Letter to the Editor

First report of CTX-M-14 -producing clinical isolates of *Salmonella* serovar Typhimurium from Egypt

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J Infect Dev Ctries 2010; 4(1):058-060.

(Received 27 July 2009 - Accepted 19 October 2009)

Salmonella serovar Typhimurium is a leading cause of food-borne diseases worldwide. Its ability to acquire new antibiotic resistance mechanisms is of increasing therapeutic concern [1]. CTX-M beta-lactamases are the most common extended spectrum β-lactamases (ESBLs) worldwide [2]. The first report of CTX-M detection in *Salmonella* was in a Tunisian outbreak caused by *Salmonella enterica* serovar Wein (*S.* Wein*)* in 1988 [3]. We report acquisition of the CTX-M-14 ESBL by three pediatric isolates of *S.* Typhimurium from Egypt. To our knowledge this is the first report of CTX-M-producing *Salmonella* in Egypt.

Three pediatric isolates 68, 94, and 111, serologically confirmed as *S.* Typhimurium, were shown to be resistant to cefotaxime and ceftriaxone [minimum inhibitory concentration (MIC) > 128 µg/ml, Table 1]. Ceftazidime MICs varied from 8 µg/ml (susceptible) to 32 µg/ml (resistant), and all isolates were ESBL-positive by the Clinical and

Table 1. Characteristics of *Salmonella* Typhimurium clinical isolates, and their transconjugants.

Strains	MIC (µg/ml)										Enzyme			
	CTX	CTX / CLAV	CAZ	CAZ / CLAV	CRO	TZP	C	GM	AMK	CIP	CTX-M-14	SHV-12	OXA-1	TEM-1-like
E.coli J53AR	0.12	≤0.06	0.12	0.25	≤0.06	1	4	0.5	2	≤0.06	-	-	-	-
Sal. 68	>128	0.25	8	1	>128	8	>128	16	4	≤0.06	+	-	-	+
SAM 68	16	≤0.06	1	0.5	32	1	4	0.25	2	≤0.06	+	-	-	-
Sal. 94	>128	1	> 128	2	>128	>128	16	>128	>128	≤0.06	+	+	-	+
SAM 94	32	0.12	1	0.12	32	1	4	0.25	128	≤0.06	+	-	-	-
Sal. 111	>128	1	32	1	>128	>128	>128	4	4	≤0.06	+	-	+	+
SAM 111	64	0.12	2	0.12	64	1	4	0.25	2	≤0.06	+	-	-	-

CTX, cefotaxime; CAZ, ceftazidime; CLAV, clavulanate; CRO, ceftriaxone; TZP , piperacillin–tazobactam ; C , chloramphenicol; GM , gentamicin; AMK, amikacin; CIP, ciprofloxacin; J53AR , azide resistance. MICs shown in bold print indicate phenotypic confirmatory resistance according to CLSI guidelines; Sal., clinical Salmonella isolate; SAM, transconjugant.

Laboratory Standards Institute (CLSI) criteria for *Escherichia coli*, *Klebsiella*, and *Proteus mirabilis* [4]. Typing by pulsed-field gel electrophoresis (PFGE) performed using the restriction enzyme *Xbal* [5] demonstrated that two of the three isolates (68 and 111) were 85% related, with a similarity of only 55% to isolate 94.

Crude enzyme preparations for isoelectric focusing (IEF) were prepared by freeze-thaw methodology. IEF utilizing the nitrocefin/cefotaxime/inhibitor overlay procedure of Moland *et al*. [6] indicated that each isolate produced multiple β-lactamases bands with pIs of 7.9 and 5.4 (all isolates), 8.2 (isolate 94), and 7.4 (isolate 111). All enzymes were inhibited by clavulanic acid. The enzyme band(s) which focused at pI 7.9 were shown to be transferable by conjugation from each host to *E. coli* J53. The characteristics transferred were the CTX-M ESBL phenotype (cefotaxime MIC was reduced at least 256-fold by clavulanic acid; see Table 1). PCR and sequencing identified the CTX-M gene as CTX-M-14 with an amino acid sequence match of 100% for the entire CTX-M14 enzyme. Genes encoding OXA-1 and SHV-12 were also

Table 2. Primers and PCR conditions used during the study

Resistance genes	Primer Name	Sequence 5'- 3'	Template product size	Size of Gene Amplicon (bp)	Primer Location on template (nucleotide numbers)	PCR conditions	GenBank Accession No.
OXA-1							
Amplification							
	OXA1F2	TGTGCAACGCAAATGGCAC	579		1545-1563	2 mM Mg, 55°C	J02967
	OXA1B14	CGACCCCAAGTTTCCTGTAAGTG			2123-2101		
Sequencing							
	OXA305F	GGAGCAGCAACGATGTTACG	989	831	1243-1262	1.5 mM Mg, 55°C	AF255921
	OXA303R	CGACTTGATTGAAGGGTTGG			2231-2212		
SHV-12							
Amplification							
	SHV prime2F	GGGAAACGGAACTGAATGAG	380		555-574	1.5 mM Mg, 55°C	AF227204
	SHV prime End R	TTAGCGTTGCCAGTGCTCG			934-916		
Sequencing							
	HSHV-2F	CGCCGGGTTATTCTTATTTGTCGC	1016	861	3-26	1.5 mM Mg, 60°C	AF227204
	HSHV-2R	TCTTTCCGATGCCGCCGCCAGTCA			1018-995		
CTX-M-14							
Amplification							
	CTXM914F	GCTGGAGAAAAGCAGCGGAG	474		1857-1876	1.5 mM Mg, 55°C	AF252622
	CTXM914R	GTAAGCTGACGCAACGTCTG			2330-2311		
Sequencing							
	CTX-M-14F2	GATGTAACACGGATTGACC	920	876	1706-1724	1.5 mM Mg, 53°C	AF252622
	CTX-M-14F3	CTGAACCTACGCTGAATACC			2243-2262		
	CTX-M-14Ra	CAAAACCAGTTACAGCCCTTC			2625-2605		
	CTX-M-14Rb	CAGCAAAAGTTCGATTTATTCAAC			2648-2625		
	CTX-M-914R	GTAAGCTGACGCAACGTCTG			2330-2311		
IS*cp1*							
Amplification							
	ISEQ*cp1*F	CTCTTCAGAATACAGACAGC	987		160-179	1.5 mM Mg, 51°C	AJ416341
	CTX-M914R	GTAAGCTGACGCAACGTCTG			1146-1127		AY899930.1

identified by sequencing. For identification of the CTX-M-14 gene, PCR was performed using primers GATGTAACACGGATTGACC for CTX-M-14F2 and CAAAACCAGTTACAGCCCTTC for CTX-M-14Ra designed from accession number AF252622. For the detection of SHV-12, HSHV-2F (CGCCGGGTTATTCTTATTTGTCGC) and HSHV-2R (TCTTTCCGATGCCGCCGCCAGTCA) were used. Sequence AF227204 was used for primer design. For the OXA-1 gene, the primers OXA305F (GGAGCAGCAACGATGTTACG) and OXA303R (CGACTTGATTGAAGGGTTGG) were used [7]. The insertion sequence IScp1 was investigated upstream of the CTX-M-14 gene in all isolates using primers ISEQcp1F (CTCTTCAGAATACAGACAGC) which corresponded to the nucleotide numbers 160 to 179, within sequence AJ416341. The resulted amplicon was 987 bp which was consistent with *IS*cp1. The PCR products were sequenced using ABI 3730 Genetic Analyzer and the deduced amino acid sequences were interpreted by Finch TV and Vector NTI Advance 10 software. Detailed PCR reactions are described in the Table 2.

In conclusion, the three *S.* Typhimurium isolates produced the plasmid encoded CTX-M-14 ESBL in addition to other β-lactamases that included either OXA-1 or SHV-12. To our knowledge this is the first report of CTX-M-14 in *Salmonella* isolated in Egypt.

Acknowledgments

We thank Chelsie N. Hurst, Philip M. Kurpiel, and Amanda L. Roth of Creighton University for their expert technical support.

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Conflict of Interest: No conflict of interest is declared.