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Prevalence and Characterization of Pathogens Responsible for Enteric Fever and Assessment of Their Antibiotic Susceptibility Pattern in Bangladesh

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Abstract

In Bangladesh, Enteric fever is a persistent health issue throughout the year, occasionally reaching epidemic levels. The growing antibiotic resistance makes treatment increasingly challenging. Therefore, studying the antibiotic resistance patterns within the population is crucial to ensure a more effective treatment strategy. This study was designed to determine the prevalence, antimicrobial susceptibility profile, and factors associated with *Salmonella spp.* infections among clinically suspected Enteric fever patients in Bangladesh. This study also aimed to investigate whether there has been a re-emergence in the susceptibility of bacterial strains to conventional drugs. Data were collected from February 2024 to July 2024, from patients suspected Enteric fever (fever less than seven days duration) in a Private Diagnostic Center of Bangladesh. A total of 195 blood samples were cultured, where 53.85% came out positive, among which 79.05% *Salmonella typhi* and 20.95% *Salmonella paratyphi* A were found. Prevalence of Typhoid fever was observed high among the school-going age group (0 - 15 years) patients. Both these organisms were susceptible to ceftazidime, cefixime, ceftriaxone and cefepime but resistant to nalidixic acid, ciprofloxacin and levofloxacin. Nalidixic Acid is resistant to all *S. paratyphi* A and sensitive to few *S. typhi*. Ciprofloxacin and levofloxacin showed delayed response (36.14% and 22.72% sensitive to *S. typhi* and *S. paratyphi* A, respectively) and resistance (63.85% and 77.27% resistant to *S. typhi*

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and *S. paratyphi* A, respectively). In case of *S. typhi*, the resistance was found against ampicillin (32.53%), chloramphenicol (27.71%), cotrimoxazole (24.09%) and the resistance of *S. paratyphi* A found against ampicillin (4.54%), chloramphenicol (0%) and cotrimoxazole (0%). This study will provide clinicians with alternative drug options and facilitate the effective treatment of Enteric fever.

Keywords

Typhoid, *Salmonella*, Nalidixic Acid, Ciprofloxacin, Chloramphenicol, Cotrimoxazole

1. Introduction

Enteric fever (typhoid and paratyphoid fever) is a serious bloodstream infection caused by *Salmonella* enteric serovar typhi (*S. typhi*) and paratyphi (*S. paratyphi*) A, B and C [1]. Enteric fever is transmitted predominantly by the fecal-oral route and manifests with several clinical outcomes including malaise, fever, chills, nausea, abdominal discomfort, transient rash and hepatosplenomegaly [2]. In spite of increased sanitation, personal hygiene, and availability of effective treatment, enteric fever remains a serious health problem in developing countries. An estimated 11.9 - 20.6 million cases of typhoid and paratyphoid fever, with recorded mortality of 129,000 - 223,000 are reported annually from developing countries [3]. Moreover, a large proportion of these cases and mortalities is concentrated in South Asia, where it exhibits seasonal variation, peaking in the rainy season, from June to August [4]. The Global Burden of Disease study estimates that typhoid has claimed the lives of more than 110,000 individuals worldwide, with approximately 9 million cases reported annually [5]. Among the countries affected, Bangladesh is significantly impacted, with an incidence rate of 252 per 100,000 people each year [6]. Antimicrobial resistance occurs when microorganisms such as bacteria, viruses, fungi, and parasites develop the ability to survive and continue to grow despite being targeted by drugs designed to eliminate them [7]. Infections caused by antimicrobial-resistant organisms are not only challenging to treat but also carry a higher risk of severe illness and death [5]. However, the prevalence of *Salmonella* varies across different regions [8]. *Salmonella* is one of the most frequently reported zoonotic pathogens, and the antimicrobial-resistant (AMR) strains of *Salmonella* pose a significant concern for public health [9]. South Asia is at high risk in terms of the emergence and spread of antimicrobial resistance. Despite the increasing knowledge of the prevalence of *Salmonella* and its AMR profile which is mostly reported by individual and local surveillance study, a comprehensive and robust understanding of the prevalence and AMR patterns in South Asia remains poorly characterized [10]. Antimicrobials such as cephalosporins, ampicillin, and fluoroquinolones are the preferred drugs for treating enteric fever and have proven to be highly effective [7]. Without antibiotic therapy, the case fatality rate is estimated to be 10% - 30%, but with proper treatment, it drops to 1% - 4% [11]. However, the extensive and irrational prescription and use of these drugs have led to the emergence and spread of drug resistance, often referred to as multidrug resistance (MDR) in pathogenic strains of *Salmonella* [12]. MDR strains contribute to treatment failures, limit drug regimen options, and increase the severity and mortality of infections [13]. The emergence of drug resistance among *Salmonella* isolates dates back to the late 1980s when traditional first-line drugs (chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole) became ineffective due to antibiotic resistance, forcing clinicians to rely on fluoroquinolones, particularly ciprofloxacin [14]. However, the recent global surge in resistance to fluoroquinolones could lead to a catastrophic rise in infectious diseases worldwide [15]. The most effective way to reduce the burden of typhoid and other waterborne diseases is by ensuring that all households have access to safe, uncontaminated drinking water. This involves not only constructing new water and sewage treatment plants but also regularly maintaining the existing ones to ensure they function properly [16]. Traditional typhoid vaccines have several limitations that have hindered their inclusion in routine immunization programs. These vaccines exhibit only modest efficacy, provide short-term protection, and are not approved for use in young children, making them unsuitable for programs like the Expanded Program on Immunization (EPI). As a result, these typhoid vaccines have not been widely used in either the public or private sectors in Bangladesh [16].

Thus, the present study was intended to determine the prevalence, antimicrobial susceptibility profile, and factors associated with *Salmonella spp*. infections among patients clinically suspected Enteric fever at Popular Medical College Hospital, Dhanmondi, Dhaka, Bangladesh. This study also aimed to investigate whether there has been a re-emergence of bacterial strain susceptibility to conventional drugs.

2. Materials and Methods

2.1. Study Area and Population

195 blood samples were collected from the patients suspected Enteric fever using aseptic techniques. A sterile syringe will be used to withdraw 20 ml of blood from each of the adult patients and 2 - 4 ml from the child and transferred into commercially prepared sterile EDTA bottles. Suspicion of Enteric fever was based on symptoms commonly reported in Bangladesh. The presence of one or more of these symptoms, such as sustained fever (103˚F - 104˚F), weakness, stomach ache, headache, diarrhea or constipation, cough, and loss of appetite were used to suspect Enteric fever. Any person with a fever was suspected of having Enteric fever unless it could be clinically attributed to other conditions. The study included suspected patients of all ages and genders who provided written informed consent for voluntary participation. However, patients with incomplete demographic information and those with ongoing or prior antibiotic therapy were excluded. A well-structured, pre-tested questionnaire was administered to each subject to

record demographic information, clinical history, and prior antibiotic use. Only the recovered *Salmonella* isolates were processed for further investigation, and duplication of isolates from the same patient was avoided.

2.2. Isolation of Pathogen

Blood (20 ml) was collected after making the venipuncture site sterile which was then put in the special blood collection bottles. Blood samples were then subjected to automated blood culture using the BD BACTEC 9120 apparatus, with aseptic conditions strictly maintained throughout the process. After collection, the bottles were placed in the BD BACTEC 9120 machine, where they were continuously incubated and agitated. When a bottle tested positive for bacterial growth, both the machine and the connected computer indicated the growth with an alarm message on the screen and a green light on the machine.

2.3. Subculture

Blood is taken from the bottle by syringe and placed into different growth media—Blood agar and MacConkey agar. Blood samples were directly inoculated onto MacConkey agar and blood agar plates. The plates were then incubated overnight at 37˚C and examined for non-lactose fermenting colonies. Blood agar plates were used to observe non-hemolytic smooth white colonies, while MacConkey agar plates were used to identify non-lactose fermenting colonies [17]. Identification of the isolates was based on colony morphology, Gram staining, and a series of biochemical tests, including the catalase test, oxidase test, citrate utilization test, triple sugar iron (TSI) test, sulfide indole motility (SIM) test, and urea hydrolysis (urease) test. Serotyping of the isolates was further performed using the agglutination method with *Salmonella* polyvalent antisera O, and monovalent O: 2, O: 9, O: 12, and Vi to confirm different serovars [17].

2.4. Antibiotic Sensitivity Testing

The susceptibility pattern was determined using the disk diffusion method. All isolated organisms were subjected to an antibiotic susceptibility test using the Kirby-Bauer disk diffusion technique. The tests were performed and interpreted following the recommendations of the Clinical and Laboratory Standards Institute [18]. All tests were conducted on Mueller-Hinton agar plates (pH 7.2 - 7.4). The agar surface was lightly and uniformly inoculated using a sterile cotton swab. Before inoculation, the swab was dipped into a bacterial suspension with turbidity visually equivalent to 0.5 McFarland standards. The swab was then removed and pressed against the tube wall to remove any excess suspension. The inoculated plates were incubated at 37˚C for 24 hours.

3. Results and Discussion

Enteric fever is one of the major endemic diseases of low-to-middle-income countries like Bangladesh. In this study, a total of 105 (53.85%) were culture-positive out of the total 195 blood cultures. This indicates that blood culture and antimicrobial susceptibility are essential for a definitive diagnosis of Enteric fever. Geographical location may be the reason for the difference in the percentage of the population infected with *Salmonella* infection, the pattern of pathogenic organisms and the resistance pattern of different organisms.

This study identified *S. typhi* as the most common isolate, accounting for 83 cases (79.05%), while *S. paratyphi* A accounted for 22 cases (20.95%) (**Table 1**). These findings align with similar studies both locally and internationally. For instance, a study conducted in Nepal reported isolation rates of 72.5% for *S. typhi* and 27.5% for *S. paratyphi* [19]. Factors contributing to the high endemicity of these diseases include densely populated urban areas with limited access to safe drinking water and sanitation, low socio-economic status, inadequate surveillance, and poor infection control [20].

Table 1. Distribution of positive isolates identified from blood samples.

Name of the Isolates	Frequency	Percentage
Salmonella typhi	83	79.05%
Salmonella paratyphi A	22	20.95%

In our study, the incidence of typhoid and paratyphoid fever was notably higher in July. Although Enteric fever cases occur sporadically throughout the year, they tend to peak during the summer and rainy seasons [21]. Multiple climate factors, such as increased rainfall, rising river levels, and temperature changes, have been shown to increase the distribution of typhoid in Bangladesh, which receives an average of 2200 mm of rainfall annually. This heavy rainfall places a significant strain on water, sanitation, and hygiene infrastructures [5]. During these periods, floods and the contamination of water sources due to seepage from treatment plants or sewers can increase the risk of typhoidal and para-typhoidal infections.

Although there was no significant association between gender and the incidence of the disease, our study observed a higher incidence in males (54.29%) compared to females (45.71%) (**Table 2**). This higher incidence among males may be attributed to their greater involvement in outdoor activities, which increases their exposure to sources of infection. Both *S. typhi* and *S. paratyphi* affected males and females nearly equally.

Table 2. Frequency of pathogens isolated from blood samples and their relationship with gender.

Name of the Isolates	Male		Female	
	No. of Isolates	(%)	No. of Isolates	(%)
Salmonella typhi	46	55.42	37	44.58
Salmonella paratyphi A	11	50	11	50
Total	57	54.29	48	45.71

In this study, 25 (23.81%) of cases of Enteric fever were in the age group (16 - 30 years old) (**Figure 1**). It may be due to the fact that pure water supply, safe sanitary disposal of excreta and improved food trade practices, or high standards in handling, processing, and storage of food are not ensured in our country. Among different age groups, prevalence was comparatively higher in the (0 - 15) age group which accounts for 36 (34.29%) positive patients. Such results might be due to the fact that school-age children had more chances of having unsafe drinking water and contaminated food at school from vendors on the streets.

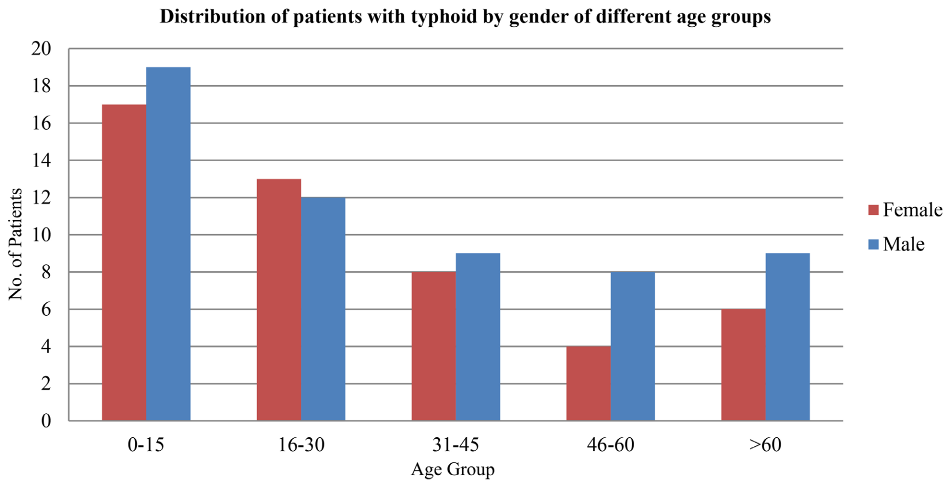

Figure 1. Distribution of patients with typhoid by gender of different age groups.

Similar results have been reported elsewhere that subjects within the age group of > 12 - 14 years are exposed to typhoid infection, reported that most infected cases occurred among patients who were below 14 years old, while less infection occurred in patients aged more than 14 years old. This fact may be related to the immunity of the children [22].

High levels of antibiotic resistance were observed among isolates to commonly used antibiotics in the study area. Notably, no resistance was found against cefixime, ceftazidime, ceftriaxone, and cefepime (**Table 3**). In South Asia, cephalosporins such as ceftriaxone and cefixime are the primary treatments for Enteric fever and are often used empirically, which may contribute to increasing resistance to typhoid causing pathogens [23].

Table 3. Antibiotic sensitivity pattern for the typhoid isolates.

Antibiotic	Sensitivity	*S. typhi* (N = 83)	*S. paratyphi* A (N = 22)
Ampicillin	S	56 (67.46)	21 (95.45)
	R	27 (32.53)	1 (4.54)
Ceftriaxone	S	83 (100)	22 (100)
	R	0 (0)	0 (0)

Continued

Cefixime	S	83 (100)	22 (100)
	R	0 (0)	0 (0)
Ceftazidime	S	83 (100)	22 (100)
	R	0 (0)	0 (0)
Cefepime	S	83 (100)	22 (100)
	R	0 (0)	0 (0)
Cotrimoxazole	S	63 (75.90)	22 (100)
	R	20 (24.09)	0 (0)
Nalidixic Acid	S	11 (13.25)	0 (0)
	R	72 (86.75)	22 (100)
Ciprofloxacin	S	30 (36.14)	5 (22.72)
	R	53 (63.85)	17 (77.27)
Levofloxacin	S	33 (39.76)	5 (22.72)
	R	50 (60.24)	17 (77.27)
Chloramphenicol	S	60 (72.29)	22 (100)
	R	23 (27.71)	0 (0)
Moxifloxacin	S	82 (98.79)	22 (100)
	R	1 (1.20)	0 (0)

A significant proportion of the isolates showed resistance to nalidixic acid 86.75%, with 63.85% and 60.24% exhibiting reduced susceptibility to ciprofloxacin and levofloxacin, respectively. Although no significant association was found between serotype and antibiotic susceptibility, *S. paratyphi* A strains demonstrated a higher rate of nalidixic acid resistance compared to *S. typhi*. Resistance to nalidixic acid is linked to decreased fluoroquinolone susceptibility and the potential development of fluoroquinolone resistance [24]. Researchers have recommended routine screening for nalidixic acid resistance in *S. typhi* isolates to alert physicians to the possible failure of ciprofloxacin therapy in patients with Enteric fever.

Fluoroquinolones, particularly nalidixic acid, are commonly used to treat enteric fever in lower-middle-income countries due to their cost-effectiveness, accessibility, and availability in oral formulations [19]. However, their effectiveness is increasingly compromised by the rise of nalidixic acid-resistant *Salmonella* strains. Genetic factors, such as mutations in the genes coding for DNA gyrase (gyrA and gyrB) and topoisomerase IV (parC and parE), are believed to contribute to the emergence of nalidixic acid resistance [25]. While genetic factors likely play a significant role in the high prevalence of nalidixic acid-resistant *Salmonella* observed in our study, investigating Genetic mechanisms contributing to resistance was beyond the scope of our study design. Consistent with several previous studies [26], we also observed a high prevalence of quinolone and nalidixic acid-resistant strains, while susceptibility rates were notably higher for conventional first-line drugs (chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole) and third-generation cephalosporins (ceftriaxone, ceftazidime, and cefixime).

For *S. typhi*, resistance was observed against ampicillin (32.53%), chloramphenicol (27.71%), and cotrimoxazole (24.09%). In contrast, *S. paratyphi* A showed resistance to ampicillin (4.54%), with no resistance to chloramphenicol or cotrimoxazole. Another study conducted in Bangladesh reported decreased resistance rates to ampicillin (100%), chloramphenicol (15.04%), and cotrimoxazole (17.27%) [27]. This rare re-emergence of susceptibility may be due to the prolonged underuse of conventional antibiotics. Additionally, the loss of high molecular weight self-transmissible plasmids that induce resistance in pathogenic strains over time due to evolution and mutation could also explain this phenomenon [26].

Recent studies have indicated that strains previously resistant to first-line drugs (chloramphenicol, ampicillin, and cotrimoxazole) are now showing decreased resistance. This reduction in resistance may be attributed to the withdrawal of selective pressure, leading to the re-emergence of sensitivity to these drugs. For instance, a study conducted in Punjab (India) reported high sensitivity rates of 93.2%, 86.2%, and 71.3% for chloramphenicol, cotrimoxazole, and ampicillin, respectively [28].

The lower cost and availability of these antibiotics in developing countries, combined with their well-established clinical efficacy, make the reuse of chloramphenicol or ampicillin advantageous. Therefore, continuous surveillance and rigorous audits of antibiotic sensitivity testing are essential to assess whether these first-line drugs can be reintegrated into treatment regimens in specific regions.

When infections develop resistance to first-line antimicrobials, treatment often shifts to second or third-line drugs, which are typically more expensive [29]. In many low-income countries, the high cost of these alternative drugs poses a significant challenge, making it difficult to treat diseases effectively in areas with widespread resistance to first-line treatments [30]. The alarming challenge facing physicians and pharmacists now is the need to develop alternative approaches in addition to the search for new antimicrobial compounds.

4. Conclusion

According to our findings, nalidixic acid is resistant to all *S. paratyphi* A and sensitive to a few *S. typhi*. Ciprofloxacin and levofloxacin showed delayed response and resistance in many cases. So, ciprofloxacin and levofloxacin cannot be potential treatment options due to their resistance. In this study, no resistance was found against cefixime, ceftazidime, ceftriaxone and cefepime. The result of this study indicates that first-line drugs (chloramphenicol, ampicillin and cotrimoxazole), which are no longer routinely used for the treatment of Enteric fever, have proved to be decreasing resistance against these isolates. This observation is that an organism that is previously resistant to a particular antibiotic may become susceptible if treatment with the antibiotic is suspended for a long time. However, more studies are recommended in this regard.

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Conflicts of Interest

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

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