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Bacterial gastroenteritis in children below five years of age: a cross-sectional study focused on etiology and drug resistance of *Escherichia coli* O157, *Salmonella* spp., and *Shigella* spp.

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Abstract

Background: Gastroenteritis is a common infectious disease in children, which results in high mortality and morbidity, especially in resource-poor countries. This study presents the selected main gastroenteritis causing bacteria, including *Escherichia coli* O157, *Salmonella* and *Shigella* species delineating their prevalence and resistance pattern to the clinically used antibiotics.

Results: A total of 346 stool specimens from children of clinically suspected gastroenteritis below five years have been obtained, and bacterial pathogens were recovered using selective media, biochemical and serologic tests. We found that 35 (10%) of them were confirmed bacterial gastroenteritis (BGE) with either *Escherichia coli* O157 (14%), *Salmonella* spp. (43%), and *Shigella* spp. (43%). Nevertheless, the prevalence of *E. coli* O157, *Salmonella* spp., and *Shigella* spp. in this study were documented as 1.45%, 4.34%, and 4.34%, respectively. Moreover, 2 (40%) of *E. coli* O157, 4 (26.67%) of *Salmonella* spp., and 14 (93%) of the *Shigella* spp. were found as multidrug-resistant. Nonetheless, *Shigella* spp. isolates showed 100%, 87%, and 73% resistance to cotrimoxazole, ciprofloxacin, and azithromycin respectively, while *E. coli* O157 showed 80% resistance to cotrimoxazole and 60% to ciprofloxacin and azithromycin. On the contrary, cotrimoxazole, ciprofloxacin, azithromycin, and tetracycline were resistant in 40%, 47%, 27%, and 53% of *Salmonella* spp. isolates respectively.

Conclusion: These findings generate significant insights on the prevalence and antibiotic resistance of the three major gastroenteritis causing bacteria in the study area. Therefore, it will help physicians and policymakers selecting the right antibiotics in essential cases, particularly, reevaluation of recommending cotrimoxazole, ciprofloxacin, and azithromycin in the management of bacterial gastroenteritis.

Keywords: Gastroenteritis, *E. coli* O157, *Shigella* spp., *Salmonella* spp., Antibiotic resistance

Background

Diarrhea or vomiting resulting from inflammation in the colon or upper small bowel could be referred as gastroenteritis. Etiologic agents might be viruses, bacteria, or parasites, but no pathogens are recovered in many cases. Bacteria cause 20–40% of diarrhea in the United

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Kingdom (Tam et al. 2012), though they mostly play a role in developing countries where childhood mortality resulting from diarrheal diseases is mostly happened (Guerrant et al. 2013). *Shigella* spp., enterotoxigenic *E. coli*, *Vibrio* spp., and *Salmonella* spp. are the most common causes of bacterial gastroenteritis in developing countries (O'Ryan et al. 2014), even though etiologic agents may vary with geographical region. World Health Organization (WHO) documented that gastroenteritis caused 477,293 (8%) deaths of children under five years in 2016 globally (WHO 2016). Worldwide, around 68% of diarrheal disease has been documented in young children taking 2.5 million lives, and it is recognized as the fifth top reason for child mortality (Hartman et al. 2019). In children under five years, acute gastroenteritis (AGE) is a principal cause of mortality and morbidity, 19% of deaths and 10% of hospital admission (Oppong et al. 2020) in resource-poor countries where proper hygiene, sanitation, potable water is deficient, and contaminations from food products are high (Farfán-García et al. 2020).

In developing countries, around half a million deaths of infants and young children happened due to AGE, where *Shigella* is the top etiologic agent (Kotloff 2017). In China, *Shigella* was found as the most frequent bacterial agent causing AGE (Wang et al. 2015), whereas, in India, *E. coli* has been recognized as the dominant (31%), followed by *Shigella* (24%) (Shrivastava et al. 2017; Bruzzese et al. 2018). Worldwide, in 2013, the deaths of children below five years due to shigellosis have been documented between 28,000 to 48,000 (Williams and Berkley 2018). *Shigella flexneri* and *Shigella sonnei* are the most frequent species causing infections. *Shigella dysenteriae,* which produces Shiga toxin recognized as a highly virulent strain, could cause epidemics with high mortality in developing countries. A high level of drug resistance, especially to ciprofloxacin and azithromycin, was observed in those outbreaks (Barrett and Fhogartaigh 2017).

According to an estimation, nontyphoidal (NTS) *Salmonella* are responsible for 93.8 million cases of gastroenteritis and 155,000 deaths globally per annum (Majowicz et al. 2010). A proportion of childhood diarrhea and travelers' diarrhea (TD) in developing countries are occurred by NTS, where *Salmonella enterica* var. Typhimurium and var. Enteritidis are frequently recovered (Barrett and Fhogartaigh 2017). A study in China revealed that diarrheagenic *E. coli* (5%) and *Salmonella* spp. (8%) were responsible for acute diarrhea in children below five years (Tian et al. 2016). Shiga toxin-producing *Escherichia coli* (STEC) O157 is a public health concern due to having potential for severity though it rarely caused gastroenteritis (Adams et al. 2016). Nevertheless, STEC O157: H7 is broadly distributed in the environment and foods, and humans typically get infected by contaminated food and drink or direct exposure with animals (Ahmed et al. 2017). Moreover, the expression of different virulence factors, extended survival capability in the environment, low infective dose, and challenges to treatment made STEC O157: H7 an enteric pathogen of principal concern around the world (Lupindu 2018).

Globally, antibiotic resistances have been increasing the risk of morbidity, mortality and economic burden, where developing countries are the primary victim due to the over and unnecessary uses of antibiotics, drugs with compromised quality, misuse of antibiotics in poultry and cattle feed, lack of continuous effective surveillance, and poverty (Chowdhury et al. 2015). The emergence of antibiotic-resistant *E. coli* O157 has been documented in different parts of the world in recent years. Over the past several decades, various strains of *Salmonella* spp. and *Shigella* spp. became resistant to the most widely used antibiotics with increasing frequency (Barrett and Fhogartaigh 2017). In bacterial gastroenteritis, investigations on causative agents and antibiotic treatment are generally not required. Recommendation of antibiotics is given to critically ill children having chronic conditions of particular risk factors. Types and regimens of antibiotics should be considered based on patient conditions, suspected bacteria, and local epidemiology (Buzesse et al. 2018). Importantly, antibacterial resistance data also have to be taken into consideration before selecting antibiotics. Thus, to reduce mortality, morbidity, and healthcare expense caused by BGE, the local pattern of bacterial resistance is considerably essential (Buzesse et al. 2018).

To delineate the prevalence and antibiotic resistance pattern of the three major gastroenteritis causing bacteria, *E. coli* O157, *Salmonella* spp., and *Shigella* spp., in children below five years of age, we have conducted a cross-sectional study in two hospitals of Chattogram district in Bangladesh from September 2017 to July 2018.

Methods

Patient selection

Children under five years of age, both male and female, exhibited acute gastroenteritis symptoms and were admitted to Chattogram Maa-O-Shishu General Hospital's diarrheal ward and Chattogram Upazila Health Complex Patiya, Chattogram were selected for this study employing a simple random sampling method. The ward's responsible physician has diagnosed the gastroenteritis in patients according to the definition of World Health Organization (WHO) as "who have diarrhea more than three times per day, with abnormal stool specimen such as watery stool, loose stool, mucus and blood in stool" (WHO 2017).

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Ethical consideration

We have taken written permission from the ethical committee of the Department of Microbiology at the University of Chittagong to conduct the study, and written consent has been taken from the patient's attended guardian with informing aims and objectives, merits, and demerits of participation in the study.

Specimen collection

Fresh stool specimens were collected in sterile plastic vials and placed in an insulated box at 4 °C and transported to the laboratory at the Microbiology department, of the University of Chittagong, or Industrial Microbiology Division, Bangladesh Council of Scientific and Industrial Research (BCSIR), Chattogram.

Isolation and identification of bacteria

The stool specimen was enriched in Luria–Bertani broth (Himedia, India) for 24 h at 37 °C, and broth culture was then inoculated onto *Salmonella-Shigella* agar (Himedia, India) and Hektoen enteric agar (Himedia, India) plates. The plates were incubated for 24 h at 37 °C. Individually recognizable colorless colonies onto *Salmonella-Shigella* agar plates were primarily selected as *Salmonella* or *Shigella* species. Motile isolates that were indole negative, citrate positive, produced red alkaline slant and yellow acidic butt with black colored H_2S generation in triple sugar iron (TSI) agar were confirmed as *Salmonella* spp. Conversely, isolates that were non-motile, indole positive, citrate negative, and produced red alkaline slant and yellow acidic butt without H_2S generation were confirmed as *Shigella* spp. (Assefa and Girma 2019). On the contrary, green or blue-green, moist, and raised colonies on Hektoen agar plates were presumptively identified as *Shigella* spp., while colonies with green or blue-green color with or without black center were recognized as *Salmonella* spp. (Assefa and Girma 2019).

Furthermore, for the isolation of *E. coli* O157, the stool specimen was inoculated in modified tryptone soya broth containing novobiocin (Lab M, UK) for 24 h at 37 °C for the selective enrichment. Then, broth culture was streaked on cefixime-tellurite sorbitol MacConkey agar (CT-SMAC) (Lab M, UK) plates and incubated at 37 °C for 24 h. Colorless bacterial colonies on CT-SMAC agar were primarily selected as *E. coli* 0157 and then confirmed with slide agglutination with the *E. coli* O157 Latex test kit (Oxoid, UK).

Antibiotic susceptibility test

Antibiotic susceptibility test (AST) was performed by standard Kirby-Bauer disc diffusion method according to Clinical and Laboratory Standards Institute guidelines (CLSI 2018). The antibiotic discs of azithromycin (15 μg), chloramphenicol (30 μg), cotrimoxazole (25 μg), tetracycline (30 μg), doxycycline (30 μg), and ciprofloxacin (5 μg) (Oxoid, UK) were used in this study. The zone of inhibition for each antibiotic was measured in millimeters, and on the basis of the inhibitory zones, isolates were classified as sensitive, intermediate, and resistant (CLSI, 2018). Isolates resistant to one or more drugs in three or more classes of antibiotics are recognized as multidrug-resistant (MDR) (Magiorakos et al. 2012; Rahman et al. 2021).

Results

In this study, a total of 346 watery stool specimens (single specimen from each child) were obtained from children below five years of age, who were clinically suspected as gastroenteritis attended in the two study hospitals, and 10% (35/346) of them were infected with either one of the three targeted bacteria, *Shigella* spp. (n = 15, 43%), *Salmonella* spp. (n = 15, 43%), and *E. coli* O157 (n = 5, 14%), and termed as "positive with bacterial gastroenteritis (BGE)." The BGE positive cases' median age was 11 months, and 28 (80%), 5 (14.29%), and 2 (5.71%) cases are aged < 1, 1–2, and > 2–4 years, respectively. Moreover, 53% (19) of them were female, and 47% (16) were male. The prevalence of BGE caused by *Shigella* spp., *Salmonella* spp., and *E. coli* O157 was documented as 4.34% (15/346), 4.34% (15/346), and 1.45% (5/346), respectively (Table 1).

Over half (57%) of the isolates causing BGE were resistant to three or more antibiotics tested in this study and recognized as multidrug-resistant. Alarmingly, 14 (93%) of the *Shigella* spp. 4 (26.67%) of *Salmonella* spp. and 2 (40%) of *E. coli* O157 were found as multidrug-resistant (Table 1). *Shigella* spp. revealed a high level of drug resistance; 100% to cotrimoxazole, 87% to ciprofloxacin, 73% to azithromycin, 40% to tetracycline, and 20% to chloramphenicol and doxycycline. Moreover, *Salmonella* spp. showed resistance to six out of six tested antibiotics

Table 1 Prevalence and distribution of multidrug-resistant *Shigella* spp., *Salmonella* spp., and *E. coli* O157 in children diagnosed with gastroenteritis in this study

Bacterial Gastroenteritis	*Shigella* spp.	*Salmonella* spp.	*E. coli* O157
Frequency			
N	15	15	5
%	43	43	14
Multidrug-resistant			
N	14	4	2
%	93.33	26.67	40
Prevalence	4.34%	4.34%	1.45%

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as 53% to tetracycline, 40% to cotrimoxazole, 47% to ciprofloxacin, 27% to azithromycin, and 7% to both chloramphenicol and doxycycline. Another isolate *E. coli* O157 showed 80% resistance to cotrimoxazole, 60% to ciprofloxacin and azithromycin, and 20% to tetracycline, chloramphenicol and doxycycline (Table 2).

Discussion

A study by Web and Starr (2005) reported that 15% of gastroenteritis were caused by *E. coli, Salmonella, Shigella*, and *Campylobacter* species, whereas, in our study, the prevalence of bacterial gastroenteritis (BGE) caused by *Shigella* spp., *Salmonella* spp., and *E. coli* O157 was recorded as 10%. *Shigella* species caused gastroenteritis in pediatric patients in central Iran has been reported by Abbasi et al. (2019); out of the 230 samples, *Shigella* spp. were identified by culture and PCR as 8.2% and 11.3%, respectively; wherein the most frequent species were *S. flexneri* (21%), and *S. sonnei* (78.9%). In acute gastroenteritis cases, the prevalence of *Shigella* has been documented in Tehran, Iran (Rahbar et al. 2007), Kerman, Iran (Nave et al. 2016), Ethiopia (Roma et al. 2000), North of Ethiopia (Kahsay and Teklemariam 2015), and Tanzania (Temu et al. 2007) as 46.5%, 9%, 34.6%, 13.3%, and 14%, respectively. Similar studies conducted in Kerman, Iran (Nave et al. 2016) and the North of Ethiopia (Kahsay and Teklemariam 2015) reported 9% and 13.3% frequencies. On the contrary, studies conducted in India reported frequencies around 2% (Aggarwal et al. 2016). In countries such as Nigeria (25%) (Abdu et al. 2014), Ethiopia (54%) (Debas et al. 2011), and Ghana (70.8%) (Opintan and Newman 2007), the most common species was *S. flexeneri* (Mahmoudi et al. 2017). These shigellosis variations could be due to age, economic growth, geographical location, temperature, and several other environmental factors (Abbasi et al. 2019). In contrast, *Shigella* spp.'s prevalence in our study is relatively low (4.34%).

Table 2 High level of resistance against six clinically used antibiotics showed by *Shigella* spp., *Salmonella* spp., and *E. coli* O157

Antibiotics	Resistance (%)		
	Shigella spp. (15)	*Salmonella* spp. (15)	*E. coli* O157 (5)
Azithromycin	73	27	60
Chloramphenicol	20	7	20
Cotrimoxazole	100	40	80
Tetracycline	40	53	20
Doxycycline	20	7	20
Ciprofloxacin	87	47	60

The prevalence of *Salmonella* spp. in our study has been recorded as 4.3%, in contrast, other studies conducted in Iran (Mahmoudi et al. 2017), Harar (eastern Ethiopia) (Reda et al. 2011), Mozambique (Mandomando et al. 2007), Palestine (Elamreen et al. 2007), and Ethiopia (Mengistu et al. 2014) reported the prevalence of *Salmonella* spp. was 42%, 42.8%, 2.5%, 2%, and 10.5%, respectively.

In parts of Europe, Japan, and North America, most outbreaks of gastroenteritis are because of enterohemorrhagic *E. coli* serotype O157: H7, though other serotypes are significantly remained health concerns in the rest of the developed countries. *E. coli* O157 and Shiga toxin-producing *E. coli* (STEC) were not commonly found in Bangladesh (Islam et al. 2007). Our study also found BGE positive cases of *E. coli* O157 were relatively lower (14%, 5/35) than the other two bacteria. The prevalence of *E. coli* O157 in this study was 1.45%, lower than the other two bacteria. In contrast, a prevalence of over 7% of STEC O157: H7 was reported in the diarrheal patient of Morogoro, Tanzania in 2006 (Raji et al. 2008), whereas 3.4% of isolates were STEC *E. coli* O157: H7 found in Tunisia (Al-Gallas et al. 2006). Similar studies in Nigeria reported a prevalence of 6% (Olorunshola et al. 2000) in Lagos and 5.4% (Chigor et al. 2010) in Zaria. The pathogen was responsible for diarrhea in children at a proportion of 1.9% in Mozambique (Mandomando et al. 2007; Lupindu 2018).

The emergence of multidrug-resistant (MDR) *Shigella* spp. is a progressive concern worldwide (Zamanlou et al. 2018). As shigelloses are highly infectious, it is necessary to know the disease's occurrence and antimicrobial susceptibility of the strains to ensure proper clinical care and patient management (Singh et al. 2011). Mild symptoms are self-limited, but antibiotics are prescribed in extreme dysentery cases to shorten the duration of diarrhea (Williams and Berkley 2018). In our study, *Shigella* spp. showed resistance to cotrimoxazole (100%), ciprofloxacin (87%), azithromycin (73%), chloramphenicol (20%), tetracycline (40%), and doxycycline (20%). Ciprofloxacin-resistant *S. dyseteriae* 1 first found in Bangladesh in 2003, but *S. boydii* and *S. flexneri* were documented in 2007 and 2008, respectively (Talukder et al. 2006; Khatun et al. 2011). Other studies from Bangladesh also revealed that ciprofloxacin-resistant *S. sonnei* was about 10% in 2007, but in 2011 it was increased by sevenfold (Ud-Din et al. 2013). Zamanlou et al. (2018) reported that 4.2% of *Shigella* spp. showed resistance to ciprofloxacin in Tabriz, Iran. In contrast, 56.2% of *S. flexneri* were also resistant to the antibiotic has been documented in India (Aggarwal et al. 2016). A study conducted in Bangladesh between 2001 to 2011 documented that *Shigella* spp. were resistant to trimethoprim-sulfamethoxazole

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(89.5%), nalidixic acid (86.5%), mecillinam (10.5%), and ampicillin (9.5%), respectively (Ud-Din et al. 2013). The other study from Bangladesh also stated that 248 (69%) *Shigella* strains showed resistance to cotrimoxazole (Talukder et al. 2003). The study conducted by Abbasi et al. (2019) revealed that cotrimoxazole (100%), ampicillin (84.2%), cefixime (68.4%), ceftriaxone (63.1%), and ciprofloxacin (10.5%) were affected by *Shigella* resistance. World Health Organization (WHO) proposed fluoroquinolone (ciprofloxacin) as first-line therapy for all patients with bloody diarrhea without age restriction, and ceftriaxone is recommended as second-line therapy or alternative antibiotics in children and adults; because of resistance to trimethoprim-sulfamethoxazole, sulfonamides, ampicillin, and tetracycline (WHO 2019). In developing countries, MDR was rising in *Shigella* spp. mainly to azithromycin, fluoroquinolones, and third-generation cephalosporins (Li et al. 2016). The raised resistance level is mostly because of the horizontal transfer via plasmids, transposons, and integrons (Barrantes and Achí 2016). Though cotrimoxazole is broadly used in the empirical management of diarrheal diseases, and its widespread application has contributed to the emergence of resistance of *Shigella* spp. (Pourakbari et al. 2010). Previously, a high level of resistance to cotrimoxazole has been reported in Iran (92.2% to 94%) (Pourakbari et al. 2010; Nikfar et al. 2017) and Turkey (95%) (Kacmaz et al. 2014). Our findings were also consistent with their results that 100% of all *Shigella* spp. found resistant to the antibiotic. Mahmoudi et al. (2017) reported that the frequency of trimethoprim/sulfamethoxazole resistance among *Shigella* spp. was high (92%), which agrees with reports from Ethiopia (Asrat 2008). These results were similar to Mengistu et al. (2014), Savadkoohi and Ahmadpour-Kacho (Barari and Ahmadpour 2007), and Jomezadeh et al. (2014), which documented trimethoprim/sulfamethoxazole resistance rates of 76.5%, 73.8%, and 80.5%, respectively. These findings may indicate inappropriate antibiotic use in the recent decade (Mahmoudi et al. 2017).

Though rates of multidrug resistance (MDR) of *Salmonella* to traditional first-line agents in both Asia and Africa is increasing (Gordon et al. 2008), Leung et al. (2013) found a marked decline in MDR and decreasing resistance to the ampicillin, chloramphenicol, and cotrimoxazole but increasing resistance to ciprofloxacin in Bangladesh. In our study, *Salmonella* spp. were found resistant to tetracycline (53%), ciprofloxacin (47%), cotrimoxazole (40%), azithromycin (27%), chloramphenicol (7%), and doxycycline (7%). Several reports from around the world also showed the occurrence of a high level of ciprofloxacin-resistant isolates. The studies conducted by Mahmoudi et al. (2017), Mamuye et al. (2015), Mengistu

et al. (2014), and Beyene et al. (2011) found the resistance of *Salmonella* isolates to trimethoprim/ sulfamethoxazole as 23%, 60%, 37.5%, and 31.5%, respectively.

We also documented that *E. coli* O157 isolates were resistant to cotrimoxazole (80%), ciprofloxacin (60%), azithromycin (60%), chloramphenicol (20%), tetracycline (20%), and doxycycline (20%). A study conducted by Chigor et al. (2010) in Nigeria found that *E. coli* O157 were resistant to cotrimoxazole (15%), ciprofloxacin (10%), chloramphenicol (5%), tetracycline (75%), ampicillin (65%), cefuroxime (50%), gentamicin (10%), nalidixic acid (55%), and nitrofurantoin (45%). Wilkerson et al. (2004) reported that 52% (15/29) of the resistant human *E. coli* O157 isolates were multidrug-resistant, including tetracycline. Another study reported that *E. coli* O157 isolates recovered from humans (n = 131) were resistant to trimethoprim-sulfamethoxazole (5%) and tetracycline (12%) (Schroeder et al. 2002).

Bacterial gastroenteritis is self-limiting; therefore, antibiotic therapy is only needed as an intervention to control BGE in infants, older people, and immunosuppressed patients, especially when bacteremia is suspected. Empiric therapy usually starts with oral cotrimoxazole or metronidazole, but parenteral treatment with ciprofloxacin or ceftriaxone could be preferred (Bruzzese et al. 2018).

Conclusions

This cross-sectional study delineated the prevalence of bacterial gastroenteritis in children below five years of age caused by the three significant pathogens *Escherichia coli* O157, *Salmonella* spp., and *Shigella* spp. in the Chattogram district of Bangladesh. The study findings revealed that the prevalence of those bacteria causing gastroenteritis is alarming. Preventive measures are highly recommended to reduce the disease burden and healthcare expense, eventually improving life quality. The findings of antibiotic resistance patterns will help physicians and policymakers select the right antibiotics where necessary, especially making a rational decision while choosing cotrimoxazole, ciprofloxacin, and azithromycin to manage bacterial gastroenteritis cases.

Authors' contributions
MSU and MMR have contributed in designing of the study. MSU, KMSI, and SD have collected samples and performed lab experiments. MSU, MMR, MOF, and AT have done data analysis and prepared the draft manuscript. MIH and MMR

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have contributed to the editing and critical revision of the manuscript. Finally, all authors have read and approved the manuscript for publication.

Declarations

Ethics approval and consent to participate
We have taken written permission from the ethical committee of the Department of Microbiology at the University of Chittagong to conduct the study, and written consent has been taken from the patient's attended guardian with informing aims and objectives, merits, and demerits of participation in the study. The permission from the ethical committee came with a letter without mentioning any reference number.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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