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Emergence of Extensively Drug-resistant *Shigella sonnei* in Bangladesh

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Abstract The objective of the study was to investigate current species distribution and growing antimicrobial resistance (AMR) of *Shigella* isolates for proper treatment. Shigellae, isolated from faecal samples in International Centre for Diarrhoeal Disease research, Bangladesh, Dhaka hospital in 2015, were tested for antimicrobial susceptibility by disc diffusion method to ampicillin, co-trimoxazole, ciprofloxacin, azithromycin, mecillinam, ceftriaxone/cefixime and meropenem. Extensively drug-resistant (XDR, resistant to 5 or 6 of 7 useful anti-*Shigella* drugs tested) *Shigella* isolates resistant to 6 drugs were analyzed for ESBL and AmpC phenotypes, plasmid profiles, R-plasmids transfer, bla_{SHV}, bla_{TEM}, bla_{CTX-M}, bla_{OXA}; and *mph*A, *mph*B, *erm*A, *erm*B, *erm*C, *ere*A, *ere*B, *mef*A and *msr*A genes by PCR; and clonality of *S. sonnei* by PFGE. Of 134 isolates cultured from 3722 (3.6%) diarrhoeal faecal samples, 46% were *S. sonnei*, 37% *S. flexneri*, 4% *S. boydii*, 5% *S. dysenteriae* and 7% non-typeable. Multidrug-resistant (MDR, resistant simultaneously to ≥3 drugs) *S. sonnei* were 95% compared to 66% (P<0.01) MDR *S. flexneri* including 18% & 14% XDR types, respectively. All isolates were susceptible to meropenem. Four (6%) *S. sonnei*, 2 (4%) *S. flexneri* and 1 (17%) *S. boydii* (total of 7 isolates) were six-drugs XDR; 5 of them had ESBL phenotypes. Three *S. sonnei* and 1 *S. flexneri* had bla_{TEM} and bla_{CTX-M}; 1 *S. boydii* had bla_{SHV}, bla_{TEM} and bla_{CTX-M}; 1 *S. sonnei* had bla_{TEM} β-lactamase. All but one *S. flexneri* had only *mph*A gene on 62-MDa conjugative-R-plasmid coding azithromycin resistance. PFGE identified MDR-*S. sonnei* Global III clade. Thus, MDR-*S. sonnei* replaced *S. flexneri* as predominant isolate in Dhaka, Bangladesh; many emerged as XDR strains requiring treatment by meropenem. The findings demand judicial use of antibiotics to contain emergence and spread of resistance locally and globally. Physicians should be informed about MDR and XDR *Shigella* for judicious prescribing of antimicrobial therapy.

Keywords *Shigella*, Antimicrobial Resistance, ESBL, *mph*A, Azithromycin

1. Introduction

Shigella is one of the significant causes of diarrhoeal diseases globally with ~80 million cases and ~700,000 deaths/year, mostly occurring in developing countries [1]. Of the four species of genus *Shigella*, *Shigella flexneri* and *Shigella sonnei* predominate. *S. sonnei,* which has historically been prevalent in developed countries, is currently undergoing an unprecedented expansion across industrializing regions in Asia, Latin America, and the Middle East replacing *S. flexneri* as the major species [2]. The current evolutionary changes in temporal distribution of *Shigella* species across the globe have altered the epidemiology, treatment and preventive strategies of bacillary dysentery (shigellosis). Antimicrobial therapy has been recommended for patients with shigellosis because it can limit the clinical course of illness and reduce the risk of complications and the duration of faecal excretion of the causative organism, reducing the spread of infection [1, 3]. The therapy also improves the growth and nutritional status of affected children, especially in developing countries [3]. A major problem, however, is the increasing resistance of *Shigella* spp. to useful antimicrobial agents [2-5]. Over the decades, *Shigella* isolates resistant to multiple first-line antimicrobial agents, such as sulphonamides, tetracycline, ampicillin, trimethoprim-sulphamethoxazole (SXT) and nalidixic acid have been reported from many countries including Bangladesh [2-5] resulting in difficulties in the selection of empirical therapy. Currently ciprofloxacin is recommended as the drug of choice by the World Health Organization for the therapy of first-line drug-resistant *Shigella* infections in both adults and children [1]. In addition, ceftriaxone, pivmecillinam (amdinocillin pivoxil) and azithromycin are considered as alternative drugs [1]. However, multidrug-resistant (MDR; resistant to three or more classes of antimicrobial agents) *Shigella* isolates have been reported worldwide and few reliable treatment options

exist, particularly in developing countries. The recent acquisition of ciprofloxacin-resistance and/or extended-spectrum-cephalosporin-resistance and/or azithromycin-nonsusceptibility by the MDR *Shigella* isolates further narrows the choice of effective antimicrobial agents for treating shigellosis [4-5].

Recently, multidrug-resistant *S. sonnei* has emerged as a serious public health threat globally [6-10]. In South and South-East Asia, and elsewhere multidrug-resistant *S. sonnei* has become highly prevalent and now partially/completely replaced *S. flexneri* probably as a part of global extension of MDR-*S. sonnei* Global III clade [4, 6-12]. International travel or recent migration of refugees from endemic areas of Asia and Africa has accelerated the global spread of drug-resistant *Shigella* to non-endemic countries by repeated introductions and autochthonous transmission [5, 7, 14, 15]. Recently MDR-*S. sonnei* resistant to ciprofloxacin with decreased susceptibility to azithromycin (MIC >16 µg/ml) caused widespread outbreaks in the USA [5, 14] resulting in difficulties in selection of antimicrobial agents for proper treatment. *Shigella* species*,* resistant to ciprofloxacin, ceftriaxone and azithromycin (MIC>32 µg/ml) caused infections in refugees and local residents in Austria and other EU countries imposing a new health problem [15]. Even the last resource meropenem was used to treat ceftriaxone-resistant *Shigella* infection because MIC values of meropenem (MIC range <0.06 µg/ml) and imipenem (MIC range <0.06–0.25 µg/ml) showed susceptibility of all resistant *Shigella* isolates tested [16, 17]. In Bangladesh, during 2001-2002 *S. sonnei* comprised only 16% of 266 *Shigella* isolates studied and a low resistance to ampicillin (16%) and high resistance to cotrimoxazole (87%) were reported among *S. sonnei* isolates [3]. However, the prevalence of *S. sonnei* increased from 7.2% in 2001 to 25% in 2011in Bangladesh [12]. In Bangladesh ESBL-mediated ceftriaxone-resistant MDR-*S. sonnei* was reported in 2004 [13] and ciprofloxacin-resistant MDR-*S. sonnei* in 2013 [12]. Currently, we do not have sufficient information on the prevalent serotypes and susceptibility patterns of *Shigella* isolates to many useful antimicrobial agents, such as mecillinam, ciprofloxacin, azithromycin, ceftriaxone/cefixime and meropenem. Given the impact of suboptimal use of antimicrobial agents in Bangladesh and the ability of *Shigella* to develop resistance after the introduction of new antimicrobial agents for treatment, it is not unlikely that antimicrobial resistance patterns in Bangladesh have changed since they were reported in the past [11-13]. Thus, the continuing changing patterns of prevalent species and resistance of *Shigella* isolates indicate the need for monitoring antimicrobial susceptibility. We aimed to study the current changing patterns of *Shigella* species and their antimicrobial resistance to explore the impact of global changes in *Shigella* infections and for determination of optimal antimicrobial therapy for shigellosis in Bangladesh.

2. Materials and Methods

2.1. Study Design, Bacterial Isolates and Antimicrobial Susceptibility Testing

During June-December, 2015, we studied consecutive *Shigella* isolates from Clinical Microbiology Laboratory, International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) Dhaka, Bangladesh. The centre's Dhaka hospital is located in the capital Dhaka that serves ~150,000 dairrhoeal patients per year from all over Bangladesh. The faecal samples of diarrheal patients were cultured and isolates were identified and serotyped by standard methods in Clinical Microbiology Laboratory [18]. Antimicrobial susceptibility to ampicillin, cotrimoxazole, ciprofloxacin, azithromycin, mecillinam, ceftriaxone/cefixime and meropenem was carried out by the disc diffusion method using CLSI-2014 guidelines except for azithromycin for which British Society of Antimicrobial Chemotherapy (BSAC) guidelines was used (S≥18 mm, R<18 mm) [19,20]. Minimum inhibitory concentration (MIC) was determined by agar dilution technique and E-test (bioMérieux, France) for selected-resistant isolates only. Extensively drug-resistant (XDR)-*Shigella* was defined as isolates resistant to 5 or 6 of 7 (ampicillin, cotrimoxazole, ciprofloxacin, azithromycin, mecillinam, ceftriaxone/cefixime and meropenem) anti-*Shigella* drugs used for the treatment of shigellosis and tested for *in-vitro* antimicrobial susceptibility of *Shigella* in our laboratory. Multidrug-resistant (MDR) *Shigella* was defined as an isolate simultaneously resistant to ≥3 anti-*Shigella* drugs. Antibiotics regarded as inappropriate for treatment of shigellosis and rarely tested for *in-vitro* susceptibility were not considered in defining XDR and MDR isolate. Six (available) XDR-*Shigella* isolates were further analyzed for MICs of ceftriaxone and azithromycin.

2.2. Biotyping of *S. sonnei*

Biotyping *of S. sonnei* was done using standard methods for fermentation of rhamnose and xylose and hydrolysis of ortho-nitrophenyl-b-Dgalactopyranoside (ONPG), and biotypes were designated according to methods described elsewhere [12].

2.3. Phenotypic and Genotypic Studies for Ceftriaxone and Azithromycin Resistance

Six (available) XDR-*Shigella* isolates were further analyzed for phenotypic and genotypic characteristics of resistance of ceftriaxone and azithromycin. One *S. flexneri* isolate was not available. The ESBL and AmpC phenotypes were detected by the double disc diffusion synergy test (DDST) and combined disc test (CDT) following CLSI guideline [19]. PCR was carried out for detection of bla_{SHV}, bla_{TEM}, $bla_{\text{CTX-M}}$, bla_{OXA} for cetriaxone resistance and *mph*A, *mph*B, *erm*A, *erm*B, *erm*C, *ere*A, *ere*B, *mef*A and *msr*A gene for azithromycin resistance [21-25]. The primer sequences used in PCR were mentioned in Table 1[21-25].

Table 1. Oligonucleotide primers used for the detection of *Shigella sonnei* ESBL and macrolide resistance genes by PCR in the study [21-25].

Target gene	Primer	Sequence, 5'→ 3'	Annealing temperature ^0C	Product size, bp
^{Bla}TEM	TEM-F	CTTCCTGTTTTTGCTCACCCA	52	717
	TEM-R	TACGATACGGGAGGGCTTAC		
^{Bla}SHV	SHV-F	TCAGCGAAAAACACCTTG	52	471
	SHV-R	TCCCGCAGATAAATCACC		
^{Bla}OXA	OXA-F	ACCAGATTCAACTTTCAA	55	598
	OXA-R	TCTTGGCTTTTATGCTTG		
^{Bla}CTX-M	CTX-M-F	TTTGCGATGTGCAGTACCAGTAA	51	544
	CTX-M-R	CGATATCGTTGGTGGTGCCATA		
mph(A)	mphA-F	GTGAGGAGGAGCTTCGCGAG	60	403
	mphA-R	TGCCGCAGGACTCGGAGGTC		
mph(B)	mphB-F	GATATTAAACAAGTAATCAGAATAG	58	494
	mphB-R	GCTCTTACTGCATCCATACG		
erm(A)	ermA-F	TCTAAAAAGCATGTAAAAGAAA	52	533
	ermA-R	CGATACTTTTTGTAGTCCTTC		
erm(B)	ermB-F	GAAAAAGTACTCAACCAAATA	45	639
	ermB-R	AATTTAAGTACCGTTACT		
erm(C)	ermC-F	TCAAAACATAATATAGATAAA	45	642
	ermC-R	GCTAATATTGTTTAAATCGTCAAT		
ere(A)	ereA-F	GCCGGTGCTCATGAACTTGAG	60	420
	ereA-R	CGACTCTATTCGATCAGAGGC		
ere(B)	ereB-F	TTGGAGATACCCAGATTGTAG	55	537
	ereB-R	GAGCCATAGCTTCAACGC		
mef(A)	mefA-F	AGTATCATTAATCACTAGTGC	54	345
	mefA-R	TTCTTCTGGTACTAAAAGTGG		
msr(A)	msrA-F	GCACTTATTGGGGGTAATGG	58	384
	msrA-R	GTCTATAAGTGCTCTATCGTG		

2.4. Plasmid Analysis and R-Plasmid Transfer by Conjugation

Plasmid DNA was prepared according to the alkaline lysis method of Kado and Liu (1981) with some modifications and plasmid sizes were analyzed by using known molecular mass plasmids in *E. coli* PDK-9, R-1and V517 in agarose gels [26]. Conjugal transfer of R-plasmids to *Escherichia coli* K-12 was carried out by the method of Neu *et al* [27] and lactose-fermenting transconjugants were selected on MacConkey agar containing azithromycin (32µg/ml). Like wild-type strains, all transconjugants were tested for antimicrobial susceptibility and plasmid profiles. 62-MDa-plasmid was extracted from agarose gel and subjected to PCR for *mph*A gene detection [24].

2.5. PFGE

PFGE of *S. sonnei* isolates was carried out for detection of clonality by XbaI restriction patterns of genomes. High-molecular-weight genomic DNA was prepared from 6 drugs-XDR *S. sonnei* study isolates (2015), 6 MDR *S. sonnei* isolates of the year 2001-2010 and 1 *S. flexneri* isolates and digests with the restriction endonuclease XbaI. Restriction fragment patterns of chromosomal DNA were demonstrated by PFGE. [28].

3. Results

3.1. Bacterial Isolates and Antimicrobial Susceptibility

Of 3722 stool cultures, 134 (3.6%) samples were positive for *Shigella*. *S. sonnei* were predominant (46%, 62 isolates) followed by *S. flexneri* 37% (50), *S. boydii* 4% (6), *S. dysenteriae* 5% (7), and atypical *Shigella* 7% (9). The overall resistance rates were: ampicillin (43%), co-trimoxazole (79%), ciprofloxacin (79%), azithromycin (87%), mecillinam (16%), ceftriaxone/cefixime (11%) and meropenem (0%). The antibiotic resistance frequencies of

co-trimoxazole (98%), azithromycin (100%), ciprofloxacin (97%), and ceftriaxone (13%) were higher in *S. sonnei* compared to those of co-trimoxazole (60%), azithromycin (72%), ciprofloxacin (76%) and ceftriaxone (10%) in *S. flexneri*. All (100%) *S. sonnei* were simultaneously resistant to two or more drugs compared to 78% and 73% in *S. flexneri* and other species (P<0.01, Table 2). MDR phenotypes (Table 2) were detected in 95% of *S. sonnei* compared to 66% in *S. flexneri* isolates (P<0.01). XDR rate was higher in *S. sonnei* than *S. flexneri* and others (18% vs. 9%, P<0.01), respectively similar to 3-drug resistance patterns (Table 2, 60%, 30% and 27%; P<0.01). Two-drug and one-drug resistance patterns were significantly more in other species than *S. sonnei* and *S. flexneri* (P<0.01). Four (6%) of the 62 *S. sonnei* and 2 of the 50 *S. flexneri* (4%) and 1 of the 6 *S. boydii* (17%) were six-drug XDR isolates.

Table 2. Antimicrobial Resistance patterns of *Shigella* isolates (N=134), June-December 2015

Resistance to number of drugs (resistance patterns)	**Resistance patterns**			
	Types	***S. sonnei* (N=62)**, No. strains (%)	***S. flexneri*** (N=50), No. strains (%)	†**Other *Shigella* spp. (N=22)**, No. strains (%)
XDR* strains (Resistance to 6 & 5 drugs)		**11 (18)§**	**7 (14)**	**2 (9)§**
*Resistance to 6 drugs (XDR)**	AAzCMCrS	***4 (6)***	***2 (4)***	***1 (5)***
*Resistance to 5 drugs (XDR)**		***7 (11)***	***5 (10)***	***1 (5)***
	AAzCMS	4 (6)	2 (4)	0 (0)
	AAzCCrS	1 (2)	2 (4)	0 (0)
	AAzCCr(i)$S	1 (2)	0 (0)	0 (0)
	AAzCM(i)S	1 (2)	0 (0)	0 (0)
	AAzCM(i)Cr	0 (0)	1 (2)	0 (0)
	AAzC(i)M(i)Cr(i)	0 (0)	0 (0)	1 (5)
Resistance to 4 drugs**		**11 (18)**	**11 (22)**	**3 (14)**
	AAzCS	4 (6)	5 (10)	1 (5)
	A(i)AzCS	4 (6)	0 (0)	0 (0)
	AAzMS	1 (2)	0 (0)	0 (0)
	AzCCr(i)S	1 (2)	0 (0)	0 (0)
	AzC(i)Cr(i)S	1 (2)	0 (0)	0 (0)
	AAzC(i)S	0 (0)	2 (4)	1 (5)
	AAzCS(i)	0 (0)	2 (4)	0 (0)
	AAzCM(i)	0 (0)	1 (2)	0 (0)
	AC(i)MS	0 (0)	1(2)	0 (0)
	AAzM(i)S	0 (0)	0 (0)	1 (5)
Resistance to 3 drugs		**37 (60)§**	**15 (30)§**	**6 (27)§**
	AzCS	32 (52)	6 (12)	2 (9)
	AzC(i)S	4 (6)	0 (0)	1 (5)
	AzCS(i)	1 (2)	1 (2)	0 (0)
	AAzC	0 (0)	3 (6)	0 (0)
	AC(i)S	0 (0)	3 (6)	1 (5)
	AAzS	0 (0)	2 (4)	1 (5)
	AAzM(i)	0 (0)	0 (0)	1 (5)
Resistance to 2 drugs		**3 (5)§**	**6 (12)§**	**5 (23)§**
Resistance to 1 drug		**0 (0)§**	**7 (14)§**	**6 (27)§**
Susceptible to all drug tested		**0 (0)**	**4 (8)**	**0 (0)**

A = ampicillin, Az = azithromycin, C = ciprofloxacin, Cr = ceftriaxone, M = mecillinam, S = cotrimoxazole. §P< 0.01, *XDR = extensively drug-resistant, overall XDR rate 15%, $(i) = intermediate.

†**Others:** *S. boydii* 4% (6), *S. dysenteriae* 5% (7), and atypical *Shigella* 7% (9), The overall resistance rates were: ampicillin (43%), co-trimoxazole (79%), ciprofloxacin (79%), azithromycin (87%), mecillinam (16%), ceftriaxone/cefixime (11%) and meropenem (0%).

3.2. Biotyping of XDR *S. sonnei*

Of 4 XDR *S. sonnei* isolates tested, all were classified as biotype g (ONPG positive, rhamnose and xylose negative).

3.3. Phenotypic and Genotypic Studies for Ceftriaxone and Azithromycin Resistance

Five (83%) of 6 six-drug-XDR strains were positive for ESBL by DDST and CDT. Three *S. sonnei* (including one ESBL-phenotype negative *S. sonnei*) and 1 *S. flexneri* had bla_{TEM} and $bla_{CTX\text{-}M}$; *S. boydii* had bla_{SHV}, bla_{TEM} and $bla_{CTX\text{-}M}$; the rest *S. sonnei* had only bla_{TEM} (Fig 2 a).

3.4. Plasmid Analysis and R-Plasmid Transfer by Conjugation

Most *Shigella* isolates had 3-4 plasmids (Fig. 1; 140, 62, 2.7, 2.1MDa). Only 62-MDa-plasmid carrying azithromycin and ampicillin resistances were transferred from 5 *Shigella* isolates (excluding *S. flexneri*) to *E. coli* K-12 (transconjugants) by conjugation (data not shown). A 403bp PCR amplicon obtained by using gel-eluted 62-MDa-plasmid DNA indicated the presence of *mph*A gene in 5 of 6 XDR *Shigella* (except *S. flexneri*) isolates (Fig. 2 b).

3.5. PFGE

XbaI restriction fragment patterns of DNA from three (S1, S2, S6) of four XDR *S. sonnei* study isolates and 3 (A1, A2 and A3) reference *S. sonnei* pulsotype A isolates were identical with minor diversifications (Fig. 3). XbaI produced approximately 21 fragments, except one isolate (S5) which produced 18 fragments showing a different pulsotype of biotype g/global III clade. All the strains were typeable that yield reproducible banding patterns. Three (S1, S3 and S6) of 4 study *S. sonnei* isolates belonged to major pulsotype A of *S. sonnei* [12] by PFGE with minor variants of A pulsotype (95% or more similarity) and belonged to biotype g group indicating Global III clade [6, 12].

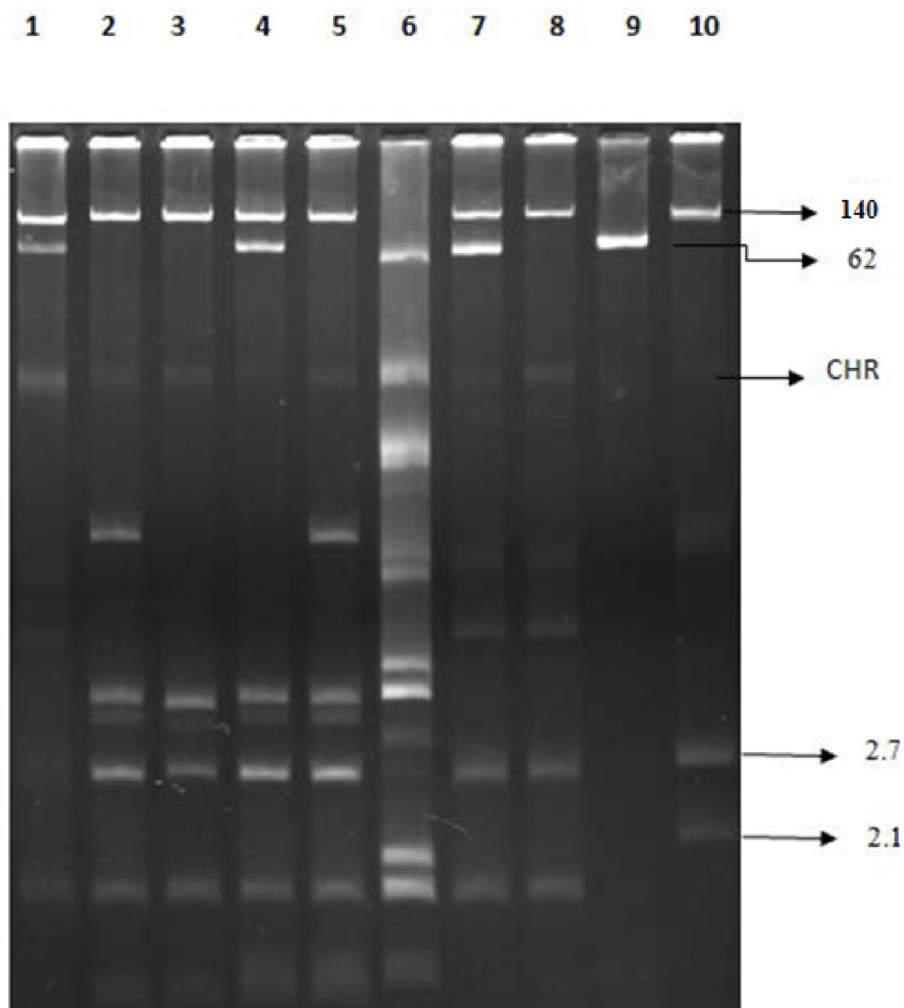

Figure 1. Agarose gel electrophoresis of plasmid DNA (MDa) showing representative patterns of *Shigella boydii and Shigella sonnei* isolates. Lane: 1, 4 (Azm^R *S. sonnei* S1 and S3); Lane: 2, 3, 5 (Azm^S *S. sonnei*); Lane: 6 *E. coli* V-517; Lane 7 (Azm^R *S. boydii* B4); Lane 8 (Azm^S *S. boydii*); Lane: 9 *E. coli* R-1; Lane: 10 PDK-9.

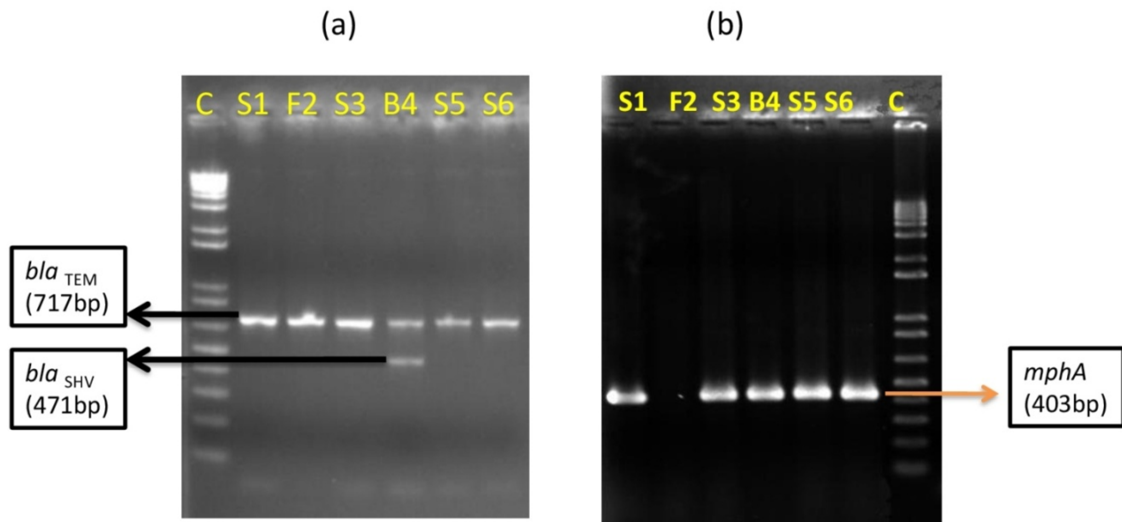

Figure 2. Agarose gel of PCR results showing *bla*$_{TEM}$ and *bla*$_{SHV}$ (gel **a**) and *mph*A genes (gel **b**) of six XDR *Shigella* isolates

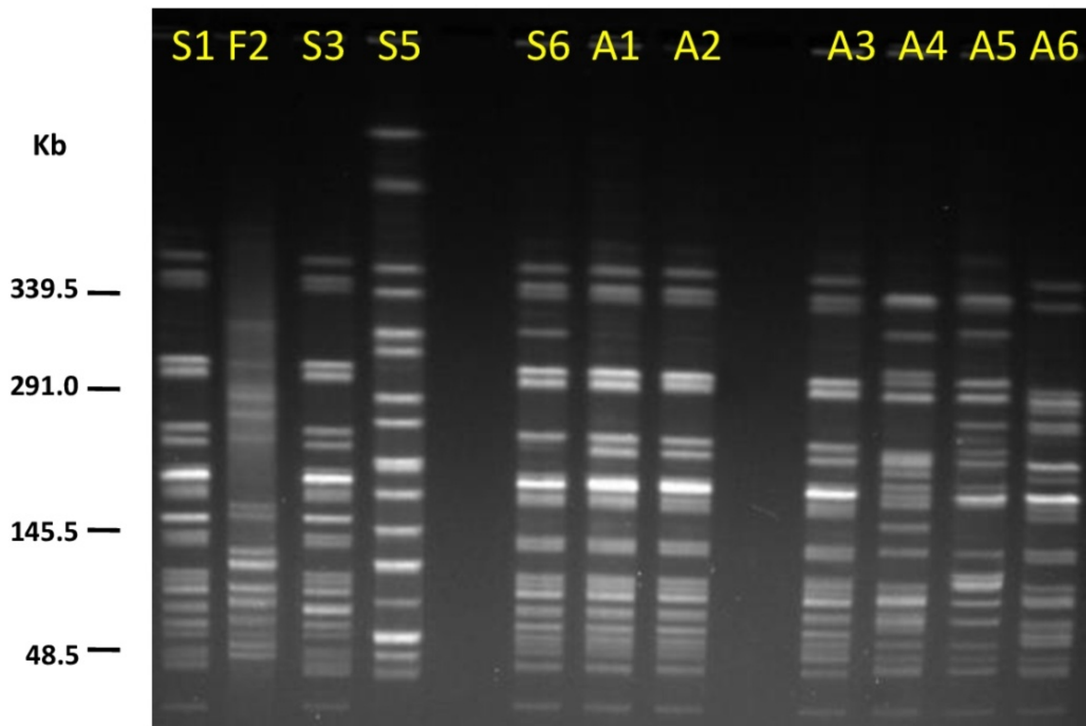

Figure 3. PFGE results showing XbaI-digested chromosomal DNA patterns of XDR *Shigella sonnei*. *S. sonnei:* Lane S1, S3, S6 belonged to pulsotype **A**, Lane S5 belonged to new pulsotype. Lane F2 *S. flexneri*; Reference *S. sonnei* strains (12): Lane A1, A2 and A3: pulsotype **A**; Lane A4 and A5: pulsotype **C** and A6: pulsotype **B** *S. sonnei* isolates were used for comparison of PFGE results.

4. Discussion

S. sonnei has been primarily responsible for bacillary dysentery in developed countries. At present it is undergoing an unprecedented spread across Asia, America, and the Middle East which represents an emerging threat to public health globally [2,7]. *S. flexneri* was the most prevalent species of *Shigella* in 2011 in Bangladesh [11]. However, an increase in the isolation rate *S. sonnei* in Dhaka and Mirpur was noted during 2010-2011 [11, 12]. We documented in the present study that *S. sonnei* replaced *S. flexneri* as the predominant isolate in Dhaka for the first time in 2015. Biotype g of *S. sonnei* is a marker of MDR Global III clade [6, 7, 12]. Biotype g of *S. sonnei* was found to comprise major PFGE pulsotype A with minor diversifications (B, C and D types) [12]. We identified six-drugs-XDR four *S. sonnei* biotype g MDR Global III clade isolates that belonged to A pulsotype/clone (3 isolates) and its variants by PFGE (a descendant of MDR Global III clade) indicating the emergence, adaptation and dissemination of XDR *S. sonnei* Global III clade for the first time in Bangladesh [6, 12].

Multidrug resistance was a problem in *S. flexneri* and *S. dysenteriae* in Bangladesh which was less common in *S. sonnei* [3, 13]. However, the highest prevalence of XDR as well as MDR phenotypes was observed in *S. sonnei* in Dhaka, Bangladesh in our study. In 2004, we reported *S. sonnei* isolates that were resistant to numerous drugs and produced R-plasmid mediated ESBL [13] which appeared to acquire from *E. coli* in the gut [29]. Five drugs-XDR-patterns were highly prevalent in all *Shigella* species being susceptible to ceftriaxone, or mecillinam along with meropenem. Six drugs-XDR *S. sonnei* can only be treated with meropenem or imipenem. Resembling Vietnam, adaptive genomic microevolution in *S. sonnei* might have occurred in Bangladesh over the last few years [6] and *S. sonnei* acquired resistance to many drugs by mutation and lateral gene transfers to emerge as a key resistant species. In the USA, XDR and multiply resistant *S. sonnei* was reported to cause dysentery outbreaks in 34 states resulting in 315 cases during 2014–2015, making treatment increasingly difficult [5,14]. XDR-*S. sonnei* was also reported to cause infections among refugees and local residents in Austria and many other EU countries in 2015 [15].

Extended-spectrum β-lactamase was detected in ceftriaxone-resistant *Shigella* by phenotypic characteristics in Bangladesh [13]. We detected multiple ESBL genes in a single *Shigella* strain for the first time in Bangladesh that will lead all third-generation cephalosporin ineffective for the treatment of shigellosis. Azithromycin is effective for the treatment of ciprofloxacin-resistant shigellosis both in children and adults. The *mph*A gene encodes a macrolide 2′-phosphotransferase that phosphorylates and inactivates azithromycin (macrolides) is spreading azithromycin-resistance in Europe and USA [14, 24]. The majority (83%) of XDR-*Shigella* carry R-plasmid mediated *mph*A gene. The conjugal transfer of *mph*A plasmid from *Shigella* to *E. coli* K-12 indicates the potential of spread of azithromycin-resistance to other bacteria in near future in Bangladesh.

Our findings have major implications for dissemination of XDR-*S. sonnei* and other species regionally and globally, and for spread of mobile drug-resistance genes among bacterial populations. Options for antimicrobial therapy for such XDR-*Shigella* infections are very limited that clearly demands judicial use of antibiotics, development of vaccines, safe food and water, improvement of hygiene and sanitation for the prevention and control of shigellosis.

In conclusion, physicians should be warned regarding the presence of XDR and high prevalence of MDR *Shigella* spp., particularly XDR-*S. sonnei* Global III clade in Dhaka that might be prevalent in other parts of Bangladesh. Further studies might explore the extent of spread in the country.

Monitoring of antimicrobial susceptibility of clinical isolates for optimum antimicrobial therapy is essential. Additionally, reduced susceptibility of *Shigella* strains to azithromycin and mecillinam should be identified by MICs determination for the early detection of the treatment failures and emergence of resistance. When indicated, pivmecillinam or ceftriaxone might be considered for treating shigellosis in Bangladesh with caution. Meropenem may be used as the last resort.

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Competing Interests

None to declare

Ethical Approval

The Ethical Review Committee of IRB of icddr,b

approved this research protocol (PR-14042) on August 21, 2014.

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