antimicrobial-resistant S. Typhi have undermined treatment effectiveness and complicated disease control efforts [7]. Resistance to conventionally used first-line agents—including ampicillin, chloramphenicol, and co-trimoxazole—has been increasingly documented in Bangladesh since the early 1990s, followed by reduced susceptibility to fluoroquinolones and other commonly prescribed antibiotics. Multidrug-resistant strains are associated with prolonged illness, higher rates of complications, increased treatment costs, and limited therapeutic options [8,9].

Blood culture remains the most widely used and practical diagnostic method for typhoid fever, particularly during the early phase of illness when bacteremia is most pronounced. Although more invasive specimens such as bone marrow provide higher diagnostic yield, blood culture continues to be the primary tool in routine clinical practice and plays a critical role in antimicrobial susceptibility testing [10]. Surveillance of resistance patterns using blood isolates is therefore essential for guiding empirical therapy and informing local treatment guidelines8.

Given the dynamic nature of antimicrobial resistance and the heterogeneity of affected populations, continuous, location-specific data are required to understand current resistance trends and their distribution across different demographic groups [11]. The Mohakhali area of Dhaka city represents a densely populated urban setting where such data are particularly relevant for public health planning and clinical decision-making. This study aimed to determine the frequency of antimicrobial-resistant Salmonella Typhi isolated from blood samples of patients across different age and sex groups in the Mohakhali area of Dhaka city, Bangladesh.

Materials and Methods

This laboratory-based cross-sectional study was conducted in the Department of Microbiology, Primeasia University, Dhaka, Bangladesh, on blood samples collected from patients clinically suspected of typhoid fever in the Mohakhali area of Dhaka city. All microbiological procedures were performed under strict aseptic conditions following standard laboratory protocols. All culture media and aqueous solutions were sterilized by autoclaving at 121 °C under 15 psi pressure for 15 minutes. Glassware was sterilized in a hot air oven at 180 °C for two hours prior to use. Prepared agar plates and slants were stored at 4 °C and brought to room temperature and dried under aseptic conditions before inoculation. MacConkey agar, Salmonella–Shigella agar, Mueller–Hinton agar, Mueller–Hinton broth, Kligler's iron agar, Simmons' citrate agar, and motility–indole–urease medium were used for the isolation and identification of bacterial isolates. All culture media were prepared according to the manufacturers' instructions. Venous blood samples were collected aseptically using sterile disposable vacutainer systems and inoculated into blood culture bottles. The bottles were loaded into an automated blood culture system following standard operating procedures after disinfecting the bottle caps with alcohol swabs. Bottles flagged positive by visual and audible alerts were promptly removed for further microbiological processing. Positive blood culture samples were subcultured onto MacConkey agar and Salmonella–Shigella agar plates and incubated aerobically at 37 °C for 18–24 hours. Plates were examined for the presence of non-lactose-fermenting pale colonies suggestive of Salmonella species. Presumptive isolates were identified using standard biochemical tests, including Kligler's iron agar reaction, citrate utilization, urease test, indole production, and motility testing. Pure cultures were maintained on MacConkey and Salmonella–Shigella agar plates and stored at 4–8°C until further analysis. Serological confirmation was performed using slide agglutination tests with commercially available Salmonella O, H, and Vi antisera. A smooth bacterial suspension was prepared in normal saline on a clean glass slide and mixed with specific antisera. The slide was gently rocked for up to two minutes, and visible agglutination was considered a positive reaction. Serotyping was carried out accordingly.

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar. Fresh bacterial colonies were inoculated into Mueller–Hinton broth and incubated to achieve turbidity equivalent to a 0.5 McFarland standard. The standardized inoculum was evenly spread over the agar surface using sterile cotton swabs, and antibiotic-impregnated discs were placed aseptically on the plates. The plates were incubated at 37 °C for 18–24 hours. After incubation, the diameters of zones of inhibition were measured in millimetres, and isolates were categorized as susceptible, intermediate, or resistant according to standardized interpretive criteria. All culture media, biochemical reactions, and antimicrobial susceptibility tests were routinely quality controlled using standard reference strains to ensure the accuracy and reproducibility of the results.

Results

A total of 80 confirmed *Salmonella Typhi* isolates obtained from blood samples of patients with clinically suspected typhoid fever were included in this study. All isolates were subjected to cultural and biochemical characterization for definitive identification. On selective and differential media, *S. Typhi* produced moderately large, moist, smooth, dome-

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shaped circular colonies. On Salmonella–Shigella (SS) agar, colonies appeared grayish-white, while on MacConkey agar they were pale or colorless, indicating non-lactose-fermenting characteristics consistent with *Salmonella* species. Biochemical identification demonstrated a uniform reaction pattern among the isolates. All tested isolates showed an acid butt and alkaline slant with hydrogen sulfide (H_2S) production on Kligler's iron agar, were methyl red positive, citrate negative, Voges–Proskauer negative, indole negative, catalase positive, and oxidase negative. These biochemical profiles confirmed the identity of the isolates as *Salmonella Typhi* (Table 1). Representative Muller Hington agar plate showing no zone of inhibition for *Salmonella typhi* & *Salmonella paratyphi A* (Figure 1).

Table 1: Biochemical characteristics of Salmonella Typhi isolates.

Test	Reaction
Kligler's Iron Agar (Butt)	Acid
Kligler's Iron Agar (Slant)	Alkaline
H_2S production	Positive / Variable
Citrate utilization	Negative
Methyl Red (MR)	Positive
Voges–Proskauer (VP)	Negative
Indole production	Negative
Catalase	Positive
Oxidase	Negative

Figure 1: Antimicrobial resistance pattern on Mueller–Hinton agar.

Table 2 shows the zone of inhibition diameter ranges (in millimeters) used in this study to interpret antimicrobial susceptibility results, based on measured inhibition zones obtained from disc diffusion testing of *Salmonella isolates*.

The emergence of multidrug-resistant *Salmonella Typhi*, particularly resistance to ampicillin, chloramphenicol, and co-trimoxazole, has led to increased reliance on fluoroquinolones for the treatment of typhoid fever. However, reduced susceptibility and resistance to fluoroquinolones were also observed, indicating declining effectiveness of this class of antibiotics. This study, conducted between January and June 2011 in Dhaka city, evaluated the prevalence and antimicrobial susceptibility patterns of *S. Typhi* isolates among pediatric patients and assessed phenotypic characteristics associated with multidrug resistance, including resistance to amoxicillin and ciprofloxacin (Figure 2).

Table 2: Standard interpretive criteria for zones of inhibition (mm).

Antibiotic (disc content)	Sensitive (mm)	Intermediate (mm)	Resistant (mm)
Amoxiclav (16 µg)	≥16	14–15	≤13
Ceftriaxone (15 µg)	≥18	14–17	≤13
Cefixime (30 µg)	≥18	16–17	≤15
Cefotaxime (30 µg)	≥21	15–20	<14
Azithromycin (30 µg)	≥18	15–17	≤14
Co-trimoxazole (10 µg)	>18	15–17	<14
Gentamicin (5 µg)	≥18	14–17	≤13
Ciprofloxacin (25 µg)	≥20	13–19	≤12
Levofloxacin (20 µg)	≥15	13–14	≤12
Meropenem (20 µg)	≥18	16–17	≤15

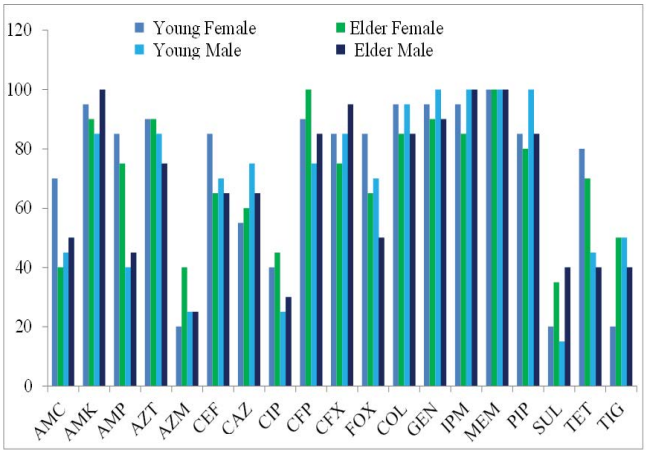

Figure 2: Percentage (%) sensitivity of *Salmonella Typhi* to tested antibiotics

Presumptive S. *Typhi* isolates were selected based on typical colony morphology and subsequently confirmed by biochemical and serological testing. Antimicrobial susceptibility analysis showed a wide variation in resistance, ranging from 0% resistance to meropenem to 68.75% resistance to sulfamethoxazole. Meropenem demonstrated 100% sensitivity, making it the most effective agent, followed by gentamicin with 93.75% sensitivity against the tested S. *Typhi* isolates (Figure 3).

In Figure 4, multidrug resistance was observed in 11 isolates (55%) among elderly females, 18 isolates (90%) among elderly males, and 12 isolates (60%) among young male and female patients, indicating a higher prevalence of MDR strains in elderly male patients.

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Figure 3: Percentage (%) sensitivity of *Salmonella typhi* to antibiotics.

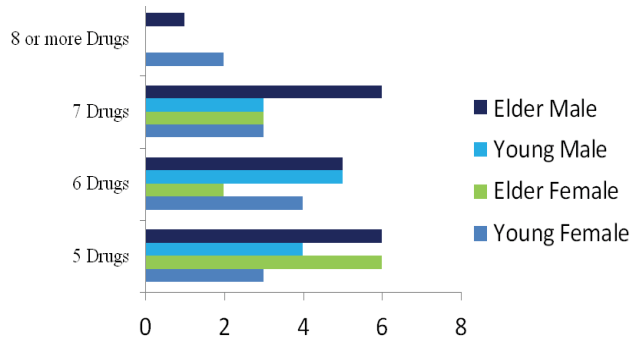

Figure 4: Multidrug resistance (MDR) pattern of *Salmonella Typhi* isolates.

Discussion

The present study revealed a substantial burden of antimicrobial resistance among *Salmonella Typhi* isolates recovered from blood samples of patients with clinically suspected typhoid fever in Dhaka city. Of the 80 confirmed isolates analyzed, 66.25% were multidrug resistant, defined as resistance to five or more of the tested antimicrobial agents. This high proportion of multidrug-resistant strains underscores the increasing difficulty in managing typhoid fever in endemic settings and reflects the progressive erosion of therapeutic efficacy of commonly used antibiotics. Antimicrobial susceptibility testing demonstrated marked variability in resistance patterns. While meropenem showed 100% sensitivity, indicating complete effectiveness against all tested isolates, resistance was highest to sulfamethoxazole (68.75%), highlighting the limited utility of several first-line and commonly prescribed agents. Gentamicin retained high activity (93.75% sensitivity), suggesting its continued effectiveness as a treatment option in severe infections. In contrast, the observed reduction in susceptibility to fluoroquinolones, including ciprofloxacin, raises concern given their widespread use as empirical therapy for typhoid fever [12,13]. The high prevalence of multidrug resistance has led to increased reliance on fluoroquinolones; however, this study demonstrated emerging resistance to this class as well. Investigation of ciprofloxacin resistance mechanisms revealed that resistance persisted following plasmid curing, indicating that resistance was not plasmid mediated. This finding suggests that chromosomal mutations or efflux pump mechanisms are likely responsible for fluoroquinolone resistance in the studied isolates, contributing to stable and clinically significant resistance [14].

Age- and sex-specific analysis of multidrug resistance patterns showed notable demographic variation. Multidrug resistance was detected in 55% of isolates from elderly female patients, 90% from elderly male patients, and 60% from younger male and female patients, indicating a particularly high burden of MDR *S. Typhi* among elderly males [15]. Additionally, a higher incidence of typhoid fever was observed among young children, consistent with increased exposure risks, immature immune responses, and environmental vulnerability in this age group. The dissemination of antimicrobial resistance in *S. Typhi* may occur through clonal expansion of resistant strains or through independent acquisition of resistance determinants mediated by mobile genetic elements such as transposons and integrons [12]. Detailed molecular epidemiological studies are essential to elucidate transmission pathways and resistance evolution. Molecular typing techniques with high discriminatory power and reproducibility would be particularly valuable for tracking resistant clones within endemic communities [16]. Control of typhoid fever requires an integrated approach that extends beyond antimicrobial therapy. Improvements in water quality, sanitation, hygiene practices, vaccination coverage, and early laboratory-based diagnosis are critical to reducing disease transmission [13]. The findings of this study further emphasize the need for rational antibiotic use, ongoing antimicrobial resistance surveillance, and regular revision of treatment guidelines based on local susceptibility data to prevent further escalation of resistance and to preserve the effectiveness of remaining therapeutic options [17].

Conclusion

Typhoid fever remains a significant public health problem in developing countries due to the emergence of multidrug-resistant *Salmonella Typhi*. Although ciprofloxacin remains largely effective, continuous antimicrobial surveillance and rational antibiotic use are essential to ensure effective treatment and limit further resistance.

Limitations

This study was conducted at a single center with a limited sample size, which may restrict the generalizability of the findings. Molecular characterization of resistance mechanisms was not performed, limiting detailed understanding of genetic determinants of antimicrobial resistance.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this study.

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