

Follow-Up Trends of Bacterial Etiology of Diarrhoea and Antimicrobial Resistance in Urban Areas of Bangladesh

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Abstract

Background: Diarrhoea is considered as the most widespread illness occurring in Bangladesh. In addition, the growing antimicrobial resistance of diarrhoeal pathogens in this developing country hardens physicians to supervise patients effectively.

Objectives: In our study, we retrospectively analysed the data of a diarrhoeal pathogens and their antimicrobial resistant patterns isolated from diarrhoeal patients attending Dhaka Hospital, International Centre for Diarrhoeal Disease Research, Bangladesh (icddr, b), Dhaka and domiciliary patients of Dhaka city of Bangladesh.

Patients and Methods: During a six-year period (2009 - 2014), a total of 90207 diarrhoeal specimens were collected from patients to screen bacterial etiology by standard culture methods. Antimicrobial susceptibility testing was performed for culture-positive selected diarrhoeal pathogens using disk diffusion method.

Results: One or more potential pathogens were identified in 20467 (23%) patients, more often from under-five children. The most predominant etiological agent was *Vibrio* spp. (33.23%), followed by *Campylobacter* spp. (26.04%), *Shigella* spp. (19.12%), *Aeromonas* spp. (12.21%), *Salmonella* spp. (6.74%) and *Plesiomonas shigelloides* (2.66%). Among children under the age of five, *Campylobacter* spp., *Vibrio* spp. and *Shigella* spp. were more common. *V. cholerae* O1 El Tor biotype was the predominant pathogen (90.75%) among all the *Vibrio* spp. isolated. High prevalence of multiple antibiotic resistances towards ampicillin (AM), ciprofloxacin (CIP) and co-trimoxazole (SXT) was common among *Vibrio* spp., *Shigella* spp. and *Campylobacter* spp. Beside, correlation with previous data from 2005 - 2008 showed that resistant percentages of *Shigella* spp., *Campylobacter* spp., *Salmonella* spp. to these antibiotics are increasing with time ($P < 0.001$).

Conclusions: These findings underscored the importance of monitoring and developing guidelines for better management of infectious diarrhoea in Bangladesh and elsewhere.

Keywords: Etiology, Drug Resistance, Bangladesh, Diarrhoea

1. Background

Infectious diarrhoea remains a very serious public health problem in low and middle-income countries (LMICs) including Bangladesh and is the leading cause of morbidity and mortality among young children less than five years of age (1). The emergence of resistance to different antimicrobial agents among bacterial pathogens associated with diarrhoea e.g. *Shigella* spp., *Campylobacter* spp., *Salmonella* spp., *Aeromonas* spp., pathogenic *Escherichia coli* and *Vibrio* spp. is a global concern. Although most diarrhoea episodes are self-limiting, the use of appropriate antibiotics can reduce the duration of diarrhoea, limit the progression of the disease, and lessen the severity of related symptoms such as fever, abdominal pain and vomiting. Another prime focus for antibiotic therapy is to reduce the chance of excretion of the causative agents and thereby the likelihood of transmission among child-care workers, medical professionals, and

workers of the catering industry or services (2).

Access to clean water supply, promoting good personal hygiene, proper nutrition, and improvement of sanitary conditions would definitely have the greatest impact on diarrhoeal diseases management. However, the development of effective vaccines against the huge number of enteric pathogens is a serious challenge (3, 4). Anti-dehydration therapy associated with adequate nutritional support and intravenous fluid therapy are the best choices of treatment for diarrhoea, regardless of the etiology and the severity of the disease (5).

The gradual increase in antimicrobial resistance among enteric pathogens in LMICs is a critical area of concern and access to current antimicrobial susceptibility data is of importance to guide treatment decisions (5). Knowledge of long-term surveillance of bacterial etiology for diarrhoea and antimicrobial resistance is essential to

make wise decisions for clinicians to prescribe effective antimicrobial therapies. Previously, we reported on bacterial etiology and their resistance patterns for the period of 2005 - 2008 (6).

2. Objectives

The aim of the present study was to follow up the trends in potential bacterial pathogens isolated from diarrhoeal patients and the antimicrobial susceptibility patterns of the isolated organisms over the six-year period from 2009 - 2014.

3. Patients and Methods

From January 2009 to December 2014, a total of 90207 stool samples and rectal swabs were received from hospitalized (inpatients) and domiciliary (outpatients) diarrhoeal patients of all ages at Clinical Laboratory Services of International Centre for Diarrhoeal Disease Research, Bangladesh (icDDR, b), Dhaka, Bangladesh. There were 14741, 16250, 13909, 14355, 14739 and 16213 samples in 2009, 2010, 2011, 2012, 2013 and 2014, respectively. All these samples were tested for the presence of *Shigella*, *Salmonella*, *Vibrio*, *Aeromonas*, and *Plesiomonas*. Antimicrobial susceptibility tests of the isolates were performed. Presence of *Campylobacter* species was screened in 71389 samples (9281, 10818, 10934, 11962, 12894 and 15500 samples in six consecutive years). All the data were analysed retrospectively for the bacterial pathogens present in the stool samples and their antibiotic resistant pattern over the study period from 2009 to 2014.

3.1. Bacteriological Isolation

The collected stool samples and rectal swabs were directly inoculated onto MacConkey agar, Salmonella-Shigella (SS) agar, Taurocholate-Tellurite-Gelatin agar (TTGA), and Brucella agar plate (BAP) with 5% sheep blood. All the plates were incubated at 37°C overnight, except for BAP which was incubated at 42°C in micro-aerophilic condition for at least 48 hours using Campygen gas pack (Oxoid, United Kingdom). For enrichment of *Salmonella* spp., *Vibrio* spp. and *Aeromonas* spp., the stool specimens were also inoculated into selenite cystine broth (Difco, BD) and bile peptone broth respectively. After overnight incubation at 37°C, the inoculated broths were subcultured onto SS agar and TTGA plates respectively. *Shigella* spp., *Salmonella* spp., *Aeromonas* spp., *Vibrio* spp., *Campylobacter* spp., *Plesiomonas* spp. were subsequently identified by standard bacteriological methods (7, 8) and API 20E biochemical profiles (bioMérieux, France) when necessary. The isolates were further confirmed serologically using commercially available antisera (Denka Seiken, Japan).

3.2. Susceptibility Testing

Antibiotic susceptibility tests of the isolates were performed using Kirby-Bauer disc diffusion method on

Muller-Hinton agar plates following the guidelines of the Clinical and Laboratory Standards Institute (7). The antimicrobial agents tested for the isolates were: ampicillin (AM; 10 µg), ciprofloxacin (CIP; 5 µg), ceftriaxone (CRO; 30 µg), chloramphenicol (C; 30 µg), erythromycin (E; 10 µg), mecillinam (MEL; 25 µg), nalidixic acid (NA; 30 µg), cotrimoxazole (SXT; 25 µg), and tetracycline (TE; 30 µg). The interpretation of antimicrobial susceptibility for *Vibrio* spp. and *Campylobacter* spp. was performed as described previously (6). *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as control strains for the susceptibility tests. The disks were obtained from Oxoid (Basingstoke, UK).

3.3. Statistical Analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 20.0 (IBM, USA). Crude odd ratios or relative risks were calculated using the χ^2 (chi-squared) method; two-tailed P values ≤ 0.05 were considered to be statistically significant. The χ^2 test for trend was considered to be statistically significant if P value was ≤ 0.05 .

4. Results

A total of 90207 stool samples or rectal swabs were received from diarrhoeal patients aged from 1 day to 102 years with a mean age of 15 years over a span of six years (January 2009-December 2014). Demographic analysis showed that 52372 (58%) patients were aged < 5 years and the male-to-female ratio was 1.46/1.0. The age distribution and the trends of gender distribution remained stable over the study period. Of the tested samples, 28.11% (4143), 29.41% (4784), 19.60% (2726), 17.59% (2525), 18.62% (2745), and 21.89% (3544) were found to be culture-positive in 2009, 2010, 2011, 2012, 2013, and 2014, respectively ($P < 0.001$) and 22.68% (20467) of all the received samples were culture-positive. The rates of isolation of different bacterial pathogens are summarized in Tables 1 and 2. Among the isolated pathogens, *Vibrio* spp. were the most predominant aetiological agents (33.23%), followed by *Campylobacter* spp. (26.04%), *Shigella* spp. (19.12%) and *Aeromonas* spp. (12.21%). Other frequently isolated pathogens included *Salmonella* spp. (6.74%) and *Plesiomonas shigelloides* (2.66%). Among the isolates from the under-five-year-old children, the incidence of *Campylobacter* spp. was 7.01%, followed by *Shigella* spp. (5.01%), *Vibrio* spp. (4.45%), *Aeromonas* spp. (2.56%), *Salmonella* spp. (1.31%), and *P. shigelloides* (0.46%) (Table 3).

Among the total bacterial isolates, the frequency of *Vibrio* spp. was 36.79%, 35.97%, 30.55%, 36.08%, 35.22% and 23.36% in 2009, 2010, 2011, 2012, 2013 and 2014, respectively (χ^2 for trend from 2009 to 2014 = 219.212, $P < 0.001$). Of these *Vibrio* spp., *V. cholerae* O1 El Tor biotype was 90.75%, followed by *V. cholerae* non-O1, non-O139 (7.08%), *V. parahaemolyticus* (1.91%), *V. fluvialis* (0.13%), and *V. alginolyticus* (0.04%). *V. cholerae* O1 affected more patients aged ≥ 5 years than children aged < 5 years (4101 [66%]: 2076 [34%];

$P < 0.001$). *V. cholerae* O1 El Tor Ogawa serotype was predominant throughout the study period (Table 2) and had distinct seasonality with peaks during March-May and in October, whereas *V. cholerae* non-O1, non-O139 was prevalent throughout the year (Figure 1).

Isolation variation was observed for *Aeromonas* spp. from 2009 to 2014. From 2009 to 2012, isolation showed a decreased pattern from 12.6% in 2009 to 6.8% in 2012 with a slight increase (13.8%) in 2010, but after 2012, isolation of *Aeromonas* spp. increased remarkably compared to the total isolates (9.7% in 2013 and 16.6% in 2014) (χ^2 for trend from 2009 to 2014 = 167.293, $P < 0.001$). A total of 5330 *Campylobacter* spp. were identified from the tested samples, and along the consecutive study years from 2009 to 2012, there was a sharp decrease in isolation of *Campylobacter* species with 9.8%, 9.7%, 7.4% and 5.2% of the tested samples. However, slight increased isolation rate of *Campylobacter* spp. was observed with 5.7% and 7.9% of the total tested samples in 2013 and 2014 (χ^2 for trend from 2009 to 2014 = 233.58, $P < 0.001$). *Salmonella* spp. showed

a rising trend of isolation rate from 2009 to 2012, except in 2010 (6.9%, 5.8%, 7.6% and 9.2% of the total isolates in 2009, 2010, 2011 and 2012, respectively), and the isolation frequency decreased sharply in 2013 (6.5%) and 2014 (5.5%) (χ^2 for trend from 2009 to 2014 = 43.66, $P < 0.001$). Non-typhoidal *Salmonella* spp. were more frequently isolated (998 [72.3%]) than typhoidal *Salmonella* spp. (382 [27.7%]) ($P < 0.002$). There was a sharp decline in the isolation of *P. shigelloides* from 4.1% in 2009 to $< 1.0\%$ in 2014 (χ^2 for trend from 2009 to 2014 = 262.81, $P < 0.001$).

Among the *V. cholerae* O1 isolates, all the isolates showed reduced susceptibility to SXT and TE was 99.0% and 61.0%, respectively. *V. cholerae* non-O1, non-O139 isolates showed reduced susceptibility to E (93.0%), but only 41.0%, 14.0% and 1.0% of the isolates were resistant to SXT, TE and CIP, respectively (Table 4). An increasing trend of resistance to CIP, NA and AM was observed in *Shigella* species. Overall, 78.0%, 64.0%, 58.0% and 31.0% of the *S. flexneri* isolates showed resistance to NA, SXT, AM, and MEL, respectively.

Table 1. Bacterial Pathogens Isolated From Diarrhoeal Patients in Bangladesh From January 2009 to December 2014 and Comparison With the Earlier Study Period From 2005 to 2008^a

Organism	2005 - 2008 (Total n = 14428) ^b	2009 - 2014 (Total n = 20467)
<i>Aeromonas</i> spp.	1848 (12.8)	2499 (12.21)
<i>Campylobacter</i> spp.	1755 (12.2)	5330 (26.04)
<i>Plesiomonas shigelloides</i>	646 (4.5)	545 (2.66)
<i>Salmonella</i> spp.	920 (6.4)	1379 (6.74)
Non-typhoidal <i>Salmonella</i> spp.	651 (4.5)	932 (4.55)
<i>S. paratyphi</i> (A and B)	48 (0.3)	207 (1.01)
<i>S. typhimurium</i>	NA	5 (0.02)
<i>S. typhi</i>	221 (1.5)	175 (0.86)
Other <i>Salmonella</i> spp.	NA	61 (0.30)
<i>Shigella</i> spp.	2925 (20.3)	3913 (19.12)
<i>S. flexneri</i>	1726 (12.0)	2093 (9.84)
<i>S. sonnei</i>	327 (2.3)	987 (4.82)
<i>S. boydii</i>	581 (4.0)	628 (3.07)
<i>S. dysenteriae</i>	213 (1.5)	179 (0.87)
<i>Shigella</i> like spp	NA	106 (0.52)
<i>Vibrio</i> spp.	6186 (42.9)	6801 (33.23)
<i>V. cholerae</i> non-O1, non-O139	233 (1.6)	482 (2.35)
<i>V. cholerae</i> O1	NA	NA
Classical Ogawa	NA	5 (0.02)
El Tor Inaba	2381 (16.5)	171 (0.83)
El Tor Ogawa	3553 (24.6)	6001 (29.32)
<i>V. parahaemolyticus</i>	14 (0.1)	130 (0.64)
<i>V. cholerae</i> O139	2 (0.01)	0
<i>V. fluvialis</i>	NA	9 (0.04)
<i>V. alginolyticus</i>	NA	3 (0.01)

Abbreviation: NA, not available.

^aValues are presented as No. (%).

^bValues in parentheses are the numbers of isolates

Table 2. Bacterial Pathogens Isolated From Diarrhoeal Patients in Bangladesh From January 2009 to December 2014

Strain Name	2009	2010	2011	2012	2013	2014	Total	Values, %
	(4143) ^a	(4784)	(2726)	(2525)	(2745)	(3544)		
<i>Aeromonas</i> spp.	523	663	288	171	267	587	2499	12.21
<i>Campylobacter</i> spp. ^b	907	1043	808	625	729	1218	5330	26.04
<i>Plesiomonas shigelloides</i>	169	243	66	25	18	24	545	2.66
<i>Salmonella</i> spp.	289	278	207	232	179	194	1379	6.74
Non-typhoidal <i>Salmonella</i> spp. (groups B, C1, C2, D, E, G)	216	168	149	169	105	125	932	4.55
<i>S. paratyphi</i> (A and B)	35	46	29	28	34	35	207	1.01
<i>S. typhimurium</i>	NA	NA	NA	1	1	3	5	.02
<i>S. typhi</i>	38	56	20	22	23	16	175	.86
Other <i>Salmonella</i> spp.	NA	8	9	12	16	16	61	.30
<i>Vibrio</i> spp.	1532	1728	835	911	967	828	6801	33.23
<i>V. cholerae</i> O1 El Tor Ogawa	1341	1570	725	806	860	699	6001	29.32
<i>V. parahaemolyticus</i>	33	44	18	23	10	2	130	.64
<i>V. cholerae</i> O1 El Tor Inaba	52	6	4	4	42	63	171	.84
<i>V. cholerae</i> O1 classical Ogawa	1	NA	2	NA	NA	2	5	.02
<i>V. cholerae</i> non-O1, non-O139	103	102	83	77	55	62	482	2.36
<i>V. alginolyticus</i>	NA	NA	3	NA	NA	NA	3	.01
<i>V. fluvialis</i>	2	6	NA	1	NA	NA	9	.04
<i>Shigella</i> spp.	723	829	522	561	585	693	3913	19.12
<i>S. boydii</i>	131	146	109	114	74	54	628	3.07
<i>S. dysenteriae</i>	59	39	37	13	15	16	179	.87
<i>S. flexneri</i>	379	429	222	262	308	413	2013	9.84
<i>S. sonnei</i>	147	202	141	142	162	193	987	4.82
<i>Shigella</i> like spp.	7	13	13	30	26	17	106	.52

Abbreviation: NA, not available.

^aValues in parentheses are the numbers of isolates.^b*Campylobacter* spp. were isolated from 9281, 10818, 10934, 11962, 12894, and 15500 tested samples in six consecutive years.

Table 3. Age Group-Wise Isolation Rates of Bacterial Pathogens in Diarrhoeal Patients in Urban Dhaka, Bangladesh During 2009 – 2014

Pathogens	Age < 5 y (n = 52835) ^a	Age ≥ 5 y (n = 37835) ^a	Crude OR (95% CI)	P Value
<i>Aeromonas</i> spp.	1357 (2.56)	1142 (3.02)	1.02 (0.94, 1.11)	.608
<i>Campylobacter</i> spp.	3705 (7.01)	1625 (4.29)	2.44 (2.28, 2.61)	< .001
<i>Plesiomonas shigelloides</i>	242 (0.46)	303 (0.8)	0.68 (0.57, 0.81)	< .001
<i>Salmonella</i> spp.	693 (1.31)	686 (1.81)	0.86 (0.77, 0.96)	.006
Non-typhoidal <i>Salmonella</i> spp.	513 (0.97)	484 (1.28)	0.91 (0.80, 1.02)	.124
<i>S. paratyphi</i> (A and B)	93 (0.17)	114 (0.3)	0.70 (0.53, 0.92)	.01
<i>S. typhi</i>	87 (0.16)	88 (0.23)	0.85 (0.63, 1.14)	.274
<i>Shigella</i> spp.	2647 (5.01)	1266 (3.35)	2.04 (1.9, 2.2)	< .001
<i>S. flexneri</i>	1353 (2.56)	660 (1.74)	1.87 (1.69, 2.06)	< .001
<i>S. sonnei</i>	777 (1.47)	210 (0.56)	3.34 (2.86, 3.9)	< .001
<i>S. boydii</i>	353 (0.67)	275 (0.73)	1.11 (0.94, 1.3)	.223
<i>S. dysenteriae</i>	102 (0.19)	77 (0.2)	1.13 (0.85, 1.53)	.394
SLO	62 (0.12)	44 (0.11)	1.21 (0.82, 1.78)	.334
<i>Vibrio</i> spp.	2353 (4.45)	6801(17.98)	0.31 (0.29, 0.33)	< .001
<i>V. cholerae</i> O1	2076 (3.93)	4101 (10.83)	0.3 (0.29, 0.32)	< .001
<i>V. cholerae</i> non-O1 non-O139	256 (0.48)	226 (0.6)	0.97 (0.81, 1.16)	.752
Other <i>Vibrio</i> spp.	21 (0.04)	121 (0.31)	0.15 (0.09, 0.23)	< .001

Abbreviations: CI, confidence interval; OR, odd ratio; SLO, *Shigella*-like organism.

^aValues are presented as No. (%).

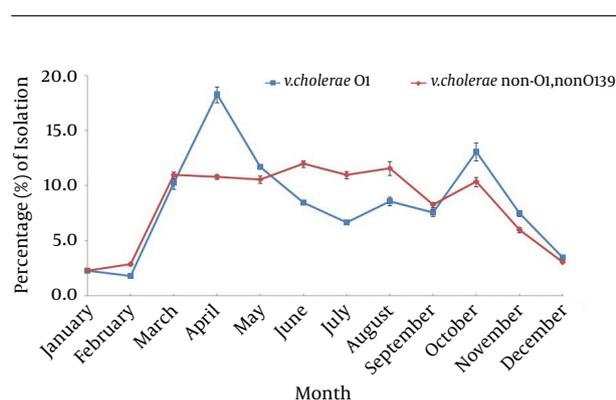


Figure 1. Distribution of *V. cholerae* O1 and *V. cholerae* Non-O1 and Non-O139 During the Study Period (January 2009 to December 2014)

Resistance to CIP in *S. flexneri* increased from 37.0% of the isolates in 2009 to 63.0% in 2014 ($P < 0.001$), whereas for *S. sonnei*, resistance increased up to 80.0% in 2014 from 18.0% in 2009 ($P < 0.001$). Overall, 96.0%, 58.0% and 57.0% of *S. sonnei*, *S. boydii*, and *S. dysenteriae* (not type 1), respectively, showed resistance to NA, while SXT resistance was 96.0%, 60.0%, and 56.0%, respectively (Table 5). Around 57.0% and 22.0% of the *Aeromonas* spp. showed resistance to SXT and TE, respectively, and resistance to CIP was relatively lower ($< 7.0%$) (Table 6). Among *Salmonella* spp., *S. paratyphi* showed increased susceptibility to AM (91%), CIP (99%), C (99%) and SXT (97%), but *S. typhi* showed 29%, 14%, 33% and 35% resistance to these antibiotics, respectively. Non-typhoidal *Salmonella* spp. were 93% susceptible to CIP and 96% to C (Table 7). Forty-six percent and 34.0% of the *Campylobacter* isolates were resistant to AM and TE, while resistance to CIP and E were above 94.0% and less than 15.0%, respectively (Table 8).

Table 4. Comparison of Percentage of Antimicrobial Resistance in *Vibrio cholerae* O1 and Non-O1, Non-O139 Isolates From Diarrhoeal Patients in Bangladesh Between the Recent (2009 - 2014) and Previous (2005 - 2008) Study Periods

Antibiotic	<i>V. cholerae</i> O1		<i>V. cholerae</i> Non-O1, Non-O139	
	2005 - 2008	2009 - 2014	2005 - 2008	2009 - 2014
	(n = 5934) ^a	(n = 6171)	(n = 233)	(n = 532)
CIP	0	0	0	1
EI	68	100	71	91
SXT	99	99	34	41
TE	61	71	8	14

Abbreviations: CIP, ciprofloxacin; EI, erythromycin intermediate; STX, cotrimoxazole; TE, tetracycline.

^aValues in parentheses are the numbers of isolates tested.

Table 5. Comparison of Percentage of Antimicrobial Resistance in *Shigella* spp. Isolates From Diarrhoeal Patients in Bangladesh Between the Recent (2009 - 2014) and Previous (2005 - 2008) Study Periods

Antibiotic	<i>S. flexneri</i>		<i>S. sonnei</i>		<i>S. boydii</i>		<i>S. dysenteriae</i>	
	2005 - 2008	2009 - 2014	2005 - 2008	2009 - 2014	2005 - 2008	2009 - 2014	2005 - 2008	2009 - 2014
	(n = 1726) ^a	(n = 2013)	(n = 327)	(n = 987)	(n = 581)	(n = 640)	(n = 213)	(n = 185)
AM	51	58	7	21	38	44	32	51
CIP	14	47	4	62	1	2	0	3
NA	83	78	84	96	55	58	52	57
SXT	70	64	97	96	53	60	73	56
MEL	17	31	2	10	16	24	5	11

Abbreviations: AM, ampicillin; CIP, ciprofloxacin; MEL, mecillinam; NA, nalidixic acid; SXT, cotrimoxazole.

^aValues in parentheses are the numbers of isolates tested.

Table 6. Comparison of Percentage of Antimicrobial Resistance in *Aeromonas* spp. Isolates From Diarrhoeal Patients in Bangladesh Between the Recent (2009 - 2014) and Previous (2005 - 2008) Study Periods

Antibiotic	2005 - 2008		2009 - 2014	
	(n = 1848) ^a		(n = 2546)	
CIP	2		7	
SXT	60		57	
TE	22		22	
ER	28		19	
EI	61		80	

Abbreviations: CIP, ciprofloxacin; EI, erythromycin intermediate; ER, erythromycin resistant; STX, cotrimoxazole; TE, tetracycline.

^aValues in parentheses are the number of isolates tested.

Table 7. Comparison of Percentage of Antimicrobial Resistance in *Salmonella* spp. Isolated From Diarrhoeal Patients in Bangladesh Between the Recent (2009 - 2014) and Previous (2005 - 2008) Study Periods

Antibiotic	2005 - 2008		2009 - 2014	
	<i>Salmonella</i> spp.	<i>Salmonella</i> group (Non-Typhoidal)	<i>Salmonella paratyphi</i>	<i>Salmonella typhi</i>
	(n = 920) ^a	(n = 931)	(n = 618)	(n = 175)
AM	30	23	9	29
CIP ^R	1	7	1	14
CIP ^I	30	24	29	54
NA	52	31	33	95
C	19	4	1	33
SXT	24	16	3	35

Abbreviations: AM, ampicillin; C, chloramphenicol; CIP^I, ciprofloxacin intermediate; CIP^R, ciprofloxacin resistant; NA, nalidixic acid; SXT, cotrimoxazole.

^aValues in parentheses are the numbers of isolates tested.

Table 8. Comparison of Percentage of Antimicrobial Resistance in *Campylobacter* spp. Isolated From Diarrhoeal Patients in Bangladesh Between the Recent (2009 - 2014) and Previous (2005 - 2008) Study Periods

Antibiotics	2005 - 2008	2009 - 2014
	(n = 1755) ^a	(n = 5331)
AM	31	46
CIP	81	94
NA	79	82
ER	2	15
TE	37	34
SXT	-	100

Abbreviations: AM, ampicillin; CIP, ciprofloxacin; ER, erythromycin resistant; NA, nalidixic acid; SXT, cotrimoxazole.

^aValues in parentheses are the numbers of isolates tested.

5. Discussion

The overall follow-up finding of the study gave in depth information regarding the trends of bacterial etiology of diarrhoea and antimicrobial resistance profiles of major diarrheagenic bacteria in urban areas of Bangladesh and a comparison with our previous study during 2005 - 2008 (6).

Overall, the frequency of culture-positive samples remained nearly equal over the period, as in our earlier study where 25% of all the received samples were culture-positive for bacterial pathogens (6, 9-11). Gender distribution was slightly biased toward male patients (60%) and the gender biasness is congruent with other studies. This gender discrimination in health care seeking behaviour is in accordance with other studies (9, 12) and this is due to the existence of strong son-preference in Bangladesh. In addition, younger children are more prone to be infected by diarrheagenic pathogens due to their dietary habits and immaturity of their immune systems. Moreover, since diarrhoeal diseases are self-limiting, adult patients are more reluctant to seek medical help or visit a doctor in small clinics or collect medicines over the counter. As a result, the data of this study might be biased, because mostly, diarrhoea cases of children aged < 5 years were included.

Overall, most of the bacterial pathogens showed unpredictable natures in isolation during the study period. In the current study, *Vibrio* spp. showed a declining trend in isolation in first three consecutive years (2009, 2010, and 2011) and after an increase in 2012, a fall in isolation was observed in later years. Likewise our earlier report during 2005 - 2008 (6) and a report from India (13), our study showed predominant isolation of *V. cholerae* serogroup O1 El Tor biotype over the six-year period and patients aged ≥ 5 years continued to have the highest incidence of *V. cholerae* O1 infection. The average frequency of isolation of *V. cholerae* O1 than non-O1, non-O139 was higher during March - May and in October in each year.

Shigella spp. was the second most predominant bacterial agent with a fluctuating increase (2009 - 2012) and decrease (2013 - 2014) trend in isolation throughout the study period. Of the isolated *Shigella* spp., *S. flexneri* was the predomi-

nant serogroup, followed by *S. sonnei*, *S. boydii* and *S. dysenteriae*. It is interesting to note that *S. sonnei* emerged as the second predominant by overtaking *S. boydii* and *S. dysenteriae* since 2009. The present study supports the findings of other investigators from Bangladesh (9, 14) and also from our neighbour countries including India (15) and Pakistan (16). Just as the isolation of atypical *Shigella* spp. from diarrhoeal patients in Bangladesh, the frequency of nontypeable *Shigella* spp. had also been reported earlier from India (11). It has been hypothesized that altering trend in *Shigella* serogroups might be due to improvement of host nutritional status (17, 18), improvement of socioeconomic status, meteorological changes, sanitation condition, change in genetic makeup of bacteria, and reduction of the chances of cross immunity by *P. shigelloides* (14, 19, 20).

A steady decreasing trend of *Campylobacter* spp. isolation from 2009 to 2012 was observed in the present study which is analogous to our previous study (6). However, in the last two years (2013, 2014) of our study, the isolation rates of *Campylobacter* spp. showed increase. Development in laboratory techniques and improvement of isolation skills might have had an indirect influence on the increased isolation of *Campylobacter* spp. over the study period.

There was a raising trend in the isolation of *Salmonella* spp. except in 2010, from 2009 to 2012. After 2012, *Salmonella* spp. isolation rates showed decreasing trend, which is similar to our earlier study during 2005 - 2008 (6). The distribution of *S. typhi* showed a decreasing trend, whereas the frequency for nontyphoidal *Salmonella* spp. and *S. paratyphi* (A and B) increased in comparison with the earlier report (6). The isolation frequency of *Aeromonas* spp. decreased in the current study period in comparison to our previous report (6). Although the real contributing factor for the observed shifting trend is unclear, it might be due to an increased awareness in the urban population of infection risks as well as consequent improvements in hygiene and maintenance of good personal hygiene (6).

Antimicrobial resistance has emerged as a serious public health problem worldwide. In the present study, *V.*

cholerae isolates were frequently resistant to SXT and TE; however, most of the isolates showed reduced susceptibility to E and were sensitive to CIP, which is in accordance with our earlier report (6). Similar resistance patterns to these antibiotics were also reported from India except for E, for which they found only 10% of the isolates resistant (11). Among all the *Shigella* isolates, 77% and 72% of *S. flexneri* were resistant to NA and SXT, respectively. The emergence of fluoroquinolone-resistant *S. sonnei* (62%) was followed by *S. flexneri* (47%), which restricted the choice of fluoroquinolone for treatment. These findings are in agreement with the data published from other parts of the world (21, 22). Susceptibility to TE, SXT and CIP in *Aeromonas* spp. was nearly stable during 2005 - 2014, whereas reduced susceptibility to E increased significantly during 2009 - 2014 ($P < 0.001$). A relatively lower level of resistance towards CIP, SXT and TE was reported from Australia (23). The resistance rates to NA, AM, SXT and C of *Salmonella* spp. generally decreased in the current period than that of 2005 - 2008, whereas the opposite trend was noted for CIP resistance (6) and our data is comparable with a report from India (24). A possible explanation for of the differences in susceptibility to different antibiotics might indicate the infrequent use of antibiotics.

CIP-resistant *Campylobacter* spp. was noted significantly higher in the present study in comparison to our previous report (6). Similar to our study, increase of CIP resistance among *Campylobacter* spp. has also been reported by other investigators (25, 26). These findings suggest the ineffectiveness of CIP as a drug of first choice for empirical treatment of campylobacteriosis. The significant increase of resistant to E among *Campylobacter* isolates limits the empirical choice of antibiotic for the treatment of campylobacteriosis in Bangladesh.

The emergence of antibiotic resistance is a continually evolving and dangerous concern which requires immediate attention and future planning to combat a global public health crisis. Multiple factors are responsible for the alarming rise in the occurrence of antimicrobial-resistant bacteria such as overuse and misuse of antibiotics, like erroneous antibiotic prescriptions for nonbacterial infections, and the incorporation of antibiotics to livestock feed and food sanitizing agents (6, 20). Prolonged survival of patients with chronic disease, nosocomial infection by multiply antibiotic-resistant (MAR) bacteria (27), increased number of immunosuppressed individuals, substandard hospital hygiene, and more international travel contribution for enhanced dissemination of resistance factors (28). The selective pressures applied by antibiotic drugs creates reservoir of antibiotic-resistant bacteria in the environment and has potentially lethal consequences for invasive diarrhoea endemic regions (20).

Versatile approaches including raising awareness about the judicious, prudent, or rational use of antibiotic are in need to prevent the spread of antibiotic resistance among enteric pathogens. Physicians should encourage patients to start antibiotic therapy after culture and sen-

sitivity results have been obtained and patients should complete the full courses of antibiotics. The use of effective infection-control practices to prevent dissemination to healthy people, surveillance of antimicrobial resistance and antimicrobial use, and improved use of vaccination may circumvent the necessities of antimicrobial therapy. Development of alternative therapies like bacterial interference using nonpathogenic (commensal) bacteria, bacteriophage therapy, use of cationic peptides, and synthetic and amphipathic cyclic D, L-a-peptides, can also be alternatives to extensive antimicrobial use.

Updated follow-up study report is very much essential in regards with promoting specific and actual line of therapy, for the diarrhoeal disease management and to reduce the health care costs in LMICs like Bangladesh. Similarly, this study will help physicians to have the overview knowledge of antimicrobial resistance of different diarrhoeal pathogens to guide the patients successfully. Although having several advantages, this data may mislead physicians who prescribe antibiotics against unusual multiple antibiotic-resistant bacteria based on this data. After all, molecular characterization of bacterial isolates and a better antibiotic susceptibility test will help to overcome the limiting factors of such study in future.

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Footnotes

Authors' Contribution: Abdullah Bashar Sami analysed the data and wrote the manuscript. Monirul Islam, Farhana Halim, Nasrin Akter, Tuhin Sadique, Saroar Hossain performed species identification and susceptibility tests. Shahriar Bin Elahi was responsible for collecting the data. Anowar Hossain and Mahbubur Rahman critically revised the manuscript, Dilruba Ahmed designed the study and reviewed the manuscript for important intellectual content. All the authors read and approved the final manuscript.

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routine management. Samples were further anonymised and anonymised for research use.

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