Ceftriaxone Resistance and Genes Encoding Extended-Spectrum β-Lactamase among Non-Typhoidal Salmonella Species from a Tertiary Care Hospital in Kuala Lumpur, Malaysia

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SUMMARY: The prevalence of ceftriaxone resistance and the associated genes encoding extended-spectrum β-lactamase (ESBL) was determined in 149 non-duplicate non-typhoidal Salmonella isolated in 2008–2009 from patients in a tertiary care hospital in Kuala Lumpur, Malaysia. The resistance rate to ceftriaxone was 2.7% (2/74) in 2008, 4.0% (3/75) in 2009, and 3.4% (5/149) overall. CTX-M ESBL genes were detected in 2 of the 5 ceftriaxone-resistant isolates. The prevalence of ceftriaxone resistance, although low, is a concern because it limits therapeutic options. Continued surveillance of ceftriaxone resistance is important to monitor its trends.

Ceftriaxone is one of the most commonly used antibiotics for treatment of invasive infections with non-typhoidal Salmonella spp. (1). Ceftriaxone resistance among Salmonella spp., however, has been reported worldwide (2–13), and common resistance mechanisms include the production of plasmid-mediated AmpC β-lactamases and extended-spectrum β-lactamases (ESBLs) (2–5,7–9). A previous study involving non-typhoidal typhoid bacteria (NTS) isolated from patients admitted to the University of Malaya Medical Centre (UMMC) between January 2007 and December 2008 showed that the ceftriaxone resistance rate was 1.3% and detected only 1 ceftriaxone-resistant (CRO-R) isolate (Salmonella Enteritidis); a putative ESBL producer was detected by the double-disk diffusion method, but the mechanism of resistance was not elucidated (11). A study from a different hospital in northern Malaysia (12) that included 80 Salmonella isolates from January 2005 to June 2006 estimated a ceftriaxone resistance rate of 6.3% among all isolates and 3.2% among invasive extra-intestinal isolates with the disk-diffusion method. On the other hand, the National Surveillance of Antimicrobial Resistance Report from the Ministry of Health, Malaysia (for 2009) (13), using data from 16 Malaysian hospitals (not including our hospital), reported a ceftriaxone resistance rate of 2.4% among Salmonella spp. This study aimed to determine the prevalence of ceftriaxone resistance among non-typhoidal Salmonella spp. (which in this study refers to all Salmonella spp., except S. Typhi and S. Paratyphi A, B, and C, and hereinafter referred to as NTS) isolated from patients admitted to the University of Malaya Medical Centre (UMMC) between January 2008 and December 2009, and to identify ESBL genes among such isolates.

All non-duplicate NTS (previously identified by standard biochemical tests and Salmonella antisera by the Diagnostic Microbiology Laboratory, UMMC) stocked during the study period were included. Only 1 isolate per patient was included, except in the case of 3 patients where a subsequent isolate was also included because it belonged to a different serogroup. If an NTS was isolated from both blood and another site of a patient, only the blood isolate was included, except in 2 cases where the blood isolate was unavailable. The minimum inhibitory concentration (MIC) of ceftriaxone was determined by Etest (AB bioMérieux, Solna, Sweden) and interpreted according to the Clinical Laboratory Standards Institute (CLSI) 2010 guidelines (sensitive, ≤1 μg/ml; resistant, ≥4 μg/ml) (14). CRO-R isolates were reconfirmed as Salmonella spp. with the API 20E system (bioMérieux SA, Marcy l’Etoile, France), and their sensitivities to other antimicrobials previously performed in the laboratory according to the CLSI guidelines (15), were retrieved from laboratory records. ESBL genes (blaCTX-M, blashv, and blatem) among CRO-R isolates were detected using previously published PCR primers and methods (16,17); the primers used were MA-1 (5'-SCS ATG TGC AGY ACC AGT AA-3') and MA-2 (5'-CCG CRA TAT GRT TGG TGG TG-3') for blaCTX-M, OS-5 (5'-TGA TCT CCC TGT TAG CCA CC-3') and OS-6 (5'-GAT TTG CGT ATT TCG CTC CC-3') for blashv, and C (5'-TCG GGG AAA TGT GCG CG-3') and D (5'-TGC TTA ATC AGT GAG GCA CC-3') for blatem (16,17). To characterize the CTX-M genes, we carried out PCR amplification as previously described (18) with primers SISnap U1 (5'-AAA AAT GAT TGA AAG GTG GTG-3') and P2D (5'-CAG CGC TTT TGC CGT CTA AG-3'). The PCR products were purified with the GeneAll PCR SV kit (General Biosystem, Seoul, Korea), and the subsequent sequencing reaction was performed with the Big Dye® Terminator V3.1 Cycle Sequencing Kit (Applied
3.4 ceftriaxone. On the other hand, a multinational study (20) was 2.9 g/ml (14). A study in Taiwan (7) showed that be-

A total of 149 non-duplicate isolates was available for the study (75 from 2009 and 74 from 2008) (Table 1). Two more isolates recorded in the stock culture collection for 2008 were excluded, because they were not retrievable for MIC testing (laboratory records showed sensitivity to ceftriaxone by disk diffusion). The MIC50 and MIC90 of the 149 isolates were 0.094 g/ml and 0.125 g/ml, respectively (Table 1). Five CRO-R NTS were detected in the study collection, and ESBL genes were detected in 2 of them. None of the isolates had an intermediate MIC. The resistance rate to ceftriaxone was 2.7% (2/74) in 2008, 4.0% (3/75) in 2009, and 3.4% (5/149) overall (Table 1). The frequency of ceftriaxone resistance among our isolates was low but is still a concern because it limits therapeutic options, and the detection of ESBL genes further raises the possibil-

Table 1. Source of NTS and ceftriaxone resistant NTS isolates (2008–2009)

<table>
<thead>
<tr>
<th>Year</th>
<th>Blood</th>
<th>Stool</th>
<th>Others</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009 (n = 75)</td>
<td>2/21 (9.52)</td>
<td>1/53 (1.89)</td>
<td>0/11 (0)</td>
<td>3/75 (4.00)</td>
</tr>
<tr>
<td>2008 (n = 74)</td>
<td>0/12 (0)</td>
<td>2/62 (3.23)</td>
<td>0/0 (—)</td>
<td>2/74 (2.70)</td>
</tr>
<tr>
<td>Total (n = 149)</td>
<td>2/33 (6.10)</td>
<td>3/115 (2.61)</td>
<td>0/1 (0)</td>
<td>5/149 (3.36)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Isolate no. (year)</th>
<th>Source</th>
<th>Identification</th>
<th>MIC of CRO (µg/ml)</th>
<th>ESBL gene detected</th>
<th>Other β-lactamase genes detected</th>
<th>Susceptibility to other antimicrobials by disk diffusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (2008) Stool</td>
<td>S. Typhimurium</td>
<td>24 (R)</td>
<td>—</td>
<td>TEM-1</td>
<td>S = IPM; I = CIP; R = AMP, SXT, Tet, CHL, NA</td>
<td></td>
</tr>
<tr>
<td>2 (2008) Stool</td>
<td>Salmonella spp. (serogroup E)</td>
<td>256 (R)</td>
<td>—</td>
<td>—</td>
<td>S = SXT, CIP, Tet, CHL, NA; R = AMP</td>
<td></td>
</tr>
<tr>
<td>3 (2009) Blood</td>
<td>S. Enteritidis</td>
<td>256 (R)</td>
<td>CTX-M-14-like</td>
<td>—</td>
<td>S = CHL, CIP, NA, IPM; R = AMP, SXT, Tet</td>
<td></td>
</tr>
<tr>
<td>4 (2009) Blood</td>
<td>S. Typhimurium</td>
<td>32 (R)</td>
<td>—</td>
<td>—</td>
<td>S = SXT, CIP, Tet, CHL, NA; R = AMP</td>
<td></td>
</tr>
<tr>
<td>5 (2009) Blood</td>
<td>Salmonella spp. (serogroup C1)</td>
<td>≥256 (R)</td>
<td>CTX-M-55/57</td>
<td>TEM-1</td>
<td>S = CIP, CHL, NA, IPM; R = AMP, SXT, Tet</td>
<td></td>
</tr>
</tbody>
</table>

R, resistant; S, sensitive; I, intermediate; CRO, ceftriaxone; CIP, ciprofloxacin; CHL, chloramphenicol; Tet, tetracycline; Amp, ampicillin; SXT, trimethoprim-sulfamethoxazole; NA, nalidixic acid; IPM, imipenem.

Table 2. Source, MIC of ceftriaxone, β-lactamase gene detected, and susceptibility to other antimicrobials determined by disk diffusion for the 5 ceftriaxone-resistant isolates

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Source</th>
<th>MIC (µg/ml)</th>
<th>ESBL gene detected</th>
<th>Other β-lactamase genes detected</th>
<th>Susceptibility to other antimicrobials by disk diffusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (2008)</td>
<td>Stool</td>
<td>24 (R)</td>
<td>—</td>
<td>TEM-1</td>
<td>S = IPM; I = CIP; R = AMP, SXT, Tet, CHL, NA</td>
</tr>
<tr>
<td>2 (2008)</td>
<td>Stool</td>
<td>256 (R)</td>
<td>—</td>
<td>—</td>
<td>S = SXT, CIP, Tet, CHL, NA; R = AMP</td>
</tr>
<tr>
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<td>Blood</td>
<td>256 (R)</td>
<td>CTX-M-14-like</td>
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<td>Blood</td>
<td>32 (R)</td>
<td>—</td>
<td>—</td>
<td>S = SXT, CIP, Tet, CHL, NA; R = AMP</td>
</tr>
<tr>
<td>5 (2009)</td>
<td>Blood</td>
<td>≥256 (R)</td>
<td>CTX-M-55/57</td>
<td>TEM-1</td>
<td>S = CIP, CHL, NA, IPM; R = AMP, SXT, Tet</td>
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R, resistant; S, sensitive; I, intermediate; CRO, ceftriaxone; CIP, ciprofloxacin; CHL, chloramphenicol; Tet, tetracycline; Amp, ampicillin; SXT, trimethoprim-sulfamethoxazole; NA, nalidixic acid; IPM, imipenem.

BioSystems, Foster City, Calif., USA) on an ABI-377 Genetic Analyzer (Applied BioSystems), using forward and reverse primers. The sequences obtained were used for a BLAST search in the GenBank database. Suscepti-

In the present study, isolate Nos. 3 and 5 were cefoxitin sensitive, whereas isolate Nos. 1, 2, and 4 were cefoxitin resistant. ESBL genes were detected in 2 (isolate Nos. 3 and 5) out of 5 (40%) CRO-R isolates. Sequence analysis of the 476-bp amplicons suggested 100% identity of the genes to those of the CRO-R isolates. Further, PCR revealed that isolate No. 5 had the partial IS481 element located in the upstream region, and sequencing of the 876-bp amplicon identified the CTX-M-55/57 genes. This isolate also had a TEM-1 gene (Table 2). The IS481 element was not detected in isolate No. 3. The CTX-M genes identified in this study have been previously found among Salmonella spp. (7,9,21), and other mechanisms or rarer ESBL genes may have been responsible for the cefoxitin resistance of 3 other CRO-R isolates (isolates Nos. 1, 2, and 4). There are no CLSI guidelines for the detection of AmpC-mediated resistance at present. Resistance to cefoxitin indicates that the resistance may be AmpC-mediated, but it can also indicate reduced outer membrane permeability or the presence of certain carbapenemases (22,23). Therefore, further phenotypic
and molecular tests (23) should be performed to confirm the type of resistance to ceftriaxone in the 3 other isolates.

In a study in Singapore (8), among 15 isolates of *Salmonella* spp. with diminished susceptibility to ceftriaxone, obtained in 2003–2006, 9 were found to express ESBL genes and 6 were found to express plasmid AmpC genes; the ESBL genes detected were *bla*<sub>SHV-5</sub>, *bla<sub>CTX-M group 1</sub>* and *bla<sub>CTX-M group 9</sub>*.

In summary, we report the prevalence of ceftriaxone resistance (3.4%) among 149 non-duplicate NTS from the UMMC over a 2-year period from January 2008 to December 2009. Two of the 5 CRO-R isolates in this study expressed CTX-M ESBL genes. Continued surveillance of ceftriaxone resistance using standardized criteria is necessary to monitor its trends.

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**Conflict of interest** None to declare.

**REFERENCES**


