

Letters to the Editor

First Report of Extended-Spectrum-β-Lactamase-Producing *Salmonella enterica* Isolates in Ireland

Production of extended-spectrum-β-lactamases (ESBLs) is predominantly associated with the *Enterobacteriaceae*, particularly *Escherichia coli* and *Klebsiella pneumoniae*. ESBL production in *Salmonella* spp. was first identified in 1988 (11) and is increasing in prevalence worldwide (3, 18). A survey of ESBL prevalence in *Enterobacteriaceae* in Ireland was previously conducted, but this did not include *Salmonella* spp. (13). We have examined a large collection of human and animal isolates of salmonella for ESBL production.

Antimicrobial susceptibility data for 4,466 isolates received between January 2001 and April 2005 was reviewed for suspected ESBL production using Clinical Laboratory Standards Institute (CLSI) ESBL screen criteria (7). This represents the complete collection of isolates received from human and animal sources by the National Salmonella Reference Laboratory of Ireland during this time period. ESBL production was confirmed by the CLSI combination disk method using cefpodoxime and cefpodoxime plus clavulanate and by the ESBL Etest method using ceftazidime/ceftazidime plus clavulanate, cefotaxime/cefotaxime plus clavulanate, and cefepime/cefepime plus clavulanate strips (AB Biodisk). Confirmed ESBL producers were screened for *bla*_{TEM} and *bla*_{SHV} by PCR using specific primers and protocols as reported previously (8). The *bla*_{CTX-M} gene was detected by PCR using universal CTX-M primers (16). The gene was characterized by PCR and sequencing as previously described for group 1 and group 9 genes (3) and using primers CTX-M-group-2F (5'-ATGATGACTCAG AGCATTC) and CTX-M-group-2R (5'-TCAGAAACCGTGGTTAC) for group 2 genes (4) or primers CTX-M-group 8/25F (5'-ATGATGAGA AAAAGC GTAAG) and CTX-M-group 8/25R (5'-TTAATAA CCGTCGGTGAC), designed from the nucleotide sequence alignment of *bla*_{CTX-M-8} (5), *bla*_{CTX-M-25} (15), and *bla*_{CTX-M-26} (6), for group 8 and group 25 genes.

Seven ESBL-producing isolates were detected (Table 1). Five were from humans, and four of these were associated

with travel outside of Ireland. Six of the seven ESBL producers were coresistant to other classes of antimicrobial agents (Table 1). The *Salmonella enterica* serovar Worthington isolate was from a 6-month-old child of Indian origin. Previous reports of serovar Worthington infection are predominantly from India, and resistance to cefotaxime and ceftriaxone has been noted (10); however, this is the first confirmation of ESBL production in serovar Worthington. The *S. enterica* serovar Concord isolate was from a patient originally from Ethiopia. CTX-M production in serovar Concord has not previously been reported. CTX-M production in *S. enterica* serovar Typhimurium is well described (9). The majority of enzymes reported in this serovar belong to CTX-M group 2; however, CTX-M-15 and CTX-M-3 (both CTX-M group 1) have also recently been reported (1, 14). Serovar Typhimurium 695/04 reported here represents the first description of *bla*_{CTX-M-14} in this serovar. Serovar Typhimurium 227/05 was associated with travel to Andorra. The isolate repeatedly was confirmed as ESBL positive, with a cefotaxime MIC of 4 μg/ml, falling to 0.094 μg/ml in the presence of clavulanic acid, and a cefepime MIC of 16 μg/ml, falling to 0.25 μg/ml with clavulanic acid, while the ceftazidime MIC was 0.5 μg/ml. PCR assays for *bla*_{CTX-M}, *bla*_{SHV}, and *bla*_{TEM} were consistently negative for this isolate, and DNA was shown to be amplifiable by the 16S/23S rRNA intergenic spacer region PCR (2) (Table 1). The most common cefotaximases encountered are the CTX-M family of β-lactamases, and several members of this group have been associated with high levels of resistance to cefepime (12, 19). Hyperproduction of SHV-5 has also been associated with elevated MICs for cefepime (17). High-level resistance to cefepime in previous studies was associated with high level resistance (>32 μg/ml) to cefotaxime, which was not observed in isolate number 227/05 (12, 17). This suggests that this isolate may harbor another type of ESBL, e.g., PER-1, PER-2, VEB-1, BEL-1, or GES-1.

TABLE 1. Extended antibiogram, PCR, and sequence analysis results for isolates confirmed as ESBL producers by phenotypic methods

Isolate no.	Yr	Serotype	Source	Extended antibiogram ^a	MIC ^b (μg/ml)			PCR (TEM, SHV, CTX-M)	Sequence analysis	Foreign travel
					TZ	CT	PM			
227	2005	Typhimurium	Human	ACSSuTMnCpd	0.5	4	16	Negative	N/A	Andorra ^c
142	2005	Concord	Human	ACSSuTTmGm MnCzCtxCpd	>256	>32	>16	TEM and CTX-M positive	CTX-M-15 and TEM-1	Ethiopia
695	2004	Typhimurium	Human	ACTxCpd	4	>32	>16	CTX-M positive	CTX-M-14	None
172	2003	Typhimurium	Human	ACSSuTTmMn SpCzCtxCpd	>256	>32	>16	CTX-M positive	CTX-M-15	Africa
174	2003	Unnamed (O4,12; H?)	Avian	ASuTmSpCzCtx	>256	>32	4	TEM positive	TEM-52	NA ^d
279	2002	Worthington	Human	ASNaKGmSpCz CtxCpd	>256	>32	1.5	TEM and SHV positive	SHV-12 and TEM-1	India
1016	2001	Java	Beef	ASTmNaSpCpd	4	6	2	TEM positive	TEM-20	NA

^a A, ampicillin; C, chloramphenicol; S, streptomycin; Su, compound sulfonamides; T, tetracycline; Tm, trimethoprim; K, kanamycin; Gm, gentamicin; Mn, minocycline; Na, nalidixic acid; Sp, spectinomycin; Cz, ceftazidime; Ctx, cefotaxime; Cpd, cefpodoxime.

^b TZ, ceftazidime; CT, cefotaxime; PM, cefepime.

^c The Principality of Andorra is located in southwestern Europe, bordered by France and Spain.

^d NA, not applicable.

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