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First Human Isolate of *Salmonella enterica* Serotype Enteritidis Harboring *bla*_{CTX-M-14} in South America

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We studied a clinical isolate of *Salmonella enterica* serotype Enteritidis showing resistance to oxyiminocephalosporins. PCR analysis confirmed the presence of *bla*_{CTX-M-14} linked to IS903 in a 95-kb IncI1 conjugative plasmid. Such a plasmid is maintained on account of the presence of a *pndAC* addiction system. Multilocus sequence typing (MLST) analysis indicated that the strain belongs to ST11. This is the first report of *bla*_{CTX-M-14} in *Salmonella* Enteritidis of human origin in South America.

During the first decade of the 21st Century, there has been a rapid worldwide dissemination of CTX-M-derived extended-spectrum β -lactamases (ESBLs) (6). Nevertheless, this process had begun as early as the 1990s in both Latin America and Europe (23). In South America, enteropathogens such as *Vibrio cholerae* and *Salmonella* spp. were among the first microorganisms found to be carrying *bla*_{CTX-M-2} (24, 25). After a period of CTX-M-2 prevalence in the region (22, 28), new CTX-M variants started to be progressively reported worldwide (1, 13, 21).

Salmonella enterica serotype Enteritidis (*Salmonella* Enteritidis) together with *Salmonella enterica* serotype Typhimurium are the main serovars associated with human salmonellosis throughout the globe, causing digestive tract infections and invasive infections linked to food-borne diseases (15). Although antibiotic treatment of gastroenterocolitis (due to microorganisms other than *Shigella* spp.) is limited to life-threatening infections in children, elderly people, or immunocompromised patients, enteropathogenic microorganisms have already been pointed out as a reservoir of antibiotic resistance genes (25, 30). In Uruguay, *Salmonella* Enteritidis and *Salmonella* Typhimurium are the most frequent agents of food-borne diseases (3, 4, 18), and historically they have remained susceptible to oxyiminocephalosporins (3, 18).

In January 2011, a 67-year-old patient with chronic renal failure but not requiring dialysis was admitted to the Hospital Pasteur of Montevideo, Uruguay, due to a gastroenterocolitis syndrome that aggravated his renal failure. Stool samples yielded *Salmonella enterica* serotype Enteritidis (strain SE1101). Serotyping was confirmed at the Centro Nacional de *Salmonella* (CNS) (18).

The antibiotic susceptibility profile was determined using the Vitek 2 Compact system (bioMérieux, Marcy l'Étoile, France), and results were interpreted according to CLSI guidelines (12). Strain SE1101 was resistant to oxyiminocephalosporins, showing a clear cefotaximase profile (MIC of cefotaxime, ≥ 64 mg/liter; MIC of ceftazidime, 8 mg/liter), but remained susceptible to other groups of antibiotics, such as aminoglycosides, trimethoprim-sulfamethoxazole, and fluoroquinolones (Etest MIC of ciprofloxacin, 0.023 mg/liter) (Table 1).

The presence of the *bla*_{CTX-M}, *bla*_{SHV}, *bla*_{TEM}, and *bla*_{PER-2} genes was determined according to García-Fulgueiras et al. (13),

obtaining positive PCR results for *bla*_{CTX-M} group 4 and *bla*_{TEM}. Sequencing of PCR products with primers for *bla*_{CTX-M-9}-related genes (1) and TEM-FH (5'-CAATAAGCTTCAAAAAGGAAGAGT-3') and TEMXhoR (5'-GTGCTCGAGCCAATGCTTAATCAGTG-3') confirmed the presence of *bla*_{CTX-M-14} and *bla*_{TEM-1}, respectively. Conjugation assays were carried out using *Escherichia coli* J53-2 (rifampin resistant) as the recipient; transconjugants were selected on MacConkey agar plates supplemented with rifampin (150 mg/liter) and ceftriaxone (1 mg/liter) (26). SE1101 transconjugants (TcSE1101) showed a resistance profile similar to that of the donor strain and positive PCR results for *bla*_{CTX-M} and *bla*_{TEM} (Table 1). Size estimation of the conjugative plasmid was carried out by treatment with S1 nuclease (Fermentas, Life Sciences) followed by pulsed-field gel electrophoresis (PFGE), as described previously (2).

The probable association of *bla*_{CTX-M-14} with insertion sequences *ISEcp1*, IS26, IS903, and *ISCR1* was sought by PCR according to García-Fulgueiras et al. (13). In this regard, *bla*_{CTX-M-14} was found upstream from IS903, which is consistent with previous reports by other authors (5). We were unable to detect any association between IS26, *ISEcp1*, or *ISCR1* upstream from *bla*_{CTX-M-14}.

Plasmid incompatibility group was determined by PCR according to Carattoli et al. (9).

Both β -lactamases were encoded in a ca. 95-kb IncI1 plasmid (data not shown). IncI plasmids are considered one of the five "epidemic resistance plasmids" due to their frequent association with ESBL genes (7). Nevertheless, only rare reports from Korea and the United Kingdom have described the association between IncI plasmids and CTX-M-14 (16, 27). As a rule, IncI plasmids

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TABLE 1 Antibiotic susceptibility of *S. enterica* serotype Enteritidis SE1101 and transconjugant TcSE1101

Antibiotic(s)	MIC (mg/liter) for ^a :		
	ST1101	TcST1101	<i>E. coli</i> J53-2
Ampicillin	≥32	≥32	4
Sulbactam-ampicillin	≥32	16	4
Tazobactam-piperacillin	≤4	≤4	≤4
Cephalothin	≥64	≥64	8
Cefotaxime	≥64	8	≤1
Ceftazidime	4	≤1	≤1
Cefepime	2	≤1	≤1
Imipenem	≤1	≤1	≤1
Meropenem	≤0.25	≤0.25	≤0.25
Amikacin	≤2	≤2	≤2
Gentamicin	≤1	≤1	≤1
Nalidixic acid	8	4	4
Ciprofloxacin	≤0.25 (0.023)	≤0.25 (0.032)	≤0.25 (0.032)
Sulfamethoxazole-trimethoprim	≤20	≤20	≤20

^a Values in parentheses were determined by Etest.

have been linked to the dissemination of CMY-2, CTX-M-1, and TEM-52 β -lactamases (7). Interestingly, IncI1 plasmids have been linked to food-borne diseases on account of the high prevalence of such plasmids in *Salmonella* isolates of avian origin (8).

The presence of addiction systems encoded in resistance plasmids was suggested to allow the maintenance of resistance genes within a given bacterial population (5, 20). Accordingly, Billard-Pomares et al. described the presence of *bla*_{CTX-M-14} in an FII-FIB plasmid with several addiction systems (*srnB*, *pemKI*, *hok mok sok*, *parB*, and *sopAB*) (5). This situation differs from what Mnif et al. reported, where plasmids bearing *bla*_{CTX-M-14} presented only one or two addiction systems, namely, the *pemKI*, *ccdAB*, and *hok sok* systems (20). In order to detect mechanisms responsible for plasmid maintenance, we also searched for addiction systems in TcSE1101, by PCR, as reported elsewhere (20); in this regard, we only detected the presence of the *pndAC* addiction system.

Recently, Mnif et al. described a clear link between IncI plasmids and *pndAC* addiction systems (20); but so far the available information concerning the combination of *bla*_{CTX-M-14}, IncI1 plasmids, *pndAC* addiction systems, and *Salmonella* spp. is, to the best of our knowledge, very rare. The search for the presence of *pndAC* in salmonellae in PubMed databases (<http://www.ncbi.nlm.nih.gov/nuccore>) yielded only 13 positive results.

However, the acquisition of *bla*_{CTX-M-14} by an “epidemic plasmid” could mark the beginning of a regional dissemination event both in salmonellae as well as in other enterobacteria.

Strain SE1101 was genetically characterized by MLST following the guidelines described in <http://mlst.ucc.ie/mlst/dbs/Senterica>, and the results indicate that it belongs to the sequence type 11 (ST11; allelic profile, 5,2,3,7,6,6,11). ST11 includes the majority of the *Salmonella* Enteritidis isolates uploaded to the *S. enterica* MLST database.

Taking into account that IncI1 plasmids are frequently found in *Salmonella* isolates of avian origin (8) and that several serovars other than Enteritidis are more frequent in poultry-derived products in our country (3), we could hypothesize that plasmid pSE1101 might have been transferred from an animal clone to a clone associated mainly with human infections.

The detection of *bla*_{CTX-M-14} in South America is a recent phenomenon (10, 19, 21); so far, there have been no reports from our continent about this ESBL in *Salmonella* spp., although *Salmonella* Enteritidis isolates of human origin bearing *bla*_{CTX-M-14} have al-

ready been reported, mainly in Spain, China, Japan, and Korea (11, 14, 17, 27).

The occurrence in Uruguay of ESBL-producing *Salmonella* isolates is an extremely rare event; in this sense, in the year 2006, we reported the presence of *bla*_{TEM-144} carried in a transferable plasmid in *Salmonella enterica* serotype Derby obtained from eggs (29), but the clinical relevance of such a finding is still unknown. Nevertheless, so far in our country, there have been no reports of ESBLs in *Salmonella* Enteritidis of human origin.

The fact that this enzyme is encoded in a plasmid that only confers resistance to β -lactamics could be explained by the recent emergence of this gene in the region and the lack of recombination events with other genetic structures, as was previously suggested for the multiresistance plasmid pTN48 (5).

The acquisition of antibiotic resistance genes by agents of gastroenteritis (which generally cause mild symptoms that do not require a medical consult) constitutes a sanitary alert due to the presence of such genes in the community setting (30). The fact that *Salmonella* Enteritidis occurs epidemically, associated with food-poisoning outbreaks, and that *bla*_{CTX-M-14} is carried in a conjugative plasmid increases the potential for dissemination of such gene.

The presence of an addiction system in plasmid pSE1101 could help with establishing *bla*_{CTX-M-14} within a given bacterial population as well as increasing the chances of its maintenance in the bacterial progeny. It is necessary to remain vigilant toward *Salmonella* antibiotic susceptibility levels in order to minimize the impact of possible outbreaks of this strain or the dissemination of its plasmid.

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