Indian J Med Res 149, February 2019, pp 247-256
DOI: 10.4103/ijmr.IJMR_2077_17

Quick Response Code:

Antimicrobial resistance, virulence & plasmid profiles among clinical isolates of *Shigella* serogroups

Dhiviya Prabaa Muthuirulandi Sethuvel[1], Susmitha Perumalla[1], Shalini Anandan[1], Joy Sarojini Michael[1], Naveen Kumar Devanga Ragupathi[1], Revathi Gajendran[1], Kamini Walia[2] & Balaji Veeraraghavan[1]

[1]Department of Clinical Microbiology, Christian Medical College, Vellore & [2]Division of Epidemiology & Communicable Diseases, Indian Council of Medical Research, New Delhi, India

Received December 29, 2017

***Background & objectives*: Bacillary dysentery caused by *Shigella* spp. remains an important cause of the crisis in low-income countries. It has been observed that *Shigella* species have become increasingly resistant to most widely used antimicrobials. In this study, the antimicrobial resistance, virulence and plasmid profile of clinical isolates of *Shigella* species were determined.**

***Methods*: Sixty clinical *Shigella* isolates were subjected to whole-genome sequencing using Ion Torrent platform and the genome sequences were analyzed for the presence of acquired resistance genes, virulence genes and plasmids using web-based software tools.**

***Results*: Genome analysis revealed more resistance genes in *Shigella flexneri* than in other serogroups. Among β-lactamases, bla_{OXA-1} was predominantly seen followed by the bla_{TEM-1B} and bla_{EC} genes. For quinolone resistance, the *qnr*S gene was widely seen. Novel mutations in *gyr*B, *par*C and *par*E genes were observed. Cephalosporins resistance gene, $bla_{CTX-M-15}$ was identified and plasmid-mediated AmpC β-lactamases genes were found among the isolates. Further, a co-trimoxazole resistance gene was identified in most of the isolates studied. Virulence genes such as *ipaD, ipaH, virF, senB, iha, capU, lpfA, sigA, pic, sepA*, *celb* and *gad* were identified. Plasmid analysis revealed that the IncFII was the most commonly seen plasmid type in the isolates.**

***Interpretation & conclusions*: The presence of quinolone and cephalosporin resistance genes in *Shigella* serogroups has serious implications for the further spread of this resistance to other enteric pathogens or commensal organisms. This suggests the need for continuous surveillance to understand the epidemiology of the resistance.**

Key words Antimicrobial resistance gene - $bla_{CTX-M-15}$ - IncF plasmid - *qnr* - *Shigella* spp. - virulence

Shigella is an important cause of diarrhoea, particularly in children less than five years of age. *Shigella* spp. is highly contagious due to its low infective dose and high transmission rate in areas with overcrowding and poor sanitary conditions[1]. Depending on the virulence potential of the strain and the nutritional status of the individual, shigellosis can progress to severe disease[2]. The Global Enteric Multicenter Study, a case-control study of moderate-to-severe paediatric diarrhoeal disease, identified enterotoxigenic

Escherichia coli and *Shigella* spp. as the most common bacterial pathogens in Sub-Saharan Africa and South Asia[3].

Although *Shigella* infection is mostly self-limiting disease, antibiotics are recommended to reduce the clinical course of illness and to prevent transmission. However, antimicrobial resistance (AMR) is an emerging concern among *Shigella* spp. particularly in Asia and Africa[4]. Over the past decades, *Shigella* species have become increasingly resistant to most widely used antimicrobials[5]. Despite the alarming increase in the AMR in bacterial pathogens in India, publicly available information concerning the molecular identity of resistance traits is minimal[6,7]. According to the WHO report, AMR pattern for *Shigella* varies with geographic location and with time[5]. The continuing changing patterns of prevalent species and resistance of *Shigella* isolates indicate the need for monitoring antimicrobial susceptibility profiles[8]. The mobile genetic elements play a significant role in transferring resistance genes horizontally to non-resistant isolates. These elements are believed to be responsible for the acquisition and dissemination of AMR among clinically relevant organisms[9].

The recent advancement in whole-genome sequencing technologies for routine microbiology is well documented[10]. However, there is limited information on the surveillance of diarrhoeagenic pathogens and their AMR pattern in developing countries. The availability of whole-genome sequences of antimicrobial-resistant pathogens enhances our knowledge of the molecular identity of resistance traits and their mechanism of dissemination within the microbial population. This study was aimed to generate the base line data of resistance, virulence and plasmid profiles of *Shigella* species isolated from clinical specimens through whole-genome sequencing.

Material & Methods

Shigella strains isolated from stool specimen from patients with diarrhoea or dysentery during the year 2011-2017 at Christian Medical College, Vellore, India were included in the study. Culture and biochemical identification of isolates was done using standard protocol[11]. Serologic confirmation was done by slide agglutination test using polyvalent somatic (O) antigen grouping sera, followed by monovalent antisera (Denka, Seiken, Japan) for *Shigella*-specific serotype identification. Antimicrobial susceptibility testing of isolates against ampicillin (10 µg), trimethoprim/sulphamethoxazole (1.25/23.75 µg), nalidixic acid (30 µg), norfloxacin (10 µg), cefotaxime (30 µg), cefixime (5 µg) and azithromycin (15 µg) was performed using Kirby-Bauer disc diffusion method[12]. The results were interpreted using breakpoints recommended by the Clinical and Laboratory Standards Institute guidelines 2017[12]. Quality control strains used were *E. coli* ATCC 35218 and *E. coli* ATCC 25922 for the antibiotics tested.

Whole-genome sequencing: Genomic DNA was extracted using the QiaSymphony DNA extraction platform (Qiagen, Hilden, Germany). Genome sequencing was performed using Ion Torrent (PGM, Life Technologies, Carlsbad, CA, USA) with 400 bp read chemistry (Life Technologies) as previously described[13].

Assembly & annotation: The raw data were assembled *de novo* using AssemblerSPAdes v.5.0.0.0 embedded in Torrent suite server v.5.0.4. The genome sequence was annotated using PATRIC, the bacterial bioinformatics database and analysis resource (*http://www.patricbrc.org*), and the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (*http://www.ncbi.nlm.nih.gov/genomes/static/Pipeline.html*)[14].

Downstream genome analysis: The whole-genome data were analyzed using open access tools at Centre for Genomic Epidemiology web-based server. AMR and virulence genes were identified using ResFinder 2.1 (*https://cge.cbs.dtu.dk//services/ResFinder/*)[15] and VirulenceFinder 1.5 (*https://cge.cbs.dtu.dk//services/VirulenceFinder/*)[16], respectively, with 90 per cent threshold for identity and with 60 per cent of minimum length coverage, where reads were mapped to a reference database of acquired genes. Furthermore, the transferable resistance genes and chromosomal mutation in the quinolone-resistant determining region were studied through PATRIC database. The presence of plasmids was analyzed using PlasmidFinder 1.3 (*https://cge.cbs.dtu.dk//services/PlasmidFinder/*) with 95 per cent threshold for identity[17]. These whole-genome shotgun sequences were deposited in DDBJ/ENA/GenBank (Table I for accession numbers).

Results

Whole-genome sequences of 60 *Shigella* isolates were analyzed in this study, which included *S. dysenteriae* (n=5), *S. flexneri* (n=23), *S. boydii* (n=17) and *S. sonnei* (n=15). Among the study isolates,

Table I. Characteristics of *Shigella* isolates analyzed in this study (n=60)

Isolate ID	Organism	Resistant pattern	Acquired resistance genes	Chromosomal mutation				Plasmid (Inc type)	Accession no.
				*gyr*A	*gyr*B	*par*C	*par*E		
FC1882	*S. boydii*	SXT-NAL	*str*A, *str*B, *aad*A1, *sul*II, *dfr*A1	D87-Y	-	-	-	IncFII	MDDI00000000
FC1764	*S. boydii*	AMP-SXT	*str*A, *str*B, bla_{TEM-1B}, *qnr*S1, *sul*II, *tet*A, *dfr*A14	-	-	-	-	IncFII, IncFIB	MDDH00000000
FC1661	*S. boydii*	SXT-NAL-FIX	*aad*A1, *sul*I, *tet*A, *dfr*A1, *dfr*A4, bla_{EC}	S83-L	-	-	*E135-V	IncA/C2, IncFII	MDGW00000000
FC2833	*S. boydii*	ALL SUSCEPTIBLE	-	-	-	-	-	IncFII	MDJL00000000
FC1567	*S. boydii*	AMP-SXT-NAL	*dfr*A3, bla_{EC}	-	-	-	-	IncFII	MIIV00000000
FC2117	*S. boydii*	AMP-SXT	*str*A, *str*B, bla_{TEM-1B}, *qnr*S1, *sul*II, *tet*A, *dfr*A14	-	-	-	-	IncFII, IncFIB	MINP00000000
FC2125	*S. boydii*	SXT-NAL-NX	*aad*A1, *dfr*A1, bla_{EC}	-	-	-	-	IncFII	MINQ00000000
FC2175	*S. boydii*	SXT	*aad*A1, *dfr*A1	-	-	-	-	IncFII	MINR00000000
FC2710	*S. boydii*	AMP-SXT-NAL (MS)	*str*A. *str*B, bla_{TEM-1B}, *qnr*S1, *sul*II, *dfr*A14	-	-	-	-	IncFII, IncFIB	MINU00000000
FC1180	*S. flexneri*	AMP-SXT-NAL-NX (MS)	*str*A, *str*B, *aad*A1, bla_{OXA-1}, *sul*II, *tet*B, *dfr*A1	S83-L	-	S80-I	-	-	MDJJ00000000
FC1139	*S. flexneri*	AMP-SXT	*dfr*A3, bla_{EC}	-	-	-	-	-	MECX00000000
FC1172	*S. flexneri*	AMP-SXT-NAL-NX (MS)	*str*A, *str*B, bla_{OXA-1}, *sul*II, *tet*B, *dfr*A1	S83-L	-	S80-I	-	-	MDJI00000000
FC1056	*S. dysenteriae* serotype 3	NAL-TAX	*str*A, *str*B, *aad*A1, *sul*II, *tet*B, *dfr*A1, bla_{EC}	-	*Q776-L	*C435-G, *S694-P	-	IncFII	MECW00000000
FC1708	*S. dysenteriae* serotype 3	SXT-NAL	*aad*A1, bla_{OXA-1}, *tet*B, *dfr*A1	-	*Q776-L	*C435-G, *S694-P	-	IncFII	MIIX00000000
FC1737	*S. dysenteriae* serotype 3	NAL	*tet*B, *dfr*A1	-	*Q776-L	*C435-G, *S694-P	-	IncFII	MIIY00000000
FC2531	*S. dysenteriae* serotype 3	AMP-NAL-TAX	*aad*A1, bla_{OXA-1}, *tet*B, *dfr*A1, bla_{EC}	-	*Q776-L	*C435-G, *S694-P	-	IncFII	MINS00000000
FC2541	*S. dysenteriae* serotype 3	AMP-NAL-TAX	*aad*A1, bla_{OXA-1}, *tet*B, *dfr*A1, bla_{EC}	-	*Q776-L	*C435-G, *S694-P	-	IncFII	MINT00000000
FC2383	*S. boydii*	AMP-SXT-NAL	*str*A, *str*B, *aad*A1, bla_{TEM-1B}, *qnr*S1, *sul*II, *dfr*A1	-	-	-	-	IncN, IncFII	MDJK00000000
FC1544	*S. boydii*	AMP-SXT-NAL	*str*A, *str*B, bla_{TEM-1B}, *qnr*S1, *sul*II, *dfr*A14	D87-Y	-	-	-	IncFII, IncFIB	MECT00000000
FC3196	*S. boydii*	AMP-SXT-NAL	*str*A, *str*B, *aad*A1, bla_{OXA-1}, *sul*II, *tet*B, *dfr*A1	S83-L	-	-	-	IncFII	MINV00000000

Contd...

Isolate ID	Organism	Resistant pattern	Acquired resistance genes	Chromosomal mutation				Plasmid (Inc type)	Accession no.
				*gyr*A	*gyr*B	*par*C	*par*E		
FC288	*S. sonnei*	AMP-SXT-NAL-NX	*str*A, *str*B, bla_{EC}, *sul*II, *dfr*A1	S83-L	-	S80-I	-	Col (BS512)	NGWI00000000
FC1373	*S. sonnei*	AMP-SXT-NAL-NX	*str*A, *str*B, bla_{EC}, *sul*II, *dfr*A1	S83-L	-	S80-I	-	Col 156	NGWH00000000
FC1417	*S. flexneri* 4	AMP-SXT-NAL-NX-TAX-FIX	*aad*A1, bla_{OXA-1}, $bla_{CTX-M-15}$, *qnr*S1, *cat*A1, *sul*II, *tet*B, *dfr*A1	S83-L	-	S80-I	-	IncFII, Col (MP18)	NGWG00000000
FC1846	*S. flexneri* 6	AMP-SXT-NAL-TAX-FIX	bla_{EC}, *aad*A1, *tet*B, *dfr*A1	D87-Y	*Q776-L	*Q506-L	-	IncFII	NGWF00000000
FC2615	*S. flexneri* 6	AMP-SXT-NAL	*aad*A1, bla_{EC}, *sul*II, *tet*B, *dfr*A1	D87-Y	*Q776-L	*Q506-L	-	IncFII	NGWE00000000
FC906	*S. flexneri* 2	AMP-SXT-NAL-NX-TAX-FIX	*str*A, *str*B, bla_{EC}, bla_{OXA-1}, *cat*A1, *sul*II, *tet*B, *dfr*A1	S83-L	-	S80-I	-	IncFII	NGWD00000000
FC1182	*S. flexneri* 1	AMP-SXT-NAL	*str*A, *str*B, *aad*A1, *sul*II, bla_{TEM-1B}, *tet*A, *dfr*A1	-	-	-	-	Col (BS512), IncFIB (K)	NGWC00000000
FC1772	*S. sonnei*	AMP-SXT-NAL-NX-TAX-FIX	bla_{EC}, *sul*I, *dfr*A5	S83-L	-	S80-I	-	Col 156	NGWB00000000
FC1659	*S. sonnei*	SXT-NAL	*str*A, *str*B, *aad*A1, bla_{OXA-1}, *cat*A1, *sul*II, *tet*B, *dfr*A1	S83-L	-	S80-I, *S542-P	-	IncFII, IncI2	NGWA00000000
FC470	*S. flexneri* 2	AMP-SXT-NAL-NX-TAX-FIX	*str*A, *str*B, bla_{TEM-1B}, bla_{DHA-1}, *qnr*B4, *qnr*S1, mphA, *sul*I, *sul*II, *tet*A, *dfr*A17	-	-	-	-	IncFII, IncFIB (K)	NGVZ00000000
FC1247	*S. flexneri* 2	AMP-SXT-NAL-NX-TAX-FIX	*str*A, *str*B, *aad*A1, bla_{EC}, bla_{TEM-1B}, *qnr*S1, *sul*II, *tet*A, *dfr*A1	S83-L	-	*Q506-L	-	IncFII, IncFIB (K)	NGVY00000000
FC1607	*S. flexneri* 4	AMP-SXT-NAL-NX-TAX-FIX	*aad*A1, *str*A, *str*B, bla_{EC}, $bla_{CTX-M\ 15}$, *qnr*S1, *cat*A1, *sul*II, *tet*B, *dfr*A1	S83-L	-	S80-I	-	IncFII, IncFIB (K)	NGVX00000000
FC1481	*S. flexneri* 4	AMP-SXT-NAL-NX-TAX-FIX	*str*A. *str*B, *aad*A1, bla_{TEM-1B}, bla_{OXA-1}, $bla_{CTX-M-15}$, *qnr*S1, *cat*A1, *sul*II, *tet*B, *dfr*A1	S83-L	-	S80-I	-	IncFII, IncFIB (K)	NGVW00000000
FC3278	*S. sonnei*	AMP-SXT-NAL	*str*A, *str*B, *bla*TEM1B, *sul*II, *dfr*A5	S83-L	-	S80-I	-	Col 156, IncB/O/K/Z	NMYB00000000
FC1244	*S. sonnei*	SXT-NAL	*str*A, *str*B, *sul*II, *dfr*A1	S83-L	-	S80-I	-	Col 156	NMYA00000000
FC3433	*S. flexneri* 2	AMP-SXT-NAL-TAX	*aad*A1, bla_{EC}, bla_{OXA-1}, *cat*A1, *tet*B, *dfr*A1	S83-L	-	S80-I	-	IncFII	NMXZ00000000
FC653	*S. sonnei*	AMP-SXT-NAL	bla_{EC}, *str*A, *str*B, *sul*II, *dfr*A1	S83-L	-	S80-I	-	Col 156	NMXY00000000

Contd...

Isolate ID	Organism	Resistant pattern	Acquired resistance genes	Chromosomal mutation				Plasmid (Inc type)	Accession no.
				*gyr*A	*gyr*B	*par*C	*par*E		
FC1170	*S. flexneri* 2	AMP-SXT-NAL	bla_{OXA-1}, *cat*A1, *tet*B, *dfr*A1	S83-L	-	S80-I	-	IncFII	NMXX00000000
FC1824	*S. flexneri* 2	AMP-SXT-NAL	*str*A, *str*B, bla_{OXA-1}, *cat*A1, *sul*II, *tet*B, *dfr*A1	S83-L	-	S80-I	-	IncFII	NMXW00000000
FC601	*S. flexneri* 1	AMP-SXT-AZM	*str*A, *str*B, *aad*A1, bla_{TEM1B}, *qnr*S1, *sul*II, *tet*A, *dfr*A1	-	-	-	-	Col 156	NMXV00000000
FC3209	*S. sonnei*	SXT-NAL-NX	*str*A, *str*B, *sul*II, *dfr*A1	S83-L	-	S80-I	-	Col 156	NMXU00000000
FC666	*S. boydii*	SXT-NAL	*aad*A1, *sul*II, *tet*B, *dfr*A1	S83-L, D87-Y	-	*Q506-L	-	IncFII	NMXT00000000
FC1747	*S. sonnei*	SXT-NAL	*str*B, *str*A, *sul*II, *dfr*A1	S83-L	-	S80-I	-	Col 156	NMXS00000000
FC15	*S. sonnei*	AMP-SXT-NAL-NX-TAX-FIX	*str*B, *str*A, $bla_{CTX-M-15}$, bla_{EC} *sul*II, *dfr*A1	S83-L	-	S80-I	-	Col (BS512), Col 156, Incl1	NMXR00000000
FC401	*S. flexneri* 1	AMP-SXT-NAL-NX	*str*A, *str*B, *aad*A1, bla_{TEM-1B}, *qnr*S1, *sul*II, *dfr*A1, *dfr*A14	-	-	-	-	IncFII, IncFIB (K)	NMXQ00000000
FC420	*S. flexneri* 2	AMP-SXT-NAL-NX	*str*A, *str*B, bla_{OXA-1}, *sul*II, *tet*B, *dfr*A1, *cat*A1	S83-L	-	S80-I	-	IncFII	NMXP00000000
FC248	*S. flexneri*	AMP-SXT-NAL-NX	bla_{OXA-1}, *tet*B, *dfr*A1, *cat*A1	S83-L	-	S80-I	-	IncFII	NMXO00000000
FC1642	*S. boydii*	SXT-NAL	*aad*A1, *tet*B, *dfr*A1, *sul*II	S83-L, D87-Y	-	*Q506-L	-	IncFII	PDYE00000000
FC1655	*S. boydii*	AMP-SXT-TAX-FIX	*str*A, *str*B, *aad*A1, bla_{EC}, $bla_{CTX-M-15}$, *qnr*S1, *sul*II, *dfr*A1	-	-	-	-	IncFII	PDYD00000000
FC1676	*S. boydii*	AMP-SXT	*str*A, *str*B, bla_{TEM-1B}, *qnr*S1, *sul*II, *tet*A, *dfr*A14	-	-	-	-	IncFII, IncFIB (K)	PDYC00000000
FC1706	*S. sonnei*	SXT-NAL	*dfr*A1	S83-L	-	S80-I	-	Incl1, Col 156	PDYB00000000
FC1628	*S. sonnei*	SXT-NAL	*str*A, *str*B, *sul*II, *dfr*A1	S83-L	-	S80-I	-	Col 156	PDYA00000000
FC1667	*S. sonnei*	NAL	*dfr*A1	S83-L	-	S80-I	-	Col 156, ColpVC	PDXZ00000000
FC1717	*S. boydii*	AMP-SXT	*str*A, *str*B, bla_{TEM-1B}, *qnr*S1, *tet*A, *sul*II	-	-	-	-	IncFII, IncFIB (K)	PDXY00000000
FC1653	*S. sonnei*	SXT-NAL	*str*A, *str*B, *sul*II, *dfr*A1	S83-L	-	S80-I	-	Col 156	PDXX00000000
FC1677	*S. sonnei*	AMP-SXT-NAL-TAX-FIX	*str*A, *str*B, bla_{EC}, $bla_{CTX-M-15}$, *sul*II, *dfr*A1	S83-L	-	S80-I	-	Col 156, Incl1	PDXW00000000
FC1405	*S. flexneri*	AMP-SXT-TET-NAL-NX	*str*A, *str*B, *aad*A1, bla_{OXA-1}, *cat*A1, *sul*II, *tet*B, *dfr*A1	S83-L	-	S80-I, *R86-C	-	IncFII	PDXV00000000
FC2101	*S. flexneri* 2	AMP-SXT-NAL-TAX-FIX	*aad*A1, bla_{EC}, bla_{CMY-4}, *dfr*A1	S83-L	-	S80-I	-	IncB/O/K/Z	PDXU00000000

Contd...

Isolate ID	Organism	Resistant pattern	Acquired resistance genes	Chromosomal mutation				Plasmid (Inc type)	Accession no.
				*gyr*A	*gyr*B	*par*C	*par*E		
FC2414	*S. flexneri* 2	AMP-SXT-NX	*str*A, *str*B, bla_{OXA-1}, *sul*II, *tet*B, *dfr*A1	S83-L	-	S80-I	-	IncFII	PDXT00000000
FC1954	*S. flexneri* 2	AMP-SXT-NAL-NX	*str*A, *str*B, bla_{OXA-1}, *sul*II, *tet*B, *dfr*A1	S83-L	-	S80-I	-	IncFII	PDXS00000000

*Novel mutations. AMP, ampicillin; SXT, trimethoprim/sulphamethoxazole; NAL, nalidixic acid; NX, norfloxacin; TAX, cefotaxime; FIX, cefixime; AZM, azithromycin

68 per cent (n=41) were resistant to more than or equal to three antimicrobials, 30 per cent (n=18) were resistant to less than three antimicrobials and two per cent (n=1) were susceptible to all tested antimicrobials. Ampicillin susceptibility was lower in *S. flexneri* compared to *S. sonnei*, while the susceptibility profile of other antibiotics remained unchanged. The susceptibility profile of the isolates is shown in Table I.

Whole genome sequencing: The genome length for the *Shigella* isolates ranged from ca. 4.2 Mbp to ca. 4.6 Mbp with coverage of 36× to 100×. Genomes were screened for known acquired genes. The presence of resistance determinants conferring resistance to β-lactams, aminoglycosides, quinolones, cephalosporins, tetracycline and sulphonamides was identified, as detailed in Table I.

Species-wise antimicrobial resistance (AMR) gene analysis

Shigella dysenteriae: Of the five *S. dysenteriae* isolates, three were found to carry bla_{OXA-1} β-lactamase gene. All the isolates carried tetracycline (*tet*) and trimethoprim (*dfr*A1) resistance genes, whereas only one isolate carried sulphonamide gene (*sul*II). An aminoglycoside resistance gene such as *str*A/B and *aad*A1 was also identified. No mutations were observed in *gyr*A and *par*E genes, but novel mutations were observed in *gyr*B (Gln776 - Leu) and *par*C (Cys435 - Gly) genes. None of the isolates harboured cephalosporin resistance gene (Tables I & II).

Shigella flexneri: All *S. flexneri* isolates were multi-drug resistant except one, which was resistant to ampicillin and trimethoprim/sulphamethoxazole alone. Among the β-lactamases, bla_{OXA-1}, bla_{TEM-1B}, $bla_{CTX-M-15}$ genes were present in 13, 5 and 3 isolates, respectively. AmpC genes such as bla_{DHA-1} and bla_{CMY-4} were found each in single isolate. For plasmid-mediated quinolone resistance, *qnr*B4 (n=1) and *qnr*S1 (n=7) genes were identified. Fifteen isolates showed two identical mutations in the *gyr*A and *par*C genes. The mutations were observed at codon 83 in the *gyr*A gene and at codon 80 in the *par*C gene which resulted in the replacement of serine by leucine and isoleucine, respectively. Two isolates had an additional mutation at codon 87 in *gyr*A gene, resulting in the replacement of aspartic acid by tyrosine. Novel mutations were observed in *gyr*B (Gln776 to Leu) and *par*C (Gln506 to Leu and Arg86 to Cys) genes. No mutation was seen in the *par*E gene (Table I). Genes encoding trimethoprim

Table II. Antimicrobial resistance genes distribution among *Shigella* serogroups % (n)

Shigella serogroup	bla_{OXA-1}	bla_{TEM-1B}	$bla_{CTX-M-15}$	bla_{DHA-1}	bla_{CMY-4}	*dfr*A1	*dfr*A14	*dfr*A17	*dfr*A4	*dfr*A5
S. dysenteriae (n=5)	60 (3)	-	-	-	-	100 (5)	-	-	-	-
S. flexneri (n=23)	56 (13)	22 (5)	13 (3)	4 (1)	4 (1)	91 (21)	4 (1)	4 (1)	-	-
S. boydii (n=17)	6 (1)	41 (7)	6 (1)	-	-	53 (9)	29 (5)	-	6 (1)	-
S. sonnei (n=15)	7 (1)	7 (1)	13 (2)	-	-	87 (13)	-	-	-	13 (2)
	*qnr*B4	*qnr*S1	*sul*I	*sul*II	*str*A	*str*B	*aad*A1	*tet*A	*tet*B	*cat*A1
S. dysenteriae (n=5)	-	-	-	20 (1)	20 (1)	20 (1)	80 (4)	-	100 (5)	-
S. flexneri (n=23)	4 (1)	30 (7)	4 (1)	74 (17)	65 (15)	65 (15)	52 (12)	17 (4)	69 (16)	43 (10)
S. boydii (n=17)	-	47 (8)	6 (1)	70 (12)	59 (10)	59 (10)	53 (9)	29 (5)	18 (3)	-
S. sonnei (n=15)	-	-	7 (1)	80 (12)	80 (12)	80 (12)	7 (1)	-	7 (1)	7 (1)

(*dfr*A1, *dfr*A14, *dfr*A17) and sulphonamide (*sul*I and *sul*II) resistance were identified. Most of the isolates carried genes such as *str*A/B, *aad*A1, *tet*A/B and *cat*A1, conferring resistance to aminoglycosides, tetracycline and chloramphenicol (Table II).

Shigella boydii: *S. boydii* isolates also carried the β-lactamase genes, bla_{OXA-1} (n=1), bla_{TEM-1B} (n=7), and $bla_{CTX-M-15}$ (n=1). AmpC genes were not detected. Among the quinolone resistant isolates, only a *qnr*S1 gene was identified in eight isolates (Tables I & II). Four isolates showed mutations in *gyr*A (S83-L and D87-Y), two in *par*C (Q506-L) and a single isolate had a mutation in the *par*E (E135-V) gene. No mutation was seen in *the gyr*B gene. Resistance genes such as *dfr*A1, *dfr*A14, *dfr*A4, *sul*I, *sul*II, *str*A/B, *aad*A1 and *tet*A/B were identified in *S. boydii* isolates.

Shigella sonnei: Like other serogroups, *S. sonnei* isolates were also found to carry bla_{OXA-1} (n=1), bla_{TEM-1B} (n=1), $bla_{CTX-M-15}$ (n=2) genes. None of the isolates carried AmpC or the *qnr* genes. However, all *S. sonnei* isolates showed two identical mutations in *gyr*A and *par*C genes, S83-L and S80-I, respectively. One isolate had additional mutation in *par*C (S542-P) gene (Table I). The isolates also carried resistance genes for sulphonamides, aminoglycoside, tetracycline and chloramphenicol (Table II).

Virulence gene analysis: The presence of virulence genes was analyzed using *E. coli* database. Most of the isolates were found to harbour virulence genes such as *ipa* involved in the entry of bacteria into epithelial cells. Other virulence genes such as *virF, senB, iha, capU, lpfA, sigA, pic, sepA, celb* and *gad* were also identified in the isolates. Distribution of these genes among *Shigella* serogroups are given in Table III.

Plasmid analysis: Plasmid distribution among *Shigella* species is given in Table IV. IncFII type was the most prevalent plasmid among all four *Shigella* serogroups. *S. dysenteriae* isolates had only the IncFII type plasmid, whereas *S. flexneri* isolates were found to have IncFIB(K), IncFII, Col156, Col(BS512), ColMP18 and IncB/O/K/Z plasmids. *S. boydii* isolates were found to have plasmids such as IncFIB, IncA/C2 and IncN. Plasmids such as IncI2, IncI1 and ColpVC were identified in *S. sonnei*.

Discussion

Shigella remains a leading cause of childhood dysentery. The clones with high virulence and multidrug resistance (MDR) have spread globally where plasmids play a major role in conferring these characteristics[18]. The pathogenesis of *Shigella* is related to various virulence factors located in the chromosome or large virulent *inv* plasmid carrying gene responsible for functions like host cell invasion and intracellular survival[2,19]. However, only a few studies have attempted to illustrate its molecular virulence profile. A recent study by Medeiros *et al*[20] showed that the presence of virulence genes in *Shigella* was associated with various clinical symptoms such as intense abdominal pain and bloody stools. They also highlighted that the higher numbers of virulence genes were associated with resistance to more antimicrobials.

In this study, vast distribution of genes was observed among all four *Shigella* serogroups, especially in *S. flexneri*. *pic* and *sep*A genes were also seen more in *S. flexneri*. The shiga toxin gene (*stx*) is an important virulence determinant related to *S. dysenteriae,* but none of the *S. dysenteriae* isolates carried this gene.

Table III. Virulence genes observed among *Shigella* serogroups % (n)

Shigella serogroup	*ipaH*	*ipaD*	*senB*	*virF*	*iha*	*capU*	*lpfA*	*sigA*	*pic*	*sepA*	*celb*	*gad*
S. dysenteriae (n=5)	-	100 (5)	100 (5)	100 (5)	100 (5)	100 (5)	100 (5)	100 (5)	-	-	-	-
S. flexneri (n=23)	4 (1)	74 (17)	9 (2)	65 (15)	9 (2)	56 (13)	69 (16)	69 (16)	48 (11)	65 (15)	-	-
S. boydii (n=17)	6 (1)	94 (16)	100 (17)	100 (17)	100 (17)	88 (15)	41 (7)	82 (14)	-	-	-	6 (1)
S. sonnei (n=15)	-	7 (1)	93 (14)	7 (1)	-	13 (2)	100 (15)	100 (15)	7 (1)	7 (1)	60 (9)	13 (2)

Table IV. Plasmids prevalence among *Shigella* serogroups % (n)

Shigella serogroup	IncFIB	IncFIB (K)	IncFII	IncA/C2	IncN	Col156	Col (BS512)	IncI2	IncI1	IncB/O/K/Z	ColpVC	Col MP18
S. dysenteriae (n=5)	-	-	100 (5)	-	-	-	-	-	-	-	-	-
S. flexneri (n=23)	-	26 (6)	74 (17)	-	-	4 (1)	4 (1)	-	-	4 (1)	-	4 (1)
S. boydii (n=17)	23 (4)	12 (2)	100 (17)	6 (1)	6 (1)	-	-	-	-	-	-	-
S. sonnei (n=15)	-	-	7 (1)	-	-	87 (13)	13 (2)	7 (1)	20 (3)	7 (1)	7 (1)	-

The pathogens capacity to rapidly acquire AMR is a major concern. Development of AMR was common in all *Shigella* species, particularly in *S. sonnei* which were known to acquire resistance genes from *E. coli* through horizontal gene transfer mechanism[21]. Furthermore, resistance in *S. flexneri* is well documented with several studies showing a high frequency of resistance to commonly used antimicrobials such as ampicillin and co-trimoxazole[21].

In the present study, increased resistance was observed to first-line antibiotics such as ampicillin, trimethoprim-sulphamethoxazole and nalidixic acid. Therefore, these drugs should not be recommended for treatment unless susceptibility is known or expected based on local surveillance. In the present study, trimethoprim-sulphamethoxazole resistance was mainly due to *dhfr*1A gene followed by the *sul*II gene. The resistance to chloramphenicol, tetracycline and streptomycin was due to the presence of *cat*A1, tetA/B and of either *str*A/B or *aad*A1 genes or both.

Among β-lactams, ampicillin resistance was usually encoded by OXA-type β-lactamase genes followed by TEM. In the present study, the resistance was predominantly due to $bla_{\text{OXA-1}}$ followed by $bla_{\text{TEM-1}}$. The predominance of OXA-1 in *Shigella* has been reported earlier[22]. Twenty one isolates in this study harboured bla_{EC} gene, a class C β-lactamase conferring resistance to β-lactam antibiotics. CTX-M-type β-lactamases $bla_{\text{CTX-M-15}}$, was identified in all serogroups except *S. dysenteriae* and plasmid-mediated AmpC β-lactamases genes were found only in *S. flexneri* isolates. Increasing number of reports of third-generation cephalosporins resistance in Asia left limited options for effective therapy[23].

The WHO has listed fluoroquinolone-resistant *Shigella* as one of its top concerns in the current international focus on AMR[24]. In general, quinolone resistance involves the accumulation of mutations in DNA gyrase and DNA topoisomerase IV; and plasmid-mediated quinolone resistance (PMQR) determinants like *qnr*A, *qnr*B, *qnr*S and *aac(6)-Ib-cr* genes which confer low-level resistance to quinolones. In this study, the plasmid-mediated *qnr*S gene was widely distributed among *S. flexneri* and *S. boydii* isolates. *qnr*B4 gene was present only in *S. flexneri* isolates. Besides, mutation analysis of DNA gyrase and topoisomerase IV genes added more information in an understanding of resistance to fluoroquinolone in *Shigella*. Novel mutations were observed in *gyr*B, *par*C and *par*E genes. However, the detailed study on the impact of these mutations in conferring quinolone resistance needs to be done.

The presence of these AMR genes in most of the isolates was related with their phenotypic profile. However, phenotypic resistance in spite of the absence of genes represents that other mechanisms might be responsible for resistance, whereas the presence of resistance genes genotypically with no phenotypic expression corresponds to non-expression of AMR genes. One susceptible isolate did not carry any resistance genes but instead carried a plasmid. Another important factor involved in the spread of resistance

was the presence of incompatible plasmid particularly, the IncF plasmid which was known to be associated with the worldwide emergence of clinically relevant extended-spectrum β-lactamases (ESBLs) and multiple AMR determinants[25]. The present study showed the dominance of IncFII plasmid among the tested isolates. Beceiro *et al*[18] have reported that IncF is a major incompatibility group involved in the co-transfer of resistance and virulence determinants. All the isolates harbouring virulence genes also harboured either single or more than one Inc type plasmid in this study, which further highlighted the significant association of these determinants in pathogenic bacteria.

The widespread emergence of MDR *Shigella* and increasing incidence with changing AMR patterns makes treatment a challenge for shigellosis. As shown here, AMR in *Shigella* spp. was serogroup-specific.

In conclusion, screening of AMR genes among *Shigella* genome showed that resistant gene distribution was variable among the *Shigella* serogroups. The findings of the present study also showed the species ability in acquiring AMR determinants and suggested the continuous surveillance of this species and its resistance profile particularly in *Shigella* endemic region.

***Financial support & sponsorship*:** This work was supported by the Indian Council of Medical Research, New Delhi (Ref. No: AMR/TF/55/13ECDII dated 23/10/2013).

***Conflicts of Interest*:** None.

References

1. Aggarwal P, Uppal B, Ghosh R, Krishna Prakash S, Chakravarti A, Jha AK, *et al.* Multi drug resistance and extended spectrum beta lactamases in clinical isolates of *Shigella*: A study from New Delhi, India. *Travel Med Infect Dis* 2016; *14* : 407-13.
2. da Cruz CB, de Souza MC, Serra PT, Santos I, Balieiro A, Pieri FA, *et al.* Virulence factors associated with pediatric shigellosis in Brazilian Amazon. *Biomed Res Int* 2014; *2014* : 539697.
3. The HC, Thanh DP, Holt KE, Thomson NR, Baker S. The genomic signatures of *Shigella* evolution, adaptation and geographical spread. *Nat Rev Microbiol* 2016; *14* : 235-50.
4. Anandan S, Muthuirulandi Sethuvel DP, Gajendiren R, Verghese VP, Walia K, Veeraraghavan B. Molecular characterization of antimicrobial resistance in clinical *Shigella* isolates during 2014 and 2015: Trends in South India. *Germs* 2017; *7* : 115-22.
5. Shakya G, Acharya J, Adhikari S, Rijal N. Shigellosis in Nepal: 13 years review of nationwide surveillance. *J Health Popul Nutr* 2016; *35* : 36.
6. Kumar P, Bag S, Ghosh TS, Dey P, Dayal M, Saha B, *et al.* Molecular insights into antimicrobial resistance traits of multidrug resistant enteric pathogens isolated from India. *Sci Rep* 2017; *7* : 14468.
7. Laxminarayan R, Chaudhury RR. Antibiotic resistance in India: Drivers and opportunities for action. *PLoS Med* 2016; *13* : e1001974.
8. Rahman M, Haque AF, Deeba IM, Ahmed D, Zahidi T, Rimu AH, *et al*. Emergence of extensively drug-resistant *Shigella sonnei* in Bangladesh. *Immunol Infect Dis* 2017; *5* : 1-9.
9. Munita JM, Arias CA. Mechanisms of antibiotic resistance. *Microbiol Spectr* 2016; *4*. doi: 10.1128/microbiolspec. VMBF-0016-2015.
10. Nair S, Ashton P, Doumith M, Connell S, Painset A, Mwaigwisya S, *et al.* WGS for surveillance of antimicrobial resistance: A pilot study to detect the prevalence and mechanism of resistance to azithromycin in a UK population of non-typhoidal salmonella. *J Antimicrob Chemother* 2016; *71* : 3400-8.
11. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC. Colour atlas and textbook of diagnostic microbiology. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 1997.
12. Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing*. 27th ed. CLSI Document M100. Wayne, PA: CLSI; 2017.
13. Dhiviya Prabaa MS, Naveen Kumar DR, Yesurajan IF, Anandan S, Kamini W, Balaji V. Identification of nonserotypeable *Shigella* spp. using genome sequencing: A step forward. *Future Sci OA* 2017; *3* : FSO229.
14. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, *et al.* NCBI prokaryotic genome annotation pipeline. *Nucleic Acids Res* 2016; *44* : 6614-24.
15. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, *et al.* Identification of acquired antimicrobial resistance genes. *J Antimicrob Chemother* 2012; *67* : 2640-4.
16. Joensen KG, Scheutz F, Lund O, Hasman H, Kaas RS, Nielsen EM, *et al.* Real-time whole-genome sequencing for routine typing, surveillance, and outbreak detection of verotoxigenic *Escherichia coli*. *J Clin Microbiol* 2014; *52* : 1501-10.
17. Carattoli A, Zankari E, García-Fernández A, Voldby Larsen M, Lund O, Villa L, *et al. In silico* detection and typing of plasmids using plasmidFinder and plasmid multilocus sequence typing. *Antimicrob Agents Chemother* 2014; *58* : 3895-903.
18. Beceiro A, Tomás M, Bou G. Antimicrobial resistance and virulence: A successful or deleterious association in the bacterial world? *Clin Microbiol Rev* 2013; *26* : 185-230.
19. Yaghoubi S, Ranjbar R, Dallal MMS, Fard SY, Shirazi MH, Mahmoudi M, *et al.* Profiling of virulence-associated factors in *Shigella* species isolated from acute pediatric diarrheal samples in Tehran, Iran. *Osong Public Health Res Perspect* 2017; *8* : 220-6.
20. Medeiros PHQ, Lima AÂM, Guedes MM, Havt A, Bona MD, Rey LC, *et al.* Molecular characterization of virulence and antimicrobial resistance profile of *Shigella* species isolated

from children with moderate to severe diarrhea in Northeastern Brazil. *Diagn Microbiol Infect Dis* 2018; *90* : 198-205.

21. Anderson M, Sansonetti PJ, Marteyn BS. *Shigella* diversity and changing landscape: Insights for the twenty-first century. *Front Cell Infect Microbiol* 2016; *6* : 45.
22. Pazhani GP, Niyogi SK, Singh AK, Sen B, Taneja N, Kundu M, *et al*. Molecular characterization of multidrug-resistant *Shigella* species isolated from epidemic and endemic cases of shigellosis in India. *J Med Microbiol* 2008; *57* : 856-63.
23. Kotloff KL. *Shigella* infection in children and adults: A formidable foe. *Lancet Glob Health* 2017; *5* : e1166-7.
24. World Health Organization. *Global antimicrobial resistance surveillance system*: *Manual for early implementation*. Geneva: WHO; 2015.
25. Ogbolu DO, Daini OA, Ogunledun A, Terry Alli OA, Webber MA. Dissemination of IncF plasmids carrying beta-lactamase genes in gram-negative bacteria from Nigerian hospitals. *J Infect Dev Ctries* 2013; *7* : 382-90.

For correspondence: Dr Balaji Veeraraghavan, Department of Clinical Microbiology, Christian Medical College, Vellore 632 004, Tamil Nadu, India
e-mail: vbalaji@cmcvellore.ac.in