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Identification of Typical Class 1 and Class 2 Integron Gene Cassettes in Clinical Isolates of MDR *Shigella flexneri* in South Indian Population

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Authors' contributions

This work was carried out in collaboration between all authors. Author VB designed the study. Authors VB, SA, DRNK performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript and managed the literature searches. Author MSDP managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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Short Communication

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ABSTRACT

Aims: Shigellosis is an acute intestinal infection caused by *Shigella spp*. Treatment with most widely used antimicrobial drugs became limited due to the emergence of multi-drug resistance (MDR). In *Shigella spp.*, antimicrobial resistance is often associated with the presence of transferable genetic elements (integrons and plasmids). Therefore, the study was aimed to identify the pattern of integron distribution in clinical isolates of *Shigella spp.* in south Indian population.
Place and Duration of Study: Department of Clinical Microbiology, Christian Medical College (CMC), Vellore, between January 2014 and December 2014.
Methodology: A total of 20 out of 67 MDR *Shigella* isolates were included in the study. All 20 isolates were characterized for the presence of integrons, gene cassettes and other resistant genes by PCR, restriction fragment length polymorphism (RFLP) and sequencing. The integron sequences were deposited in NCBI.
Results: The presence of typical class 1 integron gene cassette *dfrA12-orfF-aadA2* (KT037078) and class 2 integron gene cassette *dfr1-sat2-aadA1* (KT037079) from *S. flexneri* were first to be reported among Indian population in this study. Also, *dfrA1-sat1-aadA1* gene cassette of class 2

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integron along with *dhfr1a, sul2, bla*$_{OXA}$*, bla*$_{TEM}$, AmpC*, bla*$_{CTX-M-15}$ and *qnr* genes were identified in *Shigella spp.* in this study.
Conclusion: Association of ESBL, AmpC, *bla*$_{CTX-M}$ and *qnr* genes justified the observed phenotypic resistance of the isolates which is of major concern due to the ability of integrons to acquire resistance genes against different antibiotics, since gene cassettes exist to nearly all classes of antibiotics.

Keywords: Shigella; MDR; integron; ESBL; AmpC; bla$_{CTX-M}$*; qnr.*

1. THE STUDY

Shigellosis, caused by *Shigella spp.* remains a major health-care problem in many parts of the world, particularly in developing countries. In India the isolation rate of *Shigella* varies in different studies from 2-6% [1]. Several mechanisms have been involved in the development of resistance; the most commonly observed mechanism is the horizontal gene transfer (HGT) through integrons. Integrons are generally non-mobile and are often located on mobile genetic elements like transposons and plasmids that could serve as vehicles for the inter- and intra-species transmission of genes [2]. Independent of integrons, the organism can carry other resistance genes that could be transferred directly by plasmids or transposons, which includes Extended-spectrum β-lactamases (ESBL), AmpC β-lactamases, CTX-M, and *qnr* genes [3-5]. There are very few reports on molecular mechanism of resistance for the multi-drug resistant (MDR) *Shigella* in India. This study was aimed to perform molecular characterization of MDR *Shigella spp.* and to compare with the earlier findings and outcome.

A total of 3647 faeces specimens were processed between January to December 2014, among these 18.43% (*n* = 638) faecal pathogens were identified using standard biochemical methods [6] and serotyped by slide agglutination using group and type specific *Shigella* antisera Seiken set 1 (Denka Seiken, Japan). Of the positives, 27.5% (*n* = 176) were *Shigella spp.,* this includes *S. flexneri* (*n* = 119), *S. dysenteriae* (*n* = 5), *S. sonnei* (*n* = 35), *S. boydii* (*n* = 2) and non-agglutinable *Shigellae* (*n* = 15). The prevalence of shigellosis during the study period was 4.8% (*n* = 176).

Antimicrobial susceptibility testing of the isolates was done by Kirby-Bauer disc diffusion method using the antimicrobial agents (ampicillin 10 µg, trimethoprim-sulfamethoxazole 1.25/23.75 µg, cefotaxime 30 µg, cefixime 5 µg, nalidixic acid 30 µg, norfloxacin 10 µg - Oxoid, UK) according to Clinical and Laboratory Standards Institute (CLSI) guidelines 2014 [7]. The antimicrobial susceptibility profile of all *Shigella spp.* (*n* = 176) was depicted in Fig. 1. All MDR *Shigella spp.* (*n* = 67) (multi-drug resistant to penicillins, sulphonamides and quinolones) were identified based on phenotypic antimicrobial resistance patterns which includes 88% *S. flexneri* (*n* = 59), 5.9% *S. sonnei* (*n* = 4) and 5.9% *Shigella spp.* (*n* = 4). Of these, 20 MDR *Shigella spp.* (*Shigella flexneri* – 19 and *Shigella sonnei* – 1) were selected in random and characterized for their molecular mechanism of resistance in this study and compared with previous literature.

All the isolates were screened for the presence of class 1 and 2 integrons (*intI*1 and *intI*2) with their respective gene cassettes using specific primers as described previously [8]. In this study, all 20 MDR *Shigella* isolates carried integrons (Table 1). The distribution of integrons varies between different serotypes of *S. flexneri*, of which, the serotype 3 had class 2 integron only while the other serotypes 1, 2, 4 and 6 harboured both class 1 and 2 integrons concurrently, indicating, ability of these species in acquiring further resistance gene cassettes (Table 2). In addition, most of the remaining MDR *Shigella* isolates (*n* = 41) were positive for class 2 integrons gene cassettes (data not shown).

In *Shigella spp*., class 2 integrons were detected more frequently than class 1 integrons and their gene cassettes were relatively conservative. Totally, three types of restriction patterns were observed for class 1 and class 2 integron gene cassettes by using *PvuI* and *HinfI* enzymes respectively. One from each type of restriction pattern was sequenced (ABI Prism 3100 Genetic Analyzer - Applied Biosystems) to identify the gene cassette and the sequences were deposited in NCBI. This revealed the presence of a typical class 1 integron gene cassette, *dfrA12-orfF-aadA2* (KT037078) (in comparison with the reference in NCBI (FJ895302) in a *Shigella flexneri* isolate, known to confer resistance to trimethoprim and streptomycin. This

was rarely reported in *S. flexneri* [9-10], though this was observed frequently in clinical isolates of *S. sonnei* [11-12,8] and in other Enterobacteriaceae [13]. To the best of our knowledge, this is the first report of typical class 1 integron in *S. flexneri* in India. However the study done by Dutta et al. [14] in atypical provisional serovars of *Shigella*, showed the presence of typical class 1 integrons without gene cassettes. These typical class 1 integrons are usually present in conjugative plasmids which easily transfers resistance genes horizontally. In contrast, Ghosh et al. [15] had reported the presence of atypical class 1 integrons (without the 3'-conserved segment) in *S. flexneri* as a supporting evidence for the disease burden.

Moreover, in this study, *dfrA1-sat1-aadA1* (KT037080) of class 2 integrons was identified in 90% (*n* = 18) of the isolates and *dfr1-sat2-aadA1* (KT037079) in 10% (*n* = 2) which was first to be reported in India from *Shigella spp.* (in comparison with the NCBI reference sequences EF634237 and AB234886 respectively). Until now, the class 2 gene cassette arrays reported in *Shigella spp.* were *dfrA1-sat1-aadA1*; *dfrA1-sat1*, and *dfrA1-sat1-orfx* [16,15], which was in accordance with the present study.

Meanwhile, the co-existence of typical and atypical class 1 integrons along with class 2 integrons was reported by Zhu et al. [9] in MDR *S. flexneri* for the first time in China. Similar results were also observed by Wen et al. [16]. Such findings were yet to be observed in India.

The presence of individual resistant genes *dhfr1a, sul2* [17-18], bla_{TEM}, bla_{OXA} [5,19], $bla_{CTX\text{-}M\text{-}1}$ and AmpC (bla_{MOX}, bla_{CIT}, bla_{DHA}, bla_{ACC}, bla_{EBC} and bla_{FOX}) [20-21], and *qnr*A, *qnr*B, *qnr*S [4] were identified by previously described protocol with modifications (Table 1). The *dhfr1a* gene was identified in the genomic DNA of all 20 MDR *Shigella* strains which revealed that the integrons have been integrated into the chromosomal DNA, reflecting resistance to trimethoprim. In addition, the ESBL genes bla_{OXA} and bla_{TEM} were observed in 20% (4/20) and 60% (12/20) of the isolates. AmpC genes, CIT and FOX were also observed in two *S. flexneri* isolates, and $bla_{CTX\text{-}M\text{-}15}$ in two other *S. flexneri* and one *S. sonnei* isolates conferring resistance to cephalosporins [22]. Six out of 20 MDR *Shigella* isolates were positive for *qnr*S, out of which one *S. flexneri* had both *qnr*S and *qnr*B. In contrast, previous study [15] from India reported the presence of *aac* (6')-*1b cr* than *qnr* genes in quinolone resistant *Shigella spp.*

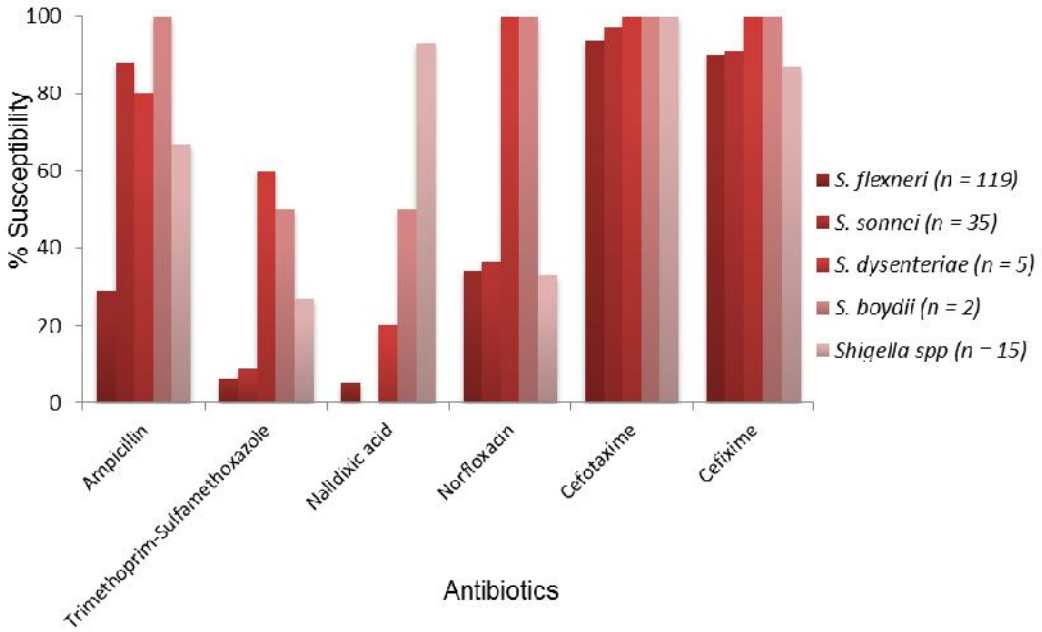

Fig. 1. Antimicrobial susceptibility pattern of *Shigella* spp. (2014)

Table 1. Phenotypic and molecular characterization of multi-drug resistant *Shigella spp*

				Antibiotic susceptibility profile						Molecular profile for antibiotic resistance									
S. No	Isolate No.	Age/Sex	Organism	AMP	SXT	NAL	NOR	CTX	FIX	*dhfr1a*	*Sul 2*	$bla_{OXA-48\ like}$	bla_{TEM}	AmpC	*Qnr*	$bla_{CTX-M-1}$	Class 1 integrons	Class 1 gene cassettes	Class 2 integron	Class 2 gene cassette
1	SF1	41/M	*S. flexneri* 2	R	R	S	R	S	R	+	-	+	-	-	-	-	+	-	+	*dfrA1-sat1-aadA1*
2	SF2	7/M	*S. flexneri* 4	R	R	R	R	S	S	+	+	+	+	-	*qnr*S	-	+	-	+	*dfrA1-sat1-aadA1*
3	SF3	2/M	*S. flexneri* 2	R	R	R	R	S	S	+	+	+	-	-	-	-	+	-	+	*dfrA1-sat1-aadA1*
4	SF4	1/M	*S. flexneri* 2	R	R	R	R	S	S	+	-	+	-	-	-	-	+	-	+	*dfrA1-sat1-aadA1*
5	SF5	21/F	*S. flexneri* 2	R	R	R	R	S	S	+	-	+	-	-	-	-	+	-	+	*dfrA1-sat1-aadA1*
6	SF6	1/M	*S. flexneri* 2	R	R	R	R	S	S	+	+	+	-	-	-	-	+	-	+	*dfrA1-sat1-aadA1*
7	SF7	55/F	*S. flexneri* 2	R	R	R	R	S	S	+	-	+	-	-	-	-	+	-	+	*dfrA1-sat1-aadA1*
8	SF8	40/F	*S. flexneri* 3	R	R	R	R	R	R	+	+	-	-	-	*qnr*S	$bla_{CTX-M-15}$	-	ND	+	*dfr1-sat2-aadA1*
9	SF9	2/F	*S. flexneri* 4	R	R	R	R	S	S	+	+	-	-	-	-	-	-	ND	+	-
10	SF10	1/F	*S. flexneri* 2	R	R	R	R	S	S	+	-	+	-	-	-	-	+	-	+	*dfrA1-sat1-aadA1*
11	SF11	15Month/M	*S. flexneri* 2	R	R	R	R	S	S	+	+	-	-	-	-	-	-	ND	+	*dfrA1-sat1-aadA1*
12	SF12	6Month/F	*S. flexneri* 2	R	R	R	R	R	R	+	-	-	-	CIT, FOX	-	-	-	ND	+	*dfrA1-sat1-aadA1*
13	SF13	6Month/M	*S. flexneri* 2	R	R	R	R	S	R	+	+	+	-	-	-	-	+	-	+	*dfrA1-sat1-aadA1*
14	SF14	16Month/M	*S. flexneri* 4	R	R	R	R	S	S	+	+	-	+	-	*qnr*S	-	+	-	+	*dfrA1-sat1-aadA1*
15	SF15	6Month/F	*S. flexneri* 2	R	R	R	R	S	S	+	+	+	-	-	-	-	+	-	+	*dfrA1-sat1-aadA1*
16	SN1	8Month/M	*S. sonnei*	R	R	R	R	R	R	+	+	-	-	-	*qnr*S	$bla_{CTX-M-15}$	-	ND	+	*dfr1-sat2-aadA1*
17	SF16	1/M	*S. flexneri* 6	R	R	R	S	R	R	+	+	-	-	-	*qnr*S	$bla_{CTX-M-15}$	+	-	+	*dfrA1-sat1-aadA1*
18	SF17	25/F	*S. flexneri* 1	R	R	R	S	S	R	+	+	-	+	-	*qnr*B *qnr*S	-	+	-	+	*dfrA1-sat1-aadA1*
19	SF18	72/M	*S. flexneri* 2	R	R	R	R	R	R	+	-	+	+	-	-	-	+	*dfrA12-orfF-aadA2*	+	*dfrA1-sat1-aadA1*
20	SF19	3/F	*S. flexneri* 2	R	R	R	R	R	R	+	+	+	-	CIT	-	-	+	-	+	*dfrA1-sat1-aadA1*

+ Positive, - Negative, ND Not done

Table 2. Class 1 and 2 integrons identified in different serogroups of *S. flexneri*

Species	No of isolates	No of positive isolates (%)		
		***intI*1 only**	***intI*2 only**	**both *intI*1 and *intI*2**
S. flexneri 1	1	0	0	1 (5)
S. flexneri 2	13	0	2 (10)	11 (55)
S. flexneri 3	1	0	1 (5)	0
S. flexneri 4	3	0	1 (5)	2 (10)
S. flexneri 6	1	0	0	1 (5)
S. sonnei	1	0	1 (5)	0
Total	20	0	5 (25%)	15 (75%)

Association of individual resistance genes with the presence of integrons 1 and 2 was also previously reported in association with increased resistance for antibiotics, this includes ampicillin (bla_{OXA1}), trimethoprim-sulfamethoxazole (*dfrA1* and *sul2*), tetracycline (*tet*A and *tet*B), chloramphenicol (*cat*A) and streptomycin (*aadA1*, *str*A and *str*B) [14]. In comparison with the previous reports, the present study correlates significantly ($P<0.05$) with the presence of integrons and MDR.

2. CONCLUSION

This study revealed the molecular mechanism behind the resistance to different class of antibiotics by *Shigella spp*. Most of which is mediated by the presence of class 2 integrons and their gene cassettes, accompanied by individual genes for resistance. This study identified the typical class 1 integron gene cassette in contrast with the atypical type reported by the previous studies, which indicates the change in trend towards the horizontal gene transfer and mechanism of acquiring resistant genes. However, with the potential of integrons to capture and collect gene cassettes it is probable that they will be widespread. This urges the need to understand and prevent the further spread of antimicrobial resistance.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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