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Short Communication

Antibiotic resistance pattern of *Salmonella* spp. isolated from stool samples of hospitalized diarrheal patients in Bangladesh

Mohammad Sharif Uddin[1], Md. Imranul Hoq[1], Mohammad Shaokat Ali[2*], Md. Mijanur Rahman[1] and K. M. Shariful Islam[1]

[1]Department of Microbiology, University of Chittagong, Bangladesh
[2]Department of Applied Chemistry and Chemical Technology, Faculty of Food Science and Technology, Chittagong Veterinary and Animal Sciences University, Khulshi, Chittagong-4225, Bangladesh

*****Corresponding author: Mohammad Shaokat Ali, Department of Applied Chemistry and Chemical Technology, Faculty of Food Science and Technology, Chittagong Veterinary and Animal Sciences University, Khulshi, Chittagong-4225. Phone: +8801825128942; E-mail: shaokat.fst@gmail.com

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Abstract: This study was carried out to investigate the drug resistance pattern of *Salmonella* spp. from diarrheal patients of under 5 years of age. Collected stool samples from the hospitalized diarrheal patients were analyzed for the presence of *Salmonella* spp. Among 350 stool samples from diarrheal patients, 15 (4%) were positive for the *Salmonella* spp. Antibiotic susceptibility test of the identified *Salmonella* spp. was performed according to Kirby-Bauer disc diffusion method. Eight commonly used antibiotics including azithromycin (15 µg), chloramphenicol (30 µg), Sulphamethaxole-trimethoprim (25 µg), metronidazole (50 µg), tetracycline (30 µg), doxycycline (30 µg), erythromycin (15 µg) and ciprofloxacin (5 µg) used to determine drug resistance pattern of the identified *Salmonella* spp. Majority of the isolates were multidrug resistant showed resistance against more than three drugs. 73% of *Salmonella* spp. was resistant to 2-4 drugs and 20% showed resistance to more than 5 drugs. We found that 100% *Salmonella* showed resistance to metronidazole and 87% were resistant to erythromycin. 7% isolates were resistant to chloramphenicol and doxycycline and 20% were resistant to ciprofloxacin. Antibiotic resistance is a serious public health problem worldwide and a leading cause of mortality and morbidity. This kind of situation, leads to great socioeconomic losses from the perspective of the patient, the hospital, and the whole society. To get rid form the drug resistance problem unnecessary, over and misuse of antibiotics should immediately prohibited and policy of judicious use of antibiotics should be strengthened.

Keywords: hospitalized diarrheal patients; stool samples; *Salmonella* spp.; antibiotic resistance pattern

1. Introduction

Salmonellosis is a growing concern day by day throughout the world and over the last several decades there have been significant shift in predominant. *Salmonella* serovars are associated with human infections. Non-typhoidal *Salmonella* serovars cause as much as an estimated 1 billion cases of gastroenteritis in humans every year (Kennedy *et al.*, 2004; Coburn *et al.*, 2007). Furthermore, they are also responsible for some serious infections such as septicemia and endocarditis (Gill, 1979), empyema (Burney *et al.*, 1977), and meningitis (Chusid *et al.*, 1980), especially in immunocompromised host. Although most *Salmonella* infections are self-limiting, effective antimicrobial therapy is needed if spread beyond the intestine occurs. The progressive increase in antimicrobial resistance in enteric pathogens is of greatest concern in the developing world. Since 1960s, several *Salmonella* spp. have been showed resistance to various common antibiotics such as ampicillin, chloramphenicol, and sulfamethoxazole-trimethoprim with increasing frequency throughout the world (Smith *et al.*, 1984). Extended-spectrum cephalosporins and fluoroquinolones have become the drugs of choice for the

treatment of infections caused by multidrug-resistant *Salmonella* serotypes due to the increased resistance to conventional antibiotics. However, since 1991, several studies from different countries have been reporting cases of infections by extended-spectrum cephalosporins resistant salmonellae (Dunne *et al.*, 2000; Fey *et al.*, 2000). According to a study by Gupta *et al.*, in the United States, from 1998 through 2001, a 5-fold increase in the prevalence of *Salmonella* species resistant to expanded-spectrum cephalosporins (Gupta *et al.*, 2003). In 10 European countries a survey conducted in 2000 identified a cefotaxime resistance rate of 0.6% in *Salmonella* isolates recovered from human sources (Threlfall *et al.*, 2000). In recent years, such a trend of increase in drug-resistant salmonellae including resistance to quinolones has been observed in many countries, particularly in Asia (Lauderdale *et al.*, 2006; Wang *et al.*, 2006). The use of antimicrobials in any purpose, including disease treatment and growth promotion in domestic livestock, can potentially lead to develop drug resistant bacteria. (Gomez-Lus, 1998; Tollefson *et al.*, 1997). Mechanisms of bacterial antimicrobial resistance includes (i) changes in bacterial cell wall permeability, (ii) removal of antimicrobials via efflux pumps mechanisms (iii) modification of the site of drug action, and (iv) destruction or inactivation of antimicrobials (Barbosa and Levy, 2000; Schwarz and Chaslus-Dancla 2001). Most often, acquired antimicrobial resistance phenotypes develop by the transfer of plasmids via conjugation process (Guerra *et al.*, 2002; Gebreyes and Altier, 2002). Plasmids may carry class I integrons, which are mobile DNA elements, play a vital role in the proliferation of bacterial multidrug resistance (MDR) (Arduino *et al.*, 2002; Di Conza *et al.*, 2002). We have conducted this study on diarrheal patients of less than five years of age in a private hospital of Chittagong district of Bangladesh.

2. Materials and Methods

2.1. Sample Collection

A total 350 fresh watery stool samples from patients of less than 5 years exhibiting symptoms of gastroenteritis were collected from different hospitals of Chittagong, Bangladesh. Then the collected samples were taken in frozen refrigerant packs and transported to the laboratory immediately.

2.2. Isolation and identification of *Salmonella* spp.

Collected samples were inoculated in Luria-Bertani (LB) broth for the enrichment of bacteria and incubated at 37°C for 24 hours. After overnigt of enrichment, cultures were aseptically inoculated onto Hektoen enteric agar and *Salmonella-Shigella* (SS) agar (Oxoid, Hampshire, England) plates and incubated at 37˚C for 24 h. Then the Hektoen agar plates were examined for characteristic green or blue-green colonies (with or without black centre), and distinct colorless colonies onto *Salmonella-Shigella* (SS) agar plates were primarily selected as species of *Salmonella.* After primary confirmation each isolates were finally confirmed as *Salmonella* by conducting several physiological and biochemical tests. Motile isolates from primary selection with negative indole tests where red violet colored ring in the top of the broth cultures after adding kovac's solution didn't formed, positive citrate tests where blue green color of the media formed and, provided red alkaline slant and yellow acidic butt with black colored H_2S production were confirmed as *Salmonella.*

2.3. Antimicrobial susceptibility test

Antibiotics susceptibility test was performed by Kirby-Bauer disc diffusion method (Hudzicki, 2009). Bacterial suspensions were prepared from fresh culture grown overnight onto nutrient agar plates by using sterile normal saline and the turbidity of the suspension was adjusted to 0.5 McFarland Standard that corresponds to approximately $1x10^8$ CFU/ml of suspension. A sterile cotton swab was dipped into the inoculum then streaked on the Mueller-Hinton agar plate properly. Then antibiotic discs impregnated with azithromycin (15 µg), chloramphenicol (30 µg), Sulphamethaxole-trimethoprim (25 µg), metronidazole (50 µg), tetracycline (30 µg), doxycycline (30 µg), erythromycin (15 µg), ciprofloxacin (5 µg) (Oxoid, UK) were dispensed onto the dried agar surface using a sterile forceps. The plates were incubated overnight at 37°C. After incubation period, the resulted zone of inhibition was compared with that of CLSI guideline (CLSI, 2010) for the interpretation of the data and categorization of the test strains as intermediate, sensitive, or resistant.

3. Results and Discussion

Among 350 collected samples from diarrheal patients, 4% (15) of them were positive for S*almonella* spp. Results of antibiotic resistance pattern of the isolated *Salmonella* spp. are shown in Table 1 and Figure 1. Majority of the isolates were multidrug resistant having resistance pattern against at least three antibiotics. We found that 100% (15) of *Salmonella* spp. showed resistance to metronidazole and 87% (13) were resistant to erythromycin. 7% (1) isolates were resistant to chloramphenicol and doxycycline and 20% (3) were resistant to

ciprofloxacin. 40% (6) of the total isolates were resistant to azithromycin and trimethoprim-sulfamethoxazole and 33% (4) were resistant to tetracycline.

In developing countries multidrug resistance in bacteria is a common problem including South Asia. This problem is most likely related to the frequent use of antibiotics without proper medical supervision (Sack *et al*., 1997).

The goal of this study was to investigate drug resistance patterns of the identified *Salmonella* spp. in Bangladesh. This study results revealed that 20% *Salmonella* were resistant to more than 5 drugs and 73% were resistant to 2-4 drugs. In another result we found that 20% *Salmonella* was resistant to ciprofloxacin which is similar to the study conducted by Xia *et al*., 2009. Reports from other countries have found an increasing frequency of ciprofloxacin-resistant isolates (Cailhol *et al.,* 2006; Davis *et al*., 1999). Ciprofloxacin-resistant isolates are involved with increased morbidity and mortality (Varma *et al.,* 2005; Helms *et al.,* 2004) and high frequency makes it difficult to use fluoroquinolones for treatment of *Salmonella* infections in humans. Due to the increasing rate of antimicrobial resistance among salmonellae, broad-spectrum agents such as imipenem or cefepime are now more likely to be used for treatment. These antibiotics are usually more expensive and toxic, and more harmful to the commensurate microflora. This kind of situation, leads to great socioeconomic losses from the perspective of the patient, the hospital, and the whole society. A recent report stated that, they have isolated a *Salmonella* strain that was resistant to imipenem, which is a most fearful indication for future (Miriagou *et al.*, 2003).

Table 1. Susceptibility of *Salmonella* isolates to various antimicrobial agents.

Antibiotics	Number of isolates		
	Resistant	Intermediate	Sensitive
Azithromycin	6	4	5
Chloramphenicol	1	0	14
Sulfamethoxazole-trimethoprim	6	1	8
Metronidazole	14	1	0
Tetracycline	4	3	8
Doxycycline	1	6	8
Erythromycin	13	1	1
Ciprofloxacin	3	5	7

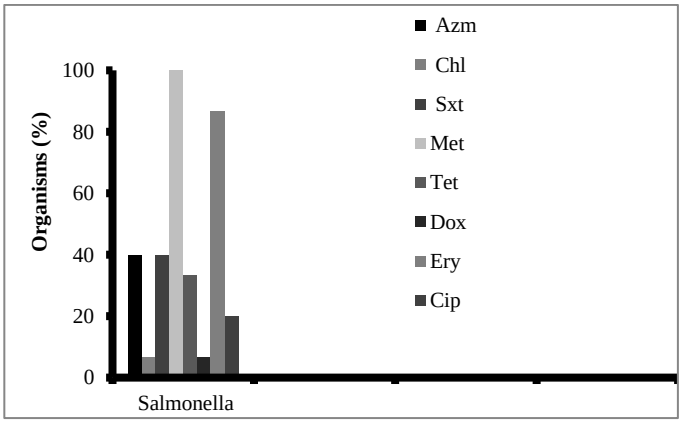

Figure 1. Distribution of isolates (*Salmonella*) according to antibiotic resistance pattern.
Note: Azm= Azithromycin, Chl= Chloramphenicol, Sxt= Sulfamethoxazole-trimethoprim, Met= Metronidazole, Tet= Tetracycline, Dox= Doxycycline, Ery= Erythromycin, Cip= Ciprofloxacin

4. Conclusions

Drug resistant *Salmonella* have constituted a serious threat to public health. To overcome the challenge of drug resistance of Salmonellae, overuse and misuse of antimicrobial agents in food animals should be stopped (McEwen *et al.,* 2002; Swartz *et al.,* 2002). Continued surveillance for resistance pattern for salmonellae is mandatory and ongoing surveillance should be expanded and well standardized. It is however expected that this study will open a new window in exploring the true condition of antibiotic resistance patterns of *Salmonella* in Bangladesh.

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Conflict of interest
None to declare.

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