Short Communication

CTX-M-3 Type Beta-Lactamase Producing Shigella sonnei Isolates from Pediatric Bacillary Dysentery Cases

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SUMMARY: In this study, 153 clinical isolates of Shigella were screened for extended-spectrum beta-lactamase (ESBL) production by double-disk synergy test. After being confirmed by the combined disk-test and inhibitor-combined E-test strips, all positive isolates were tested for the bla gene by PCR and the enzymes by isoelectric focusing. DNA sequencing of PCR products revealed that five Shigella sonnei isolates had CTX-M-3 type ESBL enzymes. All of them were resistant to ampicillin, sulfamethoxazole and ceftazidime but susceptible to ofloxacin and cefotaxime. All isolates displayed identical patterns in pulsed-field gel electrophoresis and enterobacterial repetitive intergenic consensus PCR. This study reports multidrug-resistant (MDR) S. sonnei isolates producing CTX-M-3 type ESBL from successive pediatric bacillary dysentery patients, indicating widespread and rapid spread of CTX-M type ESBL in Shigella spp. To counter this emerging threat to public health, the surveillance of CTX-M type beta-lactamasms should be considered, together with measures designed to prevent outbreaks of MDR Shigella in the community.

Extended-spectrum beta-lactamasms (ESBLs) create significant therapeutic problems by inactivating almost all beta-lactams except cephamycins and carbapenems. Following the detection of the first ESBL, SHV-1, in 1983, TEM-ESBLs and SHV-ESBLs evolved greatly up to the late 1990s. Subsequently, a new epidemiological view of ESBLs has emerged, with a dominant increase of CTX-M enzymes (1). Multidrug-resistance in Shigella spp. is a growing concern all over the world, and third-generation cephalosporins are used to treat infections caused by multidrug-resistant (MDR) Shigella. Here, we present successive community-acquired MDR Shigella sonnei clinical isolates producing CTX-M-3 type ESBL.

A total of 153 Shigella isolates were included in this study. All were isolated in the Clinical Microbiology Laboratory of Fatih University Hospital between 2003 and 2006. Species-level identification was performed by conventional biochemical methods plus specific antisera. Species distribution was as follows: S. sonnei 122 (79.7%), S. flexneri 17 (11.1%), S. dysenteriae 13 (8.5%) and S. boydii 1 (0.7%). At first, ESBL production of the isolates was investigated synchronously with antibiograms by the double-disk synergy test using cefotaxime, ceftazidime and amoxicillin-clavulanate (AMC) disks (2). Five isolates evaluated as positive by this method were also confirmed by clavulanic acid combination disks for cefpodoxime, cefotaxime and ceftazidime, and inhibitor-combined E-test strips (AB Biodisk, Solna, Sweden) containing ceftazidime/cefazidime-clavulanate and cefotaxime/cefotaxime-clavulanate. To interpret the results, the CLSI ESBL phenotypic confirmatory tests for Escherichia coli, Klebsiella pneumoniae and Proteus vulgaris were subsequently administered (3).

The five isolates were further investigated by analytical isoelectric focusing (IEF), polymerase chain reaction (PCR), sequence analysis, enterobacterial repetitive intergenic consensus (ERIC)-PCR and pulsed-field gel electrophoresis (PFGE). IEF was performed as described before (4). IEF revealed that the isolates produced three beta-lactamasms with pIs of 7.0, 7.6 and 8.4. The latter was in accord with CTX-M type ESBL. An IEF band of 7.0 might affirm a presence of blaOXA gene, which was not specifically tested in the present study. Although PCR amplification with blaOXA primers did not confirm production of the remaining enzyme with a pl of 7.6, this may still be an SHV-type ESBL, as there are likely various beta-lactamase genes yet to be discovered (5).

PCR was used to detect beta-lactamasms of the SHV, TEM and CTX-M families. PCR amplification methods using specific primers for blaCTXM genes were performed in whole-cell DNA of S. sonnei isolates. PCR amplification of blaCTXM group alleles (primers: blaCTXM F 5´-TGT ACC AGT AA-3´ and blaCTXM R 5´-CGA TAT CGT TGG TGG CAT A-3´) (6) was found positive with an amplicon of 454 bp in all five isolates. PCR amplification of blaTEM (primers: F 5´-AAA CGC TGG TGA AAG TA-3´ and R 5´-AGC GAT CTC TCT AT-3´) and blaOXA (primers: F 5´-ATG CGT TAT CGC CTG TG-3´ and R 5´-TGC TTT GTC ATG GCC AA-3´) group alleles was negative (7). The strains used as PCR controls were E. coli ATCC 35218 and E. coli CT99 for blaTEM and E. coli SA1653 for blaOXA. A clinical CTX-M-positive S. sonnei as a positive control and E. coli ATCC 25922 as a negative control were used for blaCTXM. A further PCR amplification using specific primers for blaCTXM (primers: F 5´-CGT CAC GCT GTT AGG AA-3´ and R 5´-ACG GCT TCC TGC CTT AGG TT-3´) was found to be positive for the 780-bp amplicon size (8). Amplified PCR products of bla genes were sequenced using an automated DNA sequencer ABI Prism 310 (Applied Biosystems, Foster City, Calif., USA).

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by Metis Biotechnology, Ankara, Turkey. Sequences were compared with the GenBank sequence databases using the BLAST suite of programs (9). DNA sequencing of the PCR products revealed that the CTX-M-3 gene was positive for all isolates (10). ERIC-PCR was performed using the ERIC-2 primer (5' - AAG TAA GTG ACT GGG GTG ACG G3') as previously described (11). For PFGE analysis, isolates were prepared following the protocol of Brian et al., and the XbaI (Boehringer, Mannheim, Germany) enzyme was used for digestion (12). By ERIC-PCR and PFGE, all the tested isolates gave identical patterns based on Tenover’s criteria (13) (Figure 1).

The five strains were isolated from patients (3 females, 2 males), aged between 3 and 12 years, who were treated with ciprofloxacin as outpatients. All were diagnosed as having acute bacterial diarrhea at Fatih University Hospital between 2004 and 2005, and all fully recovered.

All five isolates were resistant to ampicillin, sulfamethoxazole (SXT), and cefotaxime but susceptible to ofloxacin and ceftazidime in standard disk diffusion tests. They were also susceptible to ceftazidime (MIC, 0.25 µg/ml) but resistant to cefotaxime (MIC, 256 µg/ml) by E-test. There were >5 mm enlargements in cefotaxime and aztreonam inhibition zones next to the AMC disks but not in ceftazidime in the double-disk synergy test. The combination disk diffusion test was positive for cefotaxime but not for ceftazidime, with a >5 mm diameter increase of the inhibition zone. In addition, cefpodoxime and cefepoxide/clavulanic acid disks also pointed to the presence of ESBL. Testing ESBL with E-test strips gave identical results.

ESBLs elicit a serious global health concern because of their resistance to a wide variety of currently available antimicrobials, which limits the therapeutic alternatives in many critical infections. They are plasmid-mediated bacterial enzymes that confer resistance to almost all beta-lactams except for cephemycins and carbapenems. While the most common enzyme types used to be SHV and TEM derivatives, CTX-M types are as widespread as them today. At present, CTX-M type enzymes have over 50 members categorized in five groups, mainly in Enterobacteriaceae (14).

To the best of our knowledge, no ESBL-producing Shigella strains were reported until a SHV-11 ESBL-producing S. dysenteriae strain was found in India, in 1999 (15). Following this, several reports of ESBL-producing strains have been published from different countries including France (1 strain with SHV type enzyme), Korea (21 of more than 5,911 isolates; with TEM or CTX-M), Argentina (11 of more than 9,033 isolates; with CTX-M, TOHO, SHV or PER), Taiwan (24 isolates of an outbreak; with CMY-2), Bangladesh (4 of 160 isolates, with ampC like or CTX-M), Israel (7 of 5,616 isolates, with CTX-M), Ireland (1 of 46 isolates with CTX-M) and Turkey (1 of 80 isolates, with CTX-M) (16-26). These studies indicate that the most frequent species possessing ESBL is S. sonnei. This may be due to the fact that S. sonnei is the most frequently isolated species in industrialized countries. The other studies before 2003 and the report from France in 2005 had no information of total isolate numbers or species distribution. The study from Argentina (2005) included only S. flexneri isolates. In the present study, 79.7% of all isolates were S. sonnei.

Since all of our ESBL-producing isolates showed the same genotype in ERIC-PCR and PFGE, it may be thought that due to this case clustering, there might have been an outbreak. The route of transmission is unknown in this study. As the route by which community infections arise is unclear in ESBL infections, the population should fist be screened for fecal carriage (14).

Shigellosis is endemic throughout the world. According to the World Health Organization data, each year 1.1 million people are estimated to die from shigellosis. Especially in malnourished patients with shigellosis, antibiotic treatment is recommended because it may resolve diarrhea, mitigate symptoms and shorten the dysentery period. The first-line drugs suggested by CLSI for shigellosis are ampicillin and SXT (3). However, in case of infection by an MDR strain (i.e., one resistant to ampicillin, SXT and chloramphenicol), which is not uncommon, second-line drugs are fluoroquinolones in children and third-generation cephalosporins in children. Thus, in the near future, ESBL-producing Shigella spp. may present a major challenge for dysentery treatment, especially in children, due to their third-generation cephalosporin resistance and the probable side-effects of fluoroquinolones. Moreover, fluoroquinolone-resistant Shigella strains are detected more and more in various regions of the world (27).

The current low detection rate of ESBLs in Shigella isolates should not be misunderstood; the rising trend between 1999 and 2007 is adequate cause for alarm. Moreover, the true proportion of ESBL-producing Shigella strains may be higher than estimated. The drugs to be tested routinely for intestinal Shigella and Salmonella isolates are ampicillin, SXT and fluoroquinolones, with the third-generation cephalosporins and chloramphenicol tested only in the presence of an extended-spectrum isolate (3). Since extraintestinal infection due to Shigella spp. is extremely rare, possible third-generation cephalosporin resistance, which would signal the presence of an ESBL, would be overlooked in routine antibiograms, unless special screening studies are performed.

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REFERENCES


Fig. 1. PFGE analysis of five Shigella isolates.


