Isolation, Identification and Antibiotic Sensitivity Pattern of *Salmonella typhi* Isolated from Blood Samples of Patients in Dhaka City, Bangladesh

Mohammad Mizanur Rahman[1], Maruf Abony[1], Kaniz Fatema1, Suvamoy Datta[1*]

Abstract

In recent years, there has been a significant rise in the prevalence of multidrug resistance *Salmonella typhi* in Dhaka city. To study the prevalence rate and identification of typhoidal *Salmonella typhi* among the patients attending at a hospital by conventional test. It was seen that 88% (Amoxyclave) of the S. typhi strains are resistant to the antibiotics used against them. Gentamycin, Levofloxacin and Meropenem (100% sensitive) are the best drugs for the treatment of S. typhi infection followed by Ciprofloxacin (99% sensitive). None of the samples exhibited sensitivity to more than four drugs. Typhoid fever occurs infrequently in developed counties but massively in developing countries. Traditionally the drugs of choice were chloramphenicol, ampicillin, and cotrimoxazole but unfortunately the emergence of multidrug resistant strains of S. typhi introduced the use of Amikacin, Imipenem & Netilmycin among the patients. However, now a day's ciprofloxacin used frequently as a drug of choice but this study, isolated S. typhi are sensitive to ciprofloxacin 95 % that twisted the circumstances into different directions.

Significance | The study highlights rising multidrug-resistant *Salmonella typhi* in Dhaka, emphasizing effective antibiotic choices crucial for managing prevalent typhoid fever in developing regions.

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Introduction

Worldwide, typhoid fever affects roughly 17 million people annually, causing nearly 216,000 deaths (WHO 2007). This disease is rare in developed countries, but it continues to be a major health problem and poses the risk of emergence due to poor hygienic and sanitary conditions prevalent in developing countries (Crump et al. 2004; Crump & Mintz 2010). The causative agent, Salmonella enterica serovar Typhi, is an obligate parasite that has no known natural reservoir outside of humans (Parry et al. 2002). The increasing multi-drug resistance (MDR) of this organism is causing additional problems in the treatment and management of typhoid fever (Le Hello et al. 2011). The emergence of *Salmonella Typhi* strains resistant to multiple antibiotics has been causing enormous childhood morbidity and increasing the cost of therapy (Rowe et al. 1997). *Salmonella Typhi* and Salmonella Paratyphi A belong to the family Enterobacteriaceae. They are Gram-negative, rod-shaped, non-spore-forming, motile (with peritrichous flagella), facultative anaerobic organisms (Brenner et al. 2000). Salmonella lives in the intestinal tracts of warm and cold-blooded animals. Some species are ubiquitous, while other species are specifically adapted to a particular host (Bäumler et al. 1998). In humans, Salmonella cause two diseases called salmonellosis: enteric fever (typhoid), resulting from bacterial invasion of the bloodstream, and acute gastroenteritis, resulting from a foodborne infection/intoxication

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(Glynn et al. 1998). Until 1990, there was no evidence of clinically important MDR strains of S. Typhi in Bangladesh, and typhoid fever was treated with the first-line antibiotics like ampicillin, chloramphenicol, co-trimoxazole, and some third-generation cephalosporins and quinolones, which were most effective for the treatment of Salmonella infections (Rahman et al. 1997). But in early 1990, MDR S. Typhi was detected in the clinical microbiology laboratory of ICDDR, B, and since then it has been isolated at an increasing frequency (Rahman et al. 1997). A study conducted at the Dhaka treatment centre of ICDDR, B showed that the frequency of MDR strains increased from 8% in 1990 to 51% in 1994 (Rahman et al. 1997). However, in the succeeding two years, the frequency of resistance to these drugs decreased to 24-31% (Rahman et al. 1997). Hereafter, it has been found that resistance to multidrug further increased from 31% in 1997 to 40% in 2001 (Saha et al. 2001). The development of antimicrobial resistance among Salmonella isolates in Bangladesh has created a new problem for the management of this illness (Saha et al. 2001). Until 1989, the treatment of typhoid fever was relatively simple in Bangladesh, like in many other countries, since all S. Typhi isolates were uniformly susceptible to first-line antibiotics, i.e., ampicillin, chloramphenicol, and co-trimoxazole (Rowe et al. 1997). Although a few sporadic cases of typhoid fever caused by antibiotic-resistant S. Typhi were reported, there was no evidence of clinically important resistance among the strains of S. Typhi in Bangladesh before 1990. In the early 1990s, multidrug-resistant S. Typhi strain was detected in the clinical microbiology laboratory of ICDDR, B, and since then it has been isolated at an increasing frequency (Rahman et al. 1997). The frequency of MDR in this case increased from 8% in 1990 to 51% in 1994 (Rahman et al. 1997).

Materials and Methods

Clinical Specimens

The clinical specimens used in this study were blood samples collected from typhoid patients who attended Medinova Medical Service LTD, Dhaka during January to June 2018. Blood samples were collected using sterile syringe and aseptic conditions were maintained in all cases. Blood Samples were collected using BD Vacutainer® Blood collection Needle made in USA and aseptic conditions were maintained in all cases.

Isolation of Salmonella spp. Blood samples were inoculated directly onto MacConkey's and Salmonella-Shigella agar plates. The plates were then incubated overnight at 37°C and examined for non-lactose fermenting pale colonies. All the cultures were inoculated under aseptic conditions. Growth of microorganisms on solid media was carried out in agar plates in incubators (Eyela Incubator, Japan; member Cool Incubator, Germany).

Identification of Salmonella spp.

Biochemical tests

The isolated Salmonella spp. was further confirmed by biochemical tests following the standard procedures describe [2]. The isolates were also streaked on MacConkey's agar plate & Shigella Salmonella agar plate. The pure bacterial cultures were routinely maintained on MacConkey's Agar, Salmonella Shigella Agar plates. The cultures were stored at 4-8°C until required. The stock cultures were stored at -20°C and at -70°C.

Serological tests

Serological study was performed by a slide agglutination test. Dry and clean glass slide was marked into several parts with a wax pencil. A drop of normal saline was placed on each block. An isolated colony of Salmonella species was touched with a sterile loop and suspended in normal saline. Commercially available Salmonella antisera were added to the suspension. After back-and-forth movement of the slide for two minutes, agglutination was observed against diffuse light. Serotyping was done using specific antisera.

Antibiogram

Determination of antimicrobial susceptibility by Kirby-Bauer method

Bacterial susceptibility to antimicrobial agents was determined by using the Kirby-Bauer [3] disc-diffusion method by using antibiotic containing disks from Oxoid Ltd, UK as well as the manufacturer instructions about the clear zones of growth inhibition around the disks.

Results and Discussion

To study the prevalence of multidrug resistant *Salmonella typhi* in cases with typhoid fever, a total of 80 *Salmonella typhi* samples were tested in this study as shown in Table 1.

Colony characteristics

The colonies of were moderately large, thick, moist circular disks, dome-shaped and smooth; the opacity and size varied with different strains. Color of the colonies were grayish white on SS agar and pale or colorless in MacConkey's medium.

Biochemical characterization

The standard biochemical tests further confirmed the Salmonella isolates. All 120 isolates showed KIA (H2S production), Citrate utilization (-ve), MR (+ve), VP (-ve), Indole production (-ve), Catalase (+ve), and Oxidase (-ve), as shown in figure 1. The emergence of MDR *Salmonella typhi* (resistant to ampicillin, chloramphenicol and co-trimoxazole) initiated consistent use of fluoroquinolones that proven to be effective for the treatment of typhoid fever [4]. However, several failures of clinical treatment of typhoid patients with ciprofloxacin and other fluoroquinolones have been reported recently by [5] and some studies also confirmed the presence of fluoroquinolone resistant S. typhi [6, 7, 8]. This study has been conducted during January to June 2011 to observe the prevalence and antibiotic susceptibility pattern of S. typhi

Table 1. Result of biochemical tests.

KIA			Citrate utilization	MR	VP	Indole production	Catalase	Oxidase
Butt	Slant	H_2S						
Acid	Alkaline	+ve or -ve	-ve	+ve	-ve	-ve	+ve	-ve

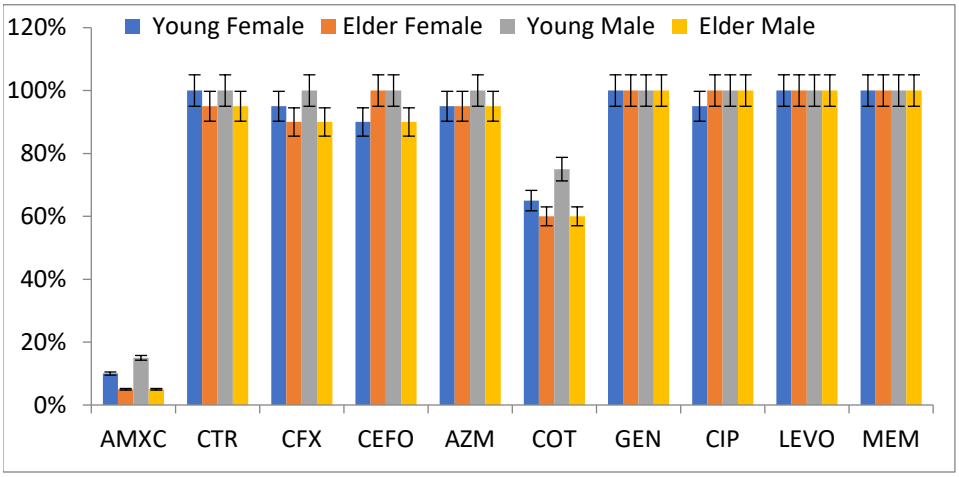

Figure 1: Percentage (%) sensitivity of *Salmonella typhi* to antibiotics.

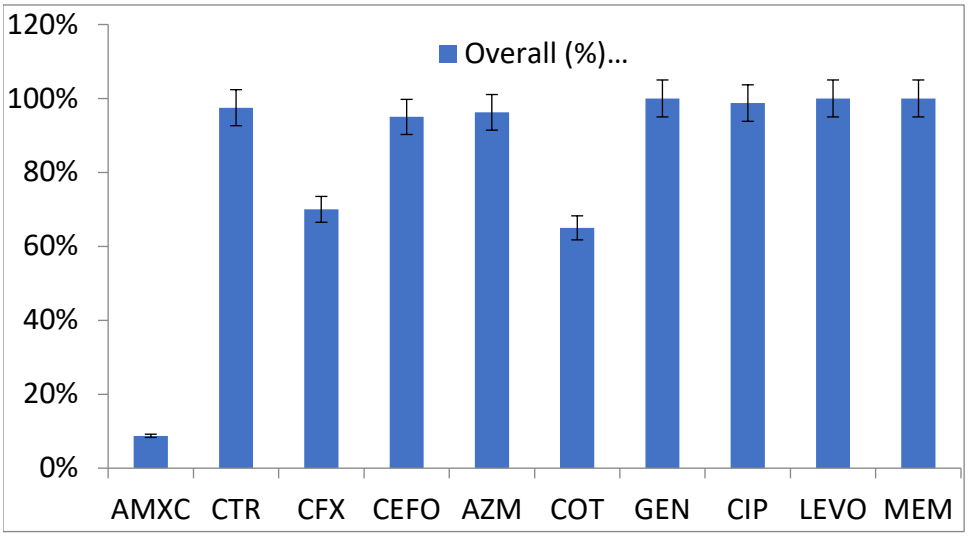

Figure 2: Overall Sensitivity (%) of *S. typhi* to each antibiotic.

among children population in Dhaka city. Furthermore, phenotypic traits of S. typhi were investigated in relation to MDR and amoxicillin & ciprofloxacin resistance mechanisms were inspected as shown in figure 2.

Since the isolation and correct identification of S. typhi is crucial for the characterization purpose, the colonies having typical cultural characteristics were selected as presumptive S. typhi, which were then subjected to biochemical and serological tests for confirmation. It was seen that 88% (Amoxyclave) of the S. typhi strains are resistant to the antibiotics used against them. Gentamycin, Levofloxacin and Meropenem (100% sensitive) are the best drugs for the treatment of S. typhi infection followed by Ciprofloxacin (99% sensitive). None of the samples exhibited sensitivity to more than four drugs. Children under age of 5 have higher incident rate of typhoid fever and this study also found a higher rate of typhoid fever. The spread of antibiotic resistance genes is usually associated with either the clonal spread of an epidemic strain or through the independent acquisition of the resistant genes on plasmids, transposons and on integrons. Clonal relationship among the strains can be achieved by using different molecular techniques. Reproducibility, typeability, discriminatory power, ease of interpretation and ease of performance are the most important criteria to be treated as good typing system (Tasnim et al., 2018). The control of typhoid fever is dependent upon provision of safe drinking water, effective sewage disposal, immunization, and identification and appropriate treatment of both cases and carriers. The widespread and injudicious use of fluoroquinolone is probably responsible for this emergence and therefore, prudent use of this class of antibacterial, surveillance for antimicrobial resistance of S. typhi and monitoring of clinical response while treating patients with typhoid fever is essential (Walid et al., 2017). There is also an urgent need to reevaluate fluoroquinolones breakpoints for S. typhi. Until then, screening of S. typhi isolates for nalidixic acid resistance should be routinely performed to alert the physician about the possibility of failure of ciprofloxacin therapy in patients with typhoid fever (Nair et al., 2018). Present resistance patterns of S. typhi and their trends should also be monitored in other neighboring countries. Further, in countries where typhoid fever is endemic, such as Bangladesh, investigation and treatment of typhoid fever cases at private laboratories and home, respectively, are the rule rather than exception. Usually, the patients are only admitted to the hospitals in case of initial treatment failure or complications. Therefore, strains isolated only from hospital in-patients, as shown in previous report may demonstrate higher rate of resistance which does not reflect the real picture in endemic areas of the particular country or community.

If these strains are widespread, as expected, a randomized, controlled trial comparing oral azithromycin to third generation cephalosporin treatment of typhoid fever due to these strains with reduced susceptibility to fluroquinolones is needed to better inform choice of optimal treatment.

Conclusion

Typhoid fever is increasingly rare in developed countries but remains widespread in developing nations. Traditionally, chloramphenicol, ampicillin, and cotrimoxazole were preferred treatments. However, the rise of multidrug-resistant S. typhi strains necessitated the use of Amikacin, Imipenem, and Netilmycin. Recently, ciprofloxacin has become a common choice due to its efficacy. A study revealed that 95% of isolated S. typhi strains are sensitive to ciprofloxacin, significantly influencing treatment strategies. This finding underscores the evolving landscape of typhoid management, reflecting the ongoing battle against antibiotic resistance.

Author contributions

S.D., conceptualized and developed the methodology, M.M.R., prepared the original draft, M.A. and K.F., collected data and reviewed and edited the writing.

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Competing financial interests

The authors have no conflict of interest.

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