

## Short Communication

Ceftriaxone Resistance and Genes Encoding Extended-Spectrum  $\beta$ -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  $\beta$ -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  $\beta$ -lactamases and extended-spectrum  $\beta$ -lactamases (ESBLs) (2–5,7–9). A previous study involving 78 clinical isolates of *Salmonella* spp. from our hospital (2007–2008) 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,  $\leq 1 \mu\text{g/ml}$ ; resistant,  $\geq 4 \mu\text{g/ml}$ ) (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 (*bla*<sub>CTX-M</sub>, *bla*<sub>SHV</sub>, and *bla*<sub>TEM</sub>) 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 *bla*<sub>CTX-M</sub>, OS-5 (5'-TTA TCT CCC TGT TAG CCA CC-3') and OS-6 (5'-GAT TTG CTG ATT TCG CTC GG-3') for *bla*<sub>SHV</sub>, and C (5'-TCG GGG AAA TGT GCG CG-3') and D (5'-TGC TTA ATC AGT GAG GCA CC-3') for *bla*<sub>TEM</sub> (16,17). To characterize the CTX-M genes, we carried out PCR amplification as previously described (18) with primers ISEcp1 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

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

Year	No. CRO-R/no. of NTS (%)				MIC <sub>50</sub> ( $\mu\text{g}/\text{ml}$ )	MIC <sub>90</sub> ( $\mu\text{g}/\text{ml}$ )
	Blood	Stool	Others	Total		
2009 ( $n = 75$ )	2/21 (9.52)	1/53 (1.89)	0/1 <sup>1)</sup> (0)	3/75 (4.00)	0.125	0.125
2008 ( $n = 74$ )	0/12 (0)	2/62 (3.23)	0/0 (—)	2/74 (2.70)	0.094	0.125
Total ( $n = 149$ )	2/33 (6.10)	3/115 (2.61)	0/1 (0)	5/149 (3.36)	0.094	0.125

<sup>1)</sup>: urine.

NTS, non-typhoidal *Salmonella* spp. except *S. Typhi* and *S. Paratyphi* A, B, and C; CRO-R, ceftriaxone resistant.

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

Isolate no. (year)	Source	Identification	MIC of CRO ( $\mu\text{g}/\text{ml}$ )	ESBL gene detected	Other $\beta$ -lactamase genes detected	Susceptibility to other antimicrobials by disk diffusion
1 (2008)	Stool	<i>S. Typhimurium</i>	24 (R)	—	TEM-1	S = IPM; I = CIP; R = AMP, SXT, Tet, CHL, NA
2 (2008)	Stool	<i>Salmonella</i> spp. (serogroup E)	256 (R)	—	—	S = SXT, CIP, Tet, CHL, NA; R = AMP
3 (2009)	Blood	<i>S. Enteritidis</i>	$\geq 256$ (R)	CTX-M-14-like	—	S = CHL, CIP, NA, IPM; R = AMP, SXT, Tet
4 (2009)	Stool	<i>S. Typhimurium</i>	32 (R)	—	—	S = SXT, CIP, Tet, CHL, NA; R = AMP
5 (2009)	Blood	<i>Salmonella</i> spp. (serogroup C1)	$\geq 256$ (R)	CTX-M-55/57	TEM-1	S = CIP, CHL, NA, IPM; R = AMP, SXT, Tet

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. Susceptibility to cefoxitin 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<sub>50</sub> and MIC<sub>90</sub> of the 149 isolates were 0.094  $\mu\text{g}/\text{ml}$  and 0.125  $\mu\text{g}/\text{ml}$ , 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 expressed in plasmids (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  $\geq 4$   $\mu\text{g}/\text{ml}$  (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 MIC = 2–8  $\mu\text{g}/\text{ml}$ ) was uncommon in Asian countries, except Taiwan (38%), and was not observed in *S. Typhimurium* (25%) in all countries. It also reported that 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 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 *bla*<sub>CTX-M-15</sub> and *bla*<sub>CTX-M-14</sub>, respectively. Further, PCR revealed that isolate No. 5 had the partial *ISEcp1* element located in the upstream region, and sequencing of the 876-bp amplicon identified the CTX-M gene as *bla*<sub>CTX-M-55/57</sub>. This isolate also had a TEM-1 gene (Table 2). The *ISEcp1* 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.

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