Ceftrioxone 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 ceftrioxone 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 ceftrioxone 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 ceftrioxone-resistant isolates. The prevalence of ceftrioxone resistance, although low, is a concern because it limits therapeutic options. Continued surveillance of ceftrioxone resistance is important to monitor its trends.

Ceftrioxone is one of the important choices for treatment of invasive infections with non-typhoidal Salmonella spp. (1). Ceftrioxone 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 78 clinical isolates of Salmonella spp. from our hospital (2007–2008) showed that the ceftrioxone resistance rate was 1.3% and detected only 1 ceftrioxone-resistant (CRO-R) isolate (Salmonella Enteritidis), a putative ESBL producer 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 reported a ceftrioxone 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 ceftrioxone resistance rate of 2.4% among Salmonella spp. This study aimed to determine the prevalence of ceftrioxone 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 University Malaya Medical Centre (UMMC) patients 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 ceftrioxone was determined by Etest (AB bioMérieux, Solna, Sweden) and interpreted according to the Clinical and 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'-TTA TCT CCC CCT TGT TAG CCA CC-3') and OS-6 (5'-GAT TTG CGT ATT TCG CTC GG-3') for blaSHV, and C (5'-TCG GGG AAA TGT GCG CG-3') and D (5'-TGC TTA ATC ATG 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 IScep1’ U1 (5'-AAA AAT GAT TGA AAG GTG GT-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 Biosystems, Foster City, Calif., USA) on an ABI-377

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A study in Taiwan (7) showed that between January 2008 and December 2009, the ceftriaxone resistance rate among our isolates was low but is still a concern because it limits therapeutic options, and ceftriaxone resistance among our isolates was 2.9% (14) involving randomly collected non-typhoid isolates of resistance or rarer ESBL genes may have been as 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 possibility of spread as many of these genes are plasmid-mediated (2,3,7,9), although they may also be found in the chromosome (19). In the United States, the ceftriaxone resistance rate among non-typhoidal Salmonella as reported by the National Antimicrobial Resistance Monitoring System (20) was 2.9% in 2008, whereas this rate was 3.3% and 3.7% in 2007 and 2006, respectively, using the revised CLSI MIC breakpoint of \( \geq 4 \mu g/ml \) (14). A study in Taiwan (7) showed that between January 1999 and December 2002, only 1.02% of non-typhoidal Salmonella isolates were resistant to ceftriaxone. On the other hand, a multinational study (10) involving randomly collected non-typhoid isolates during 2003 to 2005 from 7 Asian countries (not including Malaysia) showed that reduced susceptibility to ceftriaxone (defined in that study as an MIC of 2–8 \( \mu g/ml \)) was uncommon in Asian countries, except Taiwan (38%), and in S. Typhimurium (25%) in all countries. It also reported a ceftriaxone resistance rate of 3.0%, compared with 10.8% in Taiwan (10). When comparing ceftriaxone resistance rates, the criteria used for interpretation as “resistant” should be noted because the CLSI published revised ceftriaxone breakpoints in 2010 (14).

In the present study, isolate Nos. 3 and 5 were ceftoxitin sensitive, whereas isolate Nos. 1, 2, and 4 were ceftoxitin 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 ampicillin suggested 100% identity of the genes to those of \( \text{bla}_{\text{CTX-M-14}} \) and \( \text{bla}_{\text{CTX-M-15}} \), respectively. Further PCR revealed that isolate No. 5 had the partial \( \text{ISEcpI} \) element located in the upstream region, and sequencing of the 876-bp amplified CTX-M gene as \( \text{bla}_{\text{CTX-M-55/57}} \). This isolate also had a TEM-1 gene (Table 2). The \( \text{ISEcpI} \) 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 of resistance or rarer ESBL genes may have been responsible for the 3 other CRO-R isolates (isolates Nos. 1, 2, and 4), which were also ceftoxitin resistant. There are no CLSI guidelines for the detection of AmpC-mediated resistance at present. Resistance to ceftoxitin 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

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

<table>
<thead>
<tr>
<th>Year</th>
<th>No. CRO-R/no. of NTS (%)</th>
<th>MIC(_S) ((\mu g/ml))</th>
<th>MIC(_O) ((\mu g/ml))</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009 (n = 75)</td>
<td>2/21 (9.52)</td>
<td>0.125</td>
<td>0.125</td>
</tr>
<tr>
<td>2008 (n = 74)</td>
<td>0/12 (0)</td>
<td>0.094</td>
<td>0.125</td>
</tr>
<tr>
<td>Total (n = 149)</td>
<td>2/33 (6.10)</td>
<td>0.094</td>
<td>0.125</td>
</tr>
</tbody>
</table>

### Table 2. Source of ceftriaxone, \( \beta \)-lactamase gene detected, and susceptibility to other antimicrobials determined by disk diffusion for the 5 ceftriaxone-resistant isolates

<table>
<thead>
<tr>
<th>Isolate no. (year)</th>
<th>Source</th>
<th>Identification</th>
<th>MIC of CRO ((\mu g/ml))</th>
<th>ESBL detected</th>
<th>Other ( \beta )-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>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>
</tr>
<tr>
<td>2 (2008)</td>
<td>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>
</tr>
<tr>
<td>3 (2009)</td>
<td>Blood</td>
<td>S. Enteritidis</td>
<td>( \geq 256 ) (R)</td>
<td>CTX-M-14-like</td>
<td>—</td>
<td>S = CHL, CIP, NA, IPM; R = AMP, SXT, Tet</td>
</tr>
<tr>
<td>4 (2009)</td>
<td>Stool</td>
<td>S. Typhimurium</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>Salmonella spp. (serogroup C1)</td>
<td>( \geq 256 ) (R)</td>
<td>CTX-M-55/57</td>
<td>TEM-1</td>
<td>S = CIP, CHL, NA, IPM; R = AMP, SXT, Tet</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.

Genetic Analyzer (Applied Biosystems), using forward and reverse primers. The sequences obtained were used for a BLAST search in the GenBank database. Susceptibility to ceftoxitin for the CRO-R isolates by the CLSI disk-diffusion method (14) was also determined.

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 MIC\(_S\) and MIC\(_O\) of the 149 isolates were 0.094 \( \mu g/ml \) and 0.125 \( \mu g/ml \), 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 possibility of spread as many of these genes are plasmid-mediated (2,3,7,9), although they may also be found in the chromosome (19). In the United States, the ceftriaxone resistance rate among non-typhoidal Salmonella as reported by the National Antimicrobial Resistance Monitoring System (20) was 2.9% in 2008, whereas this rate was 3.3% and 3.7% in 2007 and 2006, respectively, using the revised CLSI MIC breakpoint of \( \geq 4 \mu g/ml \) (14). A study in Taiwan (7) showed that between January 1999 and December 2002, only 1.02% of non-typhoidal Salmonella isolates were resistant to ceftriaxone. On the other hand, a multinational study (10) involving randomly collected non-typhoid isolates during 2003 to 2005 from 7 Asian countries (not including Malaysia) showed that reduced susceptibility to ceftriaxone (defined in that study as an MIC of 2–8 \( \mu g/ml \)) was uncommon in Asian countries, except Taiwan (38%), and in S. Typhimurium (25%) in all countries. It also reported a ceftriaxone resistance rate of 3.0%, compared with 10.8% in Taiwan (10). When comparing ceftriaxone resistance rates, the criteria used for interpretation as “resistant” should be noted because the CLSI published revised ceftriaxone breakpoints in 2010 (14).

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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 have ESBL genes and 6 had 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 had CTX-M ESBL genes detected. 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


