A cross sectional study on antibiotic resistance pattern of *Salmonella typhi* clinical isolates from Bangladesh

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PEER REVIEW

ABSTRACT

Objective: To investigate and compare the resistance and sensitivity of *Salmonella typhi* samples to commonly used antibiotics in three major divisions of Bangladesh and to evaluate the gradually developing resistance pattern.
Methods: The antibiotic susceptibility of 70 clinical isolates collected from blood, sputum, urine and pus samples were identified by specific antisera and with standard biochemical tests. The patients were divided into 5 age groups. Susceptibility and resistance was also tested by Kirby–Bauer disc diffusion method using 12 regularly used antibiotics.
Results: Antibiotic susceptibility test demonstrated that 64.28% isolates of *Salmonella typhi* were multidrug resistant. Present study suggests that the clinical samples were mostly resistant against nalidixic acid with all age groups and in all three divisions with similar resistance pattern. Resistance is more common among adult people (30–40 years) and children (0–10 years). *Salmonella typhi* was mostly sensitive against gentamycin, chloramphenicol and ciprofloxacin.
Conclusions: Although the population density of Dhaka region is markedly higher than Rajshahi and Chittagong regions, no significant difference in resistance pattern was found. The rate of multidrug resistance is a matter of concern. Physicians should reconsider before prescribing nalidixic acid and cefixime. Further molecular study is needed to reveal the genomic and proteomic basis of resistance.

1. Introduction

Typhoid fever is an epidemic problem for Bangladesh, Indian subcontinent, South and Central America and Africa. Despite the use of newly developed antibacterial drugs, enteric fevers such as typhoid and paratyphoid caused by multidrug–resistant bacterial strains are one of major health problems in Bangladesh, especially for the children[1]. This disease is the most prevalent in South Central Asia and Southeast Asia with more than 100 cases per 100 000 persons per year. Regions of medium incidence (10–100 cases per 100 000 persons per year) include the rest of Asia, Latin

America, Africa and the Caribbean, and Oceania, except for Australia and New Zealand. It is estimated that there are 22 million new cases of enteric fever annually, with 200 000 deaths[2].

In early 1970s, emergence of plasmid–mediated chloramphenicol resistance was reported and the effectiveness of chloramphenicol as a first–line drug decreased gradually by outbreaks caused by resistant strains in countries as far apart as Mexico and India. Outbreaks occurred in Vietnam, Indonesia, Korea, Chile and Bangladesh in the next five years[3]. *Salmonella typhi* (*S. typhi*) is now rapidly developing resistance to cipropfloxacin and fluoroquinolone along with other conventional antibiotics as reported in different parts of the world and emerged as new challenges to the treatment of typhoid fever[4–12].

It was reported that, in Bangladesh third generation cephalosporins (ceftriaxone and cefixime) are still the effective drugs for treating typhoid fever if used in proper dose and duration. Though azithromycin is prescribed as an alternative to ciprofloxacin in resistant cases, recently it has lost the credibility due to the emergence of resistance[1,13]. According to records of the public and private hospitals, enteric fever is a major infectious disease occurring at high fluctuating incidences all over Bangladesh.

Resistance to antibiotics is mainly driven by the selective pressure imposed by their inappropriate use. Especially in developing countries like Bangladesh, people do not have the minimal awareness of resistance, antibiotics and infections. They want symptomatic relief to which the health professionals respond by prescribing antibiotics for quick recovery. Infections with drug–resistant microorganisms are associated with severity of the patient's illness, increased patient contact with healthcare personnel and length of stay in the hospital. Eventually it causes extra cost of health care, extended stay in the hospital, sudden or prolonged health complications including significant excess morbidity and mortality[14,15]. Sometimes it reaches an extreme level through the cross–infection of hospitalized patients with such drug–resistant organisms[16].

So it became inevitable to check and compare the current status of the susceptibility and resistance patterns of *S. typhi* in a country to find any regional difference. Moreover, determining the responsible compounds for antibiotic resistance by observing the correlation between *S. typhi* and some biochemical parameters may become beneficial for both the drug designers as well as the manufacturers.

This is the first comparative study reporting the assessment of antimicrobial susceptibility and resistance of *S. typhi* for two other big divisions (Rajshahi and Chittagong) of Bangladesh in addition to Dhaka that will provide ideas about the regional variations of this pattern, if any.

These observations with variations in the sensitivity and resistance patterns for *S. typhi* against particular antibiotics will provide suitable guidelines for clinicians to prescribe specific antibiotics for typhoid fevers in specific cases for the patients of Bangladesh. This information can become useful for some other tropical countries in Southeast Asia to face similar health problems.

2. Materials and methods

2.1. Sample collection

Blood, sputum, urine and pus samples were collected from 945 patients at different hospital and diagnostic centers in Dhaka, Chittagong and Rajshahi during November 2011 to November 2012. The patients were categorized in five age groups, <10, 10–20, 20–30, 30–40 and >40 years. Patients with systemic infection were selected for this study and their clinical history and examination findings were recorded on the standard form before preceding the blood culture.

2.2. Isolation of colonies

The aseptically collected samples were inoculated on nutrient agar media using a calibrated loop delivering 10 μL of the sample and incubated overnight at 37 °C. All bacterial specimens were cultured on blood agar and MacConkey agar, again incubated overnight at 37 °C and the isolates were identified by adopting standard microbiological procedure which includes colony morphology, Gram stain reaction and biochemical reaction such as oxidase, catalase, sulfide indole motility test, citrate agar slant, methyl red test, Voges Proskauer test, triple sugar iron agar test and urease test. The isolates of *Salmonella* were further confirmed by abblutination with polyvalen O antiserum A–S and individual H antisera (Denka Seiken, Japan).

2.3. Sensitivity test

Susceptibility and resistance of all isolates were checked by performing the Kirby–Bauer disc diffusion method according to the guidelines of Clinical and Laboratory Standards Institute, formerly National Committee for Clinical Laboratory Standards[17,18]. The antibiotic incorporated plates were incubated at 37 °C and zones of inhibition around the antibiotic were measured after 18 h and within 24 h of incubation.

The routinely used antibiotics, all from Hi Media, were chloramphenicol, azithromycin, cephalexin, ciprofloxacin, gentamicin, cloxacillin, azacitidine, cefixime, nitrofurantoin, nalidixic acid, levofloxacin and ampicillin. The isolates were