JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Letter to the Editor

Association of blaCTX-M-15 and qnr genes in multidrug-resistant Salmonella Typhimurium and Shigella spp from India

Dhiviya Prabaa Muthuirulandi Sethuvel[1], Shalini Anandan[1], Naveen Kumar Devanga Ragupathi[1], Balaji Veeraraghavan[1], Ohri Vinod[2], Kamini Walia[2]

[1] *Department of Microbiology, Christian Medical College, Vellore, India*
[2] *Indian Council of Medical Research, Ansari Nagar, New Delhi, India*

Key words: non-typhoidal Salmonella; *Shigella*; $bla_{CTX-M-15}$; *qnr*; AmpC; ESBL.

J Infect Dev Ctries 2015; 9(11):1294-1297. doi:10.3855/jidc.6965

(Received 04 April 2015 – Accepted 24 May 2015)

Dear Editor,

Acute infectious diarrhoea continues to be a significant cause of morbidity and mortality in children in low income countries. Among bacterial isolates, *Shigella spp* and *Salmonella spp* are known to contribute to the high burden of this illness in children. Recent literature survey reveals a sharp declining of prevalence of *S.* Typhi, while non-typhoidal *Salmonella* (NTS) and *Shigella flexneri* are increasing [1]. Among the *Shigella* isolates, > 90% are resistant to ampicillin and sulfonamides [2], 4%–10% of isolates are resistant to at least one of the third-generation cephalosporins (ceftriaxone/cefotaxime) [3,2], and 7%–33% to fluoroquinolones [2]. Whereas in NTS, 86% are resistant to ampicillin, 33%–48% to sulfonamides [4,5], 34% to third-generation cephalosporins [3], and 24%-35% to fluoroquinolones [4,5]. Monitoring of cephalosporins and fluoroquinolones resistance is particularly important because these antibiotics are among the few therapeutic options commonly used for moderate to severe *Salmonella* and *Shigella* infections.

The objective of this study was to determine the co-existence of *qnr* and extended-spectrum β lactamase (ESBL) genes in clinical isolates of multidrug-resistant (MDR) *Shigella spp* and *Salmonella spp.* (defined here as isolates resistant to ≥ 3 drugs)

The study

Faecal *Salmonella spp* and *Shigella spp* culture isolates obtained from the Christian Medical College (CMC), Vellore, India, during the year 2014 were included in this study. Totally, 3,461 faeces specimens were processed between January and December 2014, among these 18.43% (n = 638) faecal pathogens were identified. Isolation and identification of the organism was carried out using standard protocol. Isolates were serotyped by using commercial antisera (Denka Seiken, Tokyo, Japan). Of these, 25.7% (n = 164) were *Salmonella spp.* (including four *S.* Typhi) and 25.5% (n = 163) were *Shigella spp.* This included *S. flexneri* (n = 111), *S. dysenteriae* (n = 5), *S. sonnei* (n = 33), *S. boydii* (n = 2) and non-agglutinable *Shigellae* (n = 12). Of these, 2.5% (n = 4) *Salmonella spp*. and 3.6% of *Shigella spp.* (n = 6) which included *S. flexneri* (n = 5) and one *S. sonnei*, were MDR by disk diffusion method according to interpretative breakpoints recommended by the Clinical Laboratory Standards Institute (CLSI) guidelines 2014 [6]. The phenotypic antibiotic susceptibility profiles of all 10 MDR isolates are given in Table 1. Among the 10 MDR isolates, all were resistant to all antibiotics tested except one *Shigella* isolate which was susceptible to norfloxacin.

Increasing prevalence and various types of ESBLs and AmpCs are being reported in *Enterobacteriaceae* [7]. Nonetheless, in India such reports for *Shigella spp* and non-typhoidal *Salmonellae* are not often seen (Table 2). The most frequently encountered ESBLs belong to the bla_{CTX-M}, bla_{SHV}, and bla_{TEM} families.

In this study, the MDR isolates were found to harbour the genes bla_{OXA}, bla_{TEM}, *dhfr1a, sul2, qnr*A, *qnr*B, *qnr*S, AmpC and bla_{CTX-M} as shown in Table 1. Of these, two *Shigella* isolates were positive for bla_{OXA} and one for bla_{TEM}.

Table 1. Multi-drug resistant *Salmonella spp* and *Shigella spp* with phenotypic and molecular profiles

S. No	Isolate No.	Age/Sex	Organism	Antibiotic susceptibility profile									Molecular profile for antibiotic resistance										
				AMP	SXT	NAL	NOR	CIP	CTX	FIX	CTR	CHL	*dhfr1a*	*Sul 2*	bla_{OXA}	bla_{TEM}	AmpC	*qnr*	$bla_{CTX-M-1}$	Class 1 integrons	Class 1 gene cassettes	Class 2 integron	Class 2 gene cassette	Sequencing
Shigella spp																							
1	SH1	40/F	*S. flexneri*	R	R	R	R	ND	R	R	ND	ND	+	+	-	-	-	*qnrS*	+	-	-	+	+	$bla_{CTX-M-15}$
2	SH2	8m/M	*S. sonnei*	R	R	R	R	ND	R	R	ND	ND	+	+	-	-	-	*qnrS*	+	-	-	+	+	$bla_{CTX-M-15}$
3	SH3	1/M	*S. flexneri 6*	R	R	R	S	ND	R	R	ND	ND	+	+	-	-	-	*qnrS*	+	+	-	+	+	$bla_{CTX-M-15}$
4	SH4	6m/F	*S. flexneri 2*	R	R	R	R	ND	R	R	ND	ND	+	-	-	-	*CIT, FOX*	-	-	-	-	+	+	-
5	SH5	72/M	*S. flexneri 2*	R	R	R	R	ND	R	R	ND	ND	+	-	+	+	-	-	-	+	+	+	+	-
6	SH6	3/F	*S. flexneri 2*	R	R	R	R	ND	R	R	ND	ND	+	+	+	-	*CIT*	-	-	+	-	+	+	-
Non-typhoidal *Salmonella*																							
7	S1	7m/M	STM	R	R	R	ND	R	ND	ND	R	R	-	+	+	+	-	*qnrB*	+	+	+	-	-	$bla_{CTX-M-15}$
8	S2	14/F	STM	R	R	R	ND	R	ND	ND	R	R	-	+	+	+	-	*qnrB*	+	+	+	-	-	$bla_{CTX-M-15}$
9	S3	49/M	STM	R	R	R	ND	R	ND	ND	R	R	-	+	+	+	-	*qnrB*	+	+	+	-	-	$bla_{CTX-M-15}$
10	S4	35/F	STM	R	R	R	ND	R	ND	ND	R	R	-	+	+	+	-	*qnrB*	-	+	+	-	-	-

*STM – *Salmonella* Typhimurium; ND – not done

Table 2. Different resistant genes identified in *Shigella spp* and non-typhoidal *Salmonellae* in India

Reference	B-lactams	Cephalosporins	Sulfonamides	Quinolones
Shigella spp				
Ghosh et al., 2014 [37] (*n* = 91)	-	-	-	*aac(6')-Ib-cr* – 82.4%; *qnrS* – 14.3%
Taneja et al., 2012 [38] (*n* = 20)	bla_{TEM1} – 16% bla_{OXA1} – 6.7%	$bla_{CTX-M-15}$ – 8.4% bla_{CMY2} – 5.8%	-	-
Non-typhoidal *Salmonella*				
Taneja et al., 2014 [5] (*n* = 43)	bla_{TEM1} – 25.5% bla_{OXA1} – 2.3%	$bla_{CTX-M-15}$ – 11.6% bla_{CMY2} – 37%	-	-
Menezes et al., 2010 [22] (*n* = 21)	bla_{TEM1} – 48% bla_{SHV12} – 43% bla_{OXA1} – 10%	$bla_{CTX-M-15}$ – 5%	-	*aac(6')-Ib-cr* – 19%;

All six *Shigella* isolates were positive for *dhfr1a* and four for *sul2* thereby conferring resistance for trimethoprim-sulfamethoxazole, while three *Shigella* isolates were found to possess *qnr*S genes coding for quinolone resistance. Meanwhile, the four MDR STMs were all found to be positive for $bla_{OXA,}$ bla_{TEM}, *Sul2* and *qnr*B. Further, all six *Shigella* isolates harboured class 2 integrons, three of which had both class 1 and 2 integrons. All four STM were found to harbour class 1 integrons. The resistant determinants of trimethoprim-sulfamethoxazole were known to be associated with class1 and class 2 integrons [8].

Liu *et al.* [9] reported that 91.7% of the plasmid-mediated quinolone resistance (PMQR) positive *Shigella* isolates co-harboured bla_{OXA} β-lactamase gene and class 2 integrons. In contrast, none of the PMQR positive *Shigella* isolates co-harboured bla_{OXA} gene but all were positive for class 2 integrons in the present study. In addition, 100% of the PMQR positive *Shigella* isolates were also positive for $bla_{CTX\text{-}M\text{-}1}$ gene, which again differ with the 58.3% reported by Liu *et al.* [9]. Interestingly, in this study, 100% of the PMQR positive STM co-harboured both bla_{OXA} and class 1 integrons.

$bla_{CTX\text{-}M\text{-}14}$ and $bla_{CTX\text{-}M\text{-}15}$ were identified to be the most common types of cefotaximases among NTS and *Shigella spp* [10]. Recently, Menezes *et al.* [11] submitted the first report of $bla_{CTX\text{-}M\text{-}15}$ producing STM in India, which was followed by a report in other serovars by Taneja *et al.* [5].

Plasmid-mediated AmpC β-lactamases are another class of enzymes responsible for resistance to cephalosporins. The organisms over expressing ESBL and AmpC β-lactamases are of major clinical concern as they are usually resistant to all the β-lactam drugs, except for cefepime, cefpirome, and carbapenems [12].

In the present study, AmpC genes responsible for cephalosporinases were observed in two *Shigella* isolates, one with *CIT* and *FOX* genes and the other with *CIT* gene only that were phenotypically resistant to cefotaxime and cefixime. In contrast, all four MDR STM were found to be negative for AmpC genes. In addition, the $bla_{CTX\text{-}M\text{-}1}$ gene was found in three *Shigella* isolates and three STM by conferring resistance to third-generation cephalosporins. Two other *Shigella* isolates neither possessed AmpC nor $bla_{CTX\text{-}M\text{-}1}$ genes but were phenotypically resistant to cephalosporins. The resistance mechanism is not known. The co-existence of *CIT-FOX* genes was previously reported in other *Enterobacteriaceae* isolates [13]. However, the current study is the first report of *CIT-FOX* occurrence in *Shigella spp.* among Indian isolates.

Further, all six $bla_{CTX\text{-}M\text{-}1}$ amplicons were sequenced (3130 Genetic Analyzer, Applied Biosystems, Waltham, USA) and identified to be the $bla_{CTX\text{-}M\text{-}15}$ subtype with GC content 50.4%. The accession numbers for the sequences submitted to NCBI are SH1 – KP851743, SH2 – KP851744, SH3 – KP851748, S1 – KP851745, S2 – KP851746, S3 – KP851747. The amplicons sequences were 100% identical to the NCBI GenBank sequence (KJ406378.1).

The association of $bla_{CTX\text{-}M\text{-}15}$ with *qnr* and other ESBLs had been previously reported in various countries such as Denmark [14], Netherlands [15], Spain [16], and Germany [17]. However, such findings are not reported among the Indian isolates. To the best of our knowledge, this is the first Indian study to report the association of $bla_{CTX\text{-}M\text{-}15}$ with PMQR genes, *i.e., qnrS* and *qnrB* among the *Shigella spp* and NTS respectively.

$bla_{CTX\text{-}M\text{-}15}$ and *qnr*B in STM were also associated with ESBL (bla_{OXA} and bla_{TEM}) and *sul2* genes. Similar observations were documented by Geetha *et al.* [18]. In contrast, $bla_{CTX\text{-}M\text{-}15}$ and *qnrS* were associated with *dhfr1a* and *sul2* genes in MDR *Shigella spp.*

Thus, close linkage between different resistance determinants may lead to high prevalence of multi drug resistance strains under antibiotic-specific selective pressure, which in turn may limit the use of valuable antibiotics in managing non-typhoidal *Salmonella* and *Shigella* infections.

Conclusion

In the present study, the acquisition of MDR is genotypically proven by the strong association of ESBL genes (bla_{OXA}, bla_{TEM}, $bla_{CTX\text{-}M\text{-}15}$) with sulfamethoxazole resistance and PMQR genes. This multiple resistance mechanism poses a major threat for the dissemination. Especially, the co-existence of the resistance determinants on transferable plasmids may lead to the emergence and spread of MDR pathogens rapidly in various species within the country. This implies that an intense surveillance is needed to identify the trend and implement judicious use of antibiotics to minimize the selective pressure on bacteria.

Acknowledgements

The authors gratefully acknowledge the Indian Council of Medical Research, New Delhi for providing the grant for

this research (Ref. No: AMR/TF/55/13ECDII dated 23/10/2013) and the Institutional Review Board of the Christian Medical College, Vellore (83-i/11/13).

References

1. Zaidi MB, Estrada-García T, Campos FD, Chim R, Arjona F, Leon M, Michell A, Chaussabel D (2013) Incidence, clinical presentation, and antimicrobial resistance trends in Salmonella and Shigella infections from children in Yucatan, Mexico. Front Microbiol 4: 288.
2. Nath R, Saikia L, Choudhury G, Sharma D (2013) Drug resistant Shigella flexneri in & around Dibrugarh, north-east India. Indian J Med Res 137: 183–186.
3. Ashtiani MTH, Monajemzadeh M, Kashi L (2009) Trends in antimicrobial resistance of fecal shigella and Salmonella isolates in Tehran, Iran. Indian J pathol microbiol 52: 52-55.
4. Singh S, Agarwal RK, Tiwari SC, Singh H (2012) Antibiotic resistance pattern among the Salmonella isolated from human, animal and meat in India. Trop Anim Health Prod 44: 665-674.
5. Taneja N, Appannanavar SB, Kumar A, Varma G, Kumar Y, Mohan B, Sharma M (2014) Serotype profile and molecular characterization of antimicrobial resistance in non-typhoidal Salmonella isolated from gastroenteritis cases over nine years. J Med Microbiol 63: 66-73.
6. CLSI (2014) Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement M100-S24.
7. Pfeifer Y, Matten J, Rabsch W (2009) *Salmonella enterica* serovar Typhi with CTX-M beta-lactamase, Germany. Emerg Infect Dis 15: 1533–1535.
8. Chang CY, Lu PL, Lin CC, Lee TM, Tsai MY, Chang LL (2010) Integron types, gene cassettes, antimicrobial resistance genes and plasmids of Shigella sonnei isolates from outbreaks and sporadic cases in Taiwan. J Med Microbiol 60: 197-204.
9. Liu Y, Yachao LH Pan, Cheng J, Zhu Y, Ye Y, Li J (2012) Prevalence of plasmid-mediated quinolone resistance determinants in association with b-lactamases, 16S rRNA methylase genes and integrons amongst clinical isolates of Shigella flexneri. J Med Microbiol 61: 1174-1176.
10. Folster JP, Pecic G, Krueger A, Rickert R, Burger K, Carattoli A, Whichard JM (2010) Identification and Characterization of CTX-M-Producing *Shigella* Isolates in the United States. Antimicrob Agents Chemother 54: 2269–2270.
11. Menezes GA, Khan MA, Harish BN, Parija SC, Goessens W, Vidyalakshmi K, Baliga S, Hays JP (2010) Molecular characterization of antimicrobial resistance in non-typhoidal salmonellae associated with systemic manifestations from India. J Med Microbiol 59: 1477-1483.
12. Perez-Perez FJ, Hanson ND (2002) Detection of Plasmid-Mediated AmpC -Lactamase Genes in Clinical Isolates by Using Multiplex PCR. J Clin Microbiol 40: 2153–2162.
13. Manoharan A, Sugumar M, Kumar A, Jose H, Mathai D (2012) Phenotypic & molecular characterization of AmpC β-lactamases among *Escherichia coli, Klebsiella* spp. & *Enterobacter* spp. from five Indian Medical Centers. Indian J Med Res 135: 359-364.
14. Dolejska M, Villa L, Hasman H, Hansen L, Carattoli A (2013) Characterization of IncN plasmids carrying $bla_{CTX\text{-}M\text{-}1}$ and *qnr* genes in Escherichia coli and Salmonella from animals, the environment and humans. J Antimicrob Chemother 68: 333-339.
15. Veldman K, Dierikx C, Zandbergen AVE, Pelt WV, Mevius D (2010) Characterization of multidrug-resistant, qnrB2-positive and extended-spectrum-b-lactamase-producing *Salmonella* Concord and *Salmonella* Senftenberg isolates. J Antimicrob Chemother 65: 872–875.
16. Gonzalez-Lopez JJ, Carrasco NP, Salvador F, Rodriguez V, Montalva AS, Planes AM, Molina I, Larrosa MN (2014) ESBL-Producing Salmonella enterica Serovar Typhi in Traveler Returning from Guatemala to Spain. Emerg Infect Dis 20: 1918-1920.
17. Schmiedel J, Falgenhauer L, Domann E, Bauerfeind R, Berninghoff EP, Imirzalioglu C, Chakraborty T (2014) Multiresistant extended-spectrum β-lactamase producing *Enterobacteriaceae* from humans, companion animals and horses in central Hesse, Germany. BMC Microbiol 14: 187.
18. Geetha VK, Yugendran T, Srinivasan R, Harish BN (2014) Plasmid-mediated quinolone resistance in typhoidal Salmonellae: A preliminary report from South India. Ind J Med Microbiol 32: 31-34.
19. Ghosh S, Pazhani1 GP, Niyogi1 SK, James P. Nataro, Ramamurthy T (2014) Genetic Characterization of *Shigella* species Isolated from Diarrhoeal and Asymptomatic Children. J Med Microbiol 63: 903-910.
20. Taneja N, Mewara A, Kumar A, Verma G, Sharma JM (2012) Cephalosporin-resistant Shigella flexneri over 9 years (2001–09) in India. Antimicrob Chemother 67: 1347–1353.

Corresponding author

Dr. Balaji Veeraraghavan
Professor and Head
Department of Clinical Microbiology
Christian Medical College
Vellore – 632 004
Tamil Nadu, India
Phone: +91-416-2282588
Email: vbalaji@cmcvellore.ac.in

Conflict of interests: No conflict of interests is declared.