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Isolation of Multi-Drug Resistant (MDR) and Extensively Drug Resistant (XDR) *Salmonella typhi* from Blood Samples of Patients Attending Tertiary Medical Centre in Dhaka City, Bangladesh

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Abstract

Objective: Almost all year round occurrence of typhoid fever remains a major public health issue in Bangladesh. In recent years, there has been a significant rise in the prevalence of multi-drug resistance *Salmonella typhi* in Dhaka city. The major objective of this study was to observe the prevalence of multi-drug and Extended drug-resistant patterns of typhoidal *Salmonella typhi* among the patients attending a tertiary medical center by conventional culture and serologic test. **Materials and Methods:** Blood was collected by syringe and equally divided between aerobic & anaerobic BacT Alert bottle, 10 mL for paediatrics. Post isolation *Salmonella* strains were identified and were tested for antibiotic resistance. **Results:** A total of 600 blood samples of typhoid suspected patients were tested, where 32.5% came out positive, which were identified as *Salmonella typhi.* These isolates were susceptible to carbapenem drugs but highly resistant to Fluoroquinolones and Aminoglycosides antibiotics. **Discussion:** It was seen that 73.23% of isolates among male patients were extensively drug-resistant (XDR) meaning that isolates were sensitive against only one or two antibiotics while in the female it was 58.46%. Isolates from Female patients showed higher multi-drug resistant (MDR) (26.15%) isolates resistance to multiple antibiotics in comparison to isolates from the male (16.54%). 87.19% of the strains showed high drug resistance (XDR and MDR). **Conclusion:** The emergence of XDR and MDR strains of *S. typhi* in recent years is becoming a significant threat. Although no PAN-Drug resistance (resistant against all tested drugs) was observed in the current

study, a high level of XDR and MDR isolates are indicating that the scenario might take place sooner than later.

Keywords

MDR, XDR, *S. typhi*, Blood Samples, Typhoid Fever, Bangladesh

1. Introduction

Worldwide, approximately 11 - 21 million cases and 128,000 - 161,000 deaths occur due to typhoid fever, annually. Whereas, estimated 6 million cases and 54,000 deaths happen because of paratyphoid fever every year [1] [2]. Typhoid fever is a lethal illness with a case fatality rate of 10% - 30% if it is not treated. This may drop to 1% - 4% with suitable therapy [3]. Although this disease is rare in developed countries, it persists to be a major health problem and poses the risk of emergence due to the continued existence of poor hygienic & sanitary conditions in developing countries of Asia and Africa [4]. For the systemic disease of typhoid fever, the etiological agent is enterica serovar Typhi (*S. typhi*). Transmission occurs by the consumption of contaminated food or water. The pathogen *S. typhi* is specific to humans; to the best of knowledge, no animal or environmental reservoirs have been found [5]. *S. typhi* is associated with systemic infection, prolonged fever and asymptomatic carrier state [6]. It remains a great public health problem in developing countries especially in the tropics and subtropics having substandard personal hygiene and poor sanitation [7]. The increasing multi-drug resistance (MDR) of this organism is causing additional problems in the treatment and management of typhoid fever. Typhoid disease is endemic in many countries and the emergence of the multi-drug resistance (MDR) typhoid has become a huge global health threat [6]. Furthermore, in the last 20 years, multi-drug resistant (MDR) *S. typhi* strains have developed and spread worldwide, resulting in high rates of illness and death [8].

The vast majority of cases occur in South East Asia, Africa and South America but both the case fatality rate and the spectrum of clinical complications of typhoid vary considerably in different areas where the disease is endemic [9]. In sub-Saharan Africa, hospital-based studies indicate that non-*typhi* serotypes of *Salmonella*, particularly *S. enterica* serotype *enteritidis* and *S. enterica* serotype *typhimurium*, greatly outnumber *S. typhi* and *S. paratyphi* as causes of blood stream infection [10]. In Asia, a large population-based prospective study was done to inform typhoid fever vaccine policy using standardized surveillance methods, which estimated typhoid fever incidence in China, India, Indonesia, Pakistan and Vietnam. According to this study, the high incidence of typhoid fever in these regions was predominantly among children and adolescents but also demonstrated the considerable difference in incidence occurrence between surveillance sites in the same regions [11]. According to the Global Burden of Disease study (GBD), in the year 2000, 22 million new cases of typhoid fever, 210, 000

typhoid fever-related deaths, and 5.4 million cases of paratyphoid fever were reported [12].

In Bangladesh, which is situated in South Asia, the population is mostly penurious; therefore, it is likely that typhoid incidence will be high [13]. In 2005, Brook *et al.* had done a community-based study in an urban slum in Bangladesh, where it was suggested that the overall incidence was 3.9/1000 persons/year and the rate was higher in preschool children aged between 0 and 4 years old (18.7 per 1000 persons/years) [14]. A recent study revealed that typhoid fever was endemic in urban areas with a high incidence of multi-drug resistant strains. In this study, it was also found that the incidence rate was higher in children aged < 5 years (10.5/1000 persons/year) with an overall incidence rate of 2.0/1000 persons/year. Age-wise data demonstrated that the infection was lower in an older group (0.9/1000 person-years) than that of children. Both of these studies reported that the incidence of typhoid fever among populations in the urban slums of Dhaka was higher [13] [14] [15].

Due to increasing resistance to the antimicrobial drugs used traditionally for therapy, the use of fluoroquinolones, such as ciprofloxacin, for the treatment of typhoid has become more common in developing countries [16]. In Asia, disease burden estimates have usually relied on routinely reported, clinically diagnosed cases of typhoid fever compiled by governments or hospitals, usually with uncertain denominators. A study conducted by Chiou *et al.* [17] indicated that before 1995, MDR typhoid was caused by a diverse range of *S. typhi* and MDR plasmids [16]. Clinical diagnosis of typhoid is not specific because the presenting signs and symptoms are diverse and similar to those of other common febrile illnesses, such as malaria and dengue fever. A specific diagnosis of typhoid requires access to a competent laboratory that can process blood cultures [11]. In this study, we conducted blood culture surveillance in a tertiary medical centre of a densely populated urban community in Dhaka to estimate the incidence and determine the antimicrobial susceptibility patterns of *S. typhi*. Antimicrobial sensitivity pattern by antibiogram and multi-drug resistance of clinically isolated *S. typhi* was done to further support the findings.

2. Materials and Methods

2.1. Collecting and Processing Clinical Samples

Six hundred patients suspected of suffering from enteric fever were selected and blood samples were drawn from tertiary care hospitals in Dhaka city of Bangladesh. Blood samples were collected from patients belonging to different age groups in sterile stop corked special bottles. Samples were put in BACTEC machine where it was incubated at 37˚C and agitated continuously. Samples with positive growth were subcultured [18].

2.2. Isolation of Clinical *Salmonella Species*

Samples from patients with a positive result were sub-cultured on sterile XLD

and MacConkey agar plates. *Salmonella* spp. produced red colonies with black centers and off-white colonies on XLD agar and MacConkey agar, respectively [18].

2.3. Identification of *Salmonella* Isolates

Following biochemical tests were conducted for identification of *Salmonella typhi*: Catalase, Citrate, Flagella, Gelatin Hydrolysis, Gram Staining, H_2S, Indole, Motility, Methyl Red, Nitrate Reduction, Oxidase, Spore, Triple Sugar Iron Agar, Urease, Voges Proskauer, Fermentation of Glucose, Glycerol, Inositol, Lactose, Mannitol, Mannose, MyoInositol, Raffinose, Rhamnose, Salicin, Sorbitol, Sucrose, Xylose and Lysine Decarboxylase [19] [20].

2.4. Determination of Antibiotic Resistance Using Kirby Bauer Method

The antibacterial susceptibility testing was performed by the Kirby Bauer disk diffusion method against different antibiotics according to Clinical Laboratory and Standards Institute (CLSI) recommendations [21]. Antibiotics used for this purpose were Amikacin, Gentamicin, Imipenem, Meropenem, Cephradin, Cefuroxime, Cefixime, Ceftazidime, Cefotaxime, Ceftriaxone, Nalidixic acid, Ciprofloxacin, Levofloxacin, Azithromycin, Nitrofurantoin, Amoxyclave, Amoxicillin, Co-trimoxazole. Inoculum adjusted to 0.5 McFarland standards was used to seed the culture media and antibiotics discs were placed on the heavily seeded inoculum on a solid Mueller-Hinton agar plate that was incubated at 37˚C for 24 h. The zone of inhibition was documented in millimetres (mm) [20] [22] [23].

2.5. Statistical Analysis

The positive prevalence rates of typhoid to male and female patients were analyzed. The results in different ages were descriptive analysis and ANOVA two factors without replication was conducted for statistical analysis of derived data using Microsoft excel. The results were considered statistically significant differences if $p < 0.05$.

3. Results and Discussion

In case of the total number of positive patients of both sex and different age groups attending tertiary care hospitals during the study period, the majority of patients with enteric fevers are male and it was observed the most in younger male children while in females it was in the age range of 21 - 40 (**Table 1**). **Table 2** exhibited the statistical analysis of data revealed mean of female patients with typhoid was half of male patients where median of data, standard deviation and kurtosis were higher in male patients. Female patients showed more sample variance and skewness in comparison to male patients. After conducting ANOVA two factors without replication, p-value of the data were 0.019 signifying the data were statistically significant (p-value < 0.05). Efluoroquinolones group exhibited

Table 1. Total number of positive patients in different age and sex groups.

Age	Male patients with typhoid	Female patients with typhoid
1 - 20	62	24
21 - 40	47	27
41 - 60	9	11
61 - 80	8	3
≥81	1	0
Sum	127	65

Table 2. Statistical analysis of male and female patients with typhoid.

Patients with typhoid	Mean	Standard Error	Median	Standard Deviation	Sample Variance	Kurtosis	Skewness	Confidence Level (95.0%)
Male	42.33333	19.64292	28	48.11514	2315.067	1.205065	1.256433	50.49374
Female	21.66667	9.735388	17.5	23.84673	568.6667	2.152259	1.408	25.02561

the most drug-resistant. All 3 antibiotics of varying generations from the fluoroquinolones group (Nalidixic acid, ciprofloxacin and Levofloxacin) showed little to no effect against test isolates where isolates were most sensitive against antibiotics from carbapenem groups (**Figure 1**). **Figure 2** exhibits the prevalence of MDR and XDR among isolated pathogenic *Salmonella* strains. It was observed a majority of isolates showed an extended drug-resistant pattern where only 19% of isolates showed multi-drug resistant. Out of all isolates, only 12% of samples showed antibiotic resistance to 1 or 2 antibiotics. **Figure 3** shows the prevalence of XDR and MDR isolates in different age ranges of both sex groups. MDR and XDR were both high in the age group of 1 - 20 years in the case of male patients. Whereas in female patients high XDR was observed in 21 - 40 years old and MDR was seen in 1 - 20 years old age range.

Antimicrobial drugs are the main foundation of therapy for typhoid patients. Even then, the thorough use of first-line antimicrobials, such as ampicillin, chloramphenicol and cotrimoxazole, has directed to the rise and global spread of multi-drug resistant (MDR) *S. typhi* strains. In recent years multiple isolates have been observed in different countries which are sensitive to only handful of antibiotics and they are being termed as extended drug-resistant (XDR) *S. typhi* [24]. In the current study, 32% of clinical samples showed the presence of *Salmonella* spp. in blood samples, which is higher than other studies conducted in Dhaka city [19]. Multiple studies showed the presence of MDR *S. typhi* in Pakistan in recent years, which were resistant to the first-line antibiotics including chloramphenicol, ampicillin, and sulfamethoxazole [25]. In this study, isolates were most resistant against ciprofloxacin (99.52%) followed by nalidixic acid (81.60%). High fluoroquinolones resistance, as well as high cephalosporin resistance, was observed in the research conducted by Klemm *et al.* [26]. The current study exhibited cephalosporin resistance for old generation cephalosporin drugs. The

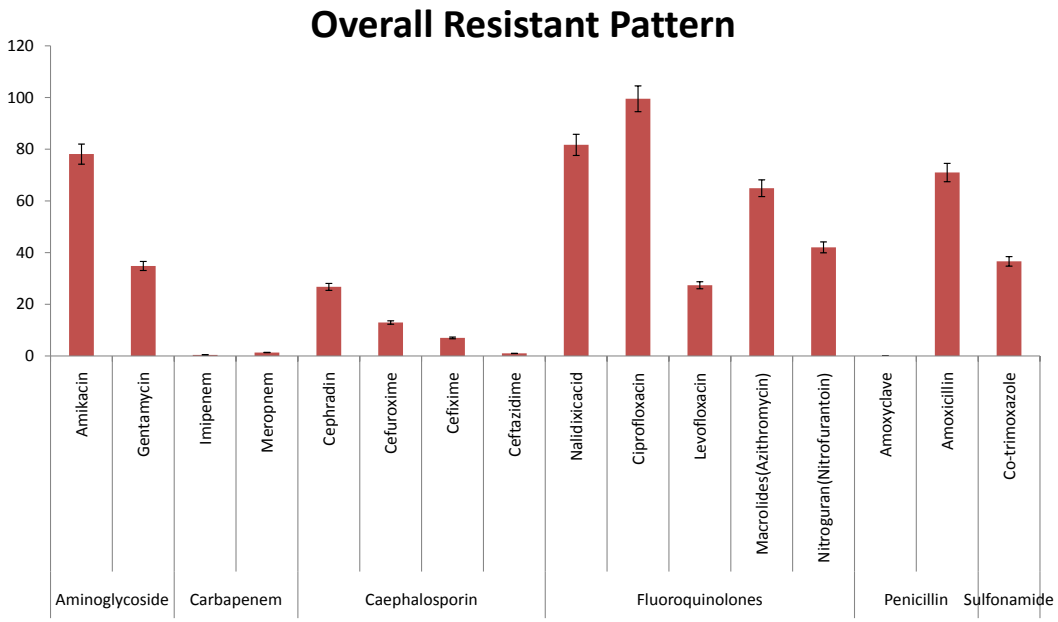

Figure 1. Determination of the overall resistance pattern of test isolates.

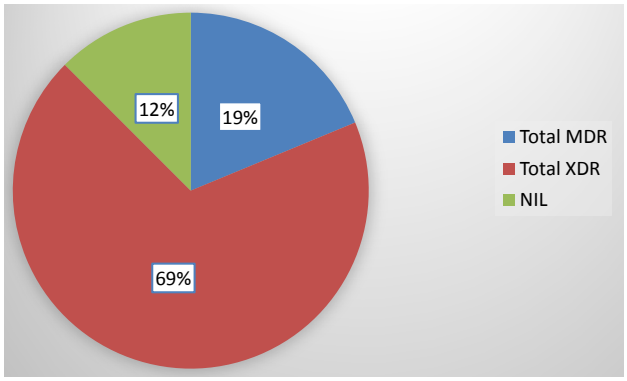

Figure 2. Prevalence of drug resistance in *S. typhi*.

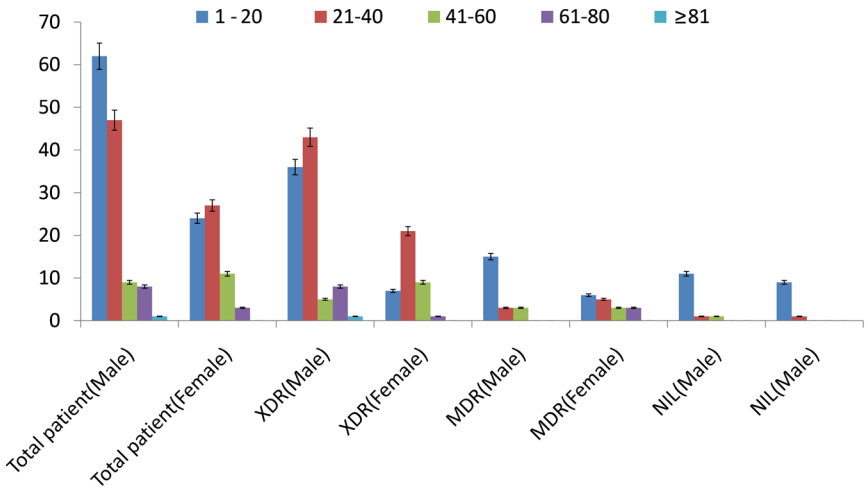

Figure 3. MDR and XDR pattern among the different age ranges of male and female patients.

carbapenem-resistant pattern in this study (Imipenem 0.4% and Meropenem 1.33% resistant) was less than findings obtained in a study conducted in South Africa (23.7%) [27] [28].

In 2017, study by Ali *et al.* showed *S. typhi* (64/197 patients) and *S. paratyphi* (5/197) MDR isolates but did not show any cephalosporin resistance [29]. Child patients have been most affected by the disease according to the age distribution data observed in most studies [25] [26] [30] [31]. In this present study, patients between the ages of 21 - 40 years showed a high prevalence of XDR isolates as well as MDR in both male and female patients. However, in male patients, a high prevalence of MDR *Salmonella* was seen in age ranging between 1 - 20 years. A study from tertiary care hospitals of Pakistan exhibited a high load of fluoroquinolone-resistant and MDR *S. typhi* strains but did not report any XDR*S. typhi*-cases [32]. Aside from Pakistan, cases of XDR *S. typhi* have been reported in 23 different countries including Canada which was reported by Wong *et al.* in pediatric settings [31], countries of Europe including United Kingdom in which Klemm *et al.* reported XDR *S. typhi* serovar resistant against Fluoroquinolones and third generation Cephalosporins [26]. In Denmark, Engsbro *et al.* reported XDR *S. typhi* in pregnant travelers [33] while Chatham-Stephens teams reported XDR *S. typhi* among travelers from Pakistan to USA [34].

The multi-country Typhoid Fever Surveillance in Africa Program (TSAP) conducted studies in countries including Burkina Faso, Ghana, Ethiopia, Kenya, Guinea-Bissau, Madagascar, Senegal, South Africa, Tanzania and Sudan. Both TSAP & Se Eun-Park of the International Vaccine Institute and the Surveillance of Enteric Fever in Asia Project (SEAP) showed the presence of 52% MDR typhoidal *Salmonella* strains [35] [36]. In this present paper, out of 192 positive isolates of *Salmonella* spp., 169 isolates (88.02%) were drug-resistant including both MDR (19.79%) and XDR (68.23%) strains. This high amount of drug resistance might be contributed to the migration of drug-resistant strains from Pakistan and other surrounding countries due to international travel. High prevalence of multi-drug resistant isolate can also be seen in other highly infectious organisms such as *Mycobacterium tuberculosis* in Bangladesh in recent years. This finding indicates available antibiotics may soon cease to inhibit bacterial growth and act as proper medication for infected patients [37]. Many studies have confirmed the transfer of drug-resistant *Salmonella* serovars via the farm to fork method [38] [39]. Unsupervised and unnecessary use of antibiotics in farm settings contributes to the development of antibiotic-resistant genes.

4. Conclusion

The presence of XDR *S. typhi* and disease transmission via food and water continues to be a severe concern for public health in Bangladesh. Current MDR and XDR isolates indicate selective pressure on the use of available antibiotics and they are soon to be ineffective against drug resistant *S. typhi* isolates. In recent years, discovery of new antimicrobials against pathogens has been very slow and

the future in this sector is not very promising. If not addressed properly, the rise of PAN-DR (resistant to all available drugs) in *Salmonella* spp. may not be all that surprising, rendering us defenseless highly resistant pathogen. The Covid-19 situation taught us that a single incident may cause a global pandemic if situations are correct and the antibiotic resistant issue is a long-term threat we have been facing and had ample time to prepare for. The health care system in Bangladesh as well as in global settings should keep surveillance on developing drug resistance of microorganisms, especially in clinical sectors. The focus should be placed on public awareness, regarding the responsible use of antibiotics and availability of typhoid vaccination as well as prevention of the disease by improving sanitation and water supply.

Conflicts of Interest

Authors have declared that no competing interests exist.

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