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

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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 *Shigella* (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 AmpC 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 *blaCTX-M*, *blaSHV*, and *blaTEM* families.

In this study, the MDR isolates were found to harbour the genes *blaOXA*, *blaTEM*, *dhfr1a*, sul2, *qnrA*, *qnrB*, *qnrS*, AmpC and *blaCTX-M* as shown in Table 1. Of these, two *Shigella* isolates were positive for *blaOXA* and one for *blaTEM*. 

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

<table>
<thead>
<tr>
<th>S. No</th>
<th>Isolate No.</th>
<th>Age/Sex</th>
<th>Organism</th>
<th>Antibiotic susceptibility profile</th>
<th>Molecular profile for antibiotic resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>AMP</td>
<td>SXT</td>
</tr>
<tr>
<td>1</td>
<td>SH1</td>
<td>40/F</td>
<td><em>S. flexneri</em></td>
<td>R R R R R ND R R ND</td>
<td>+ + + - - qnrS</td>
</tr>
<tr>
<td>2</td>
<td>SH2</td>
<td>8m/M</td>
<td><em>S. sonnei</em></td>
<td>R R R S ND R R ND</td>
<td>+ + + - - qnrS</td>
</tr>
<tr>
<td>3</td>
<td>SH3</td>
<td>1/M</td>
<td><em>S. flexneri</em></td>
<td>R R R R ND R R ND</td>
<td>+ + + - - qnrS</td>
</tr>
<tr>
<td>4</td>
<td>SH4</td>
<td>6m/F</td>
<td><em>S. flexneri</em></td>
<td>R R R R ND R R ND</td>
<td>+ - + - - CIT, FOX</td>
</tr>
<tr>
<td>5</td>
<td>SH5</td>
<td>72/M</td>
<td><em>S. flexneri</em></td>
<td>R R R R R ND R R ND</td>
<td>+ + + + +</td>
</tr>
<tr>
<td>6</td>
<td>SH6</td>
<td>3/F</td>
<td><em>S. flexneri</em></td>
<td>R R R R R ND R R ND</td>
<td>+ + + + +</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Reference</th>
<th>B-lactams</th>
<th>Cephalosporins</th>
<th>Sulfonamides</th>
<th>Quinolones</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Shigella spp</em></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Ghosh et al., 2014 [37]</td>
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<td></td>
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<tr>
<td><em>Taneja et al., 2012</em> [38]</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td><em>Non-typhoidal Salmonella</em></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><em>Taneja et al., 2014</em> [5]</td>
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<td></td>
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<tr>
<td><em>Menezes et al., 2010</em> [22]</td>
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</tbody>
</table>
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 qnrS genes coding for quinolone resistance. Meanwhile, the four MDR STMs were all found to be positive for blaOXA, blaTEM, Sul2 and qnrB. 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 blaOXA β-lactamase gene and class 2 integrons. In contrast, none of the PMQR positive Shigella isolates co-harboured blaOXA 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 blaCTX-M-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 blaOXA and class 1 integrons.

blaCTX-M-14 and blaCTX-M-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 blaCTX-M-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 blaCTX-M-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 blaCTX-M-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 blaCTX-M-1 amplicons were sequenced (3130 Genetic Analyzer, Applied Biosystems, Waltham, USA) and identified to be the blaCTX-M-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 blaCTX-M-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 blaCTX-M-15 with PMQR genes, i.e., qnrS and qnrB among the Shigella spp and NTS respectively.

blaCTX-M-15 and qnrB in STM were also associated with ESBL (blaOXA and blaTEM) and sul2 genes. Similar observations were documented by Geetha et al. [18]. In contrast, blaCTX-M-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 (blaOXA, blaTEM, blaCTX-M-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.

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References


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