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Letter to the Editor

Occurrence of plasmidic AmpC β-lactamase in a *Salmonella* Typhimurium isolate of equine origin: First report of CMY-2 in animals in Argentina

Sir,

The increase in transferable resistance to third-generation cephalosporins in zoonotic *Salmonella* constitutes a serious public health threat. Resistance to third-generation cephalosporins is mainly due to acquisition of genes encoding extended-spectrum β-lactamases or AmpC β-lactamases. These genes are mainly plasmid-encoded and are transferable into various serovars of *Salmonella* and other enteric bacteria. CMY-type enzymes are the most common plasmid-mediated AmpC β-lactamase among *Enterobacteriaceae* [1]. A variety of plasmids have been shown to carry the bla_{CMY-2} gene and some of them may be associated with particular *Salmonella* serotypes from environmental sources. Although CMY-2-producing *Salmonella* have been found in humans and animals in many countries and regions, isolates from animals has not been previously reported in Argentina.

Here we isolated CMY-2-producing *Salmonella* Typhimurium strain 16-10 (ST10-16) from a diarrhoeal stool sample of a race horse in 2010 in Buenos Aires, and the bla_{CMY-2}-bearing plasmid was characterised. Identification was carried out using conventional methods, and serotyping was conducted using the Kauffmann–White scheme. The susceptibility profile was determined by disk diffusion test according to Clinical and Laboratory Standards Institute (CLSI) documents M100-S25 and M31-A3. Phenotypic screening for β-lactamases was performed by synergy testing using disks containing 10 μg clavulanic acid and 300 μg phenylboronic acid (PBA). A multiplex PCR assay was conducted for the detection of family-specific plasmid-mediated AmpC β-lactamases, and specific primers were used to achieve the complete bla_{CMY} gene [2].

To assess the plasmid transferability and the co-transferred resistance determinants, both conjugation and transformation were attempted by liquid mating and electroporation, respectively. Minimum inhibitory concentrations (MICs) were also determined for the transconjugants and electrotransformants using the broth microdilution assay and were interpreted according to CLSI document M100-S25. Characterisation of the plasmid incompatibility (Inc) group was conducted by PCR-based replicon typing, whilst its molecular weight was estimated by pulsed-field gel electrophoresis (PFGE) analysis of S1 nuclease-digested DNA. PCR mapping and sequencing were used to determine the genetic environment of bla_{CMY-2} [2,3]. The sequence type (ST) was determined according to the *Salmonell*a multilocus sequence typing (MLST) database (http://mlst.ucc.i.e./mlst/dbs/Senterica) [accessed 4 September 2015].

By disk diffusion test, ST10-16 was resistant to ampicillin, cefalotin, cefuroxime, cefotaxime, ceftazidime, cefoxitin and ceftiofur, intermediate to tetracycline, ciprofloxacin, nalidixic acid and aztreonam, and susceptible to cefepime, imipenem, meropenem, kanamycin, gentamicin, amikacin, streptomycin, trimethoprim/sulfamethoxazole, chloramphenicol and enrofloxacin. Synergy was only observed between PBA and both cefotaxime and ceftazidime disks, suggesting the presence of an AmpC type β-lactamase. Determination of the AmpC β-lactamase alleles rendered a 462 bp amplicon, which suggested the presence of a coding gene for a CIT cluster. Amplification of the complete bla_{CMY} gene resulted in a 1146 bp amplicon identical to bla_{CMY-2}. This gene was located on a plasmid of 130 kb, named pST10-16, which was classified as belonging into the IncI1 incompatibility group. The pST10-16 could be transferred by conjugation and electroporation, and a similar resistance profile to β-lactams was observed in the transconjugant and electrotransformant obtained (Table 1). MLST analysis showed that ST10-16 displayed the following allelic profile: 10, 7, 12, 9, 5, 9, 2, which corresponds to ST19. bla_{CMY-2} is the prevalent plasmid-mediated AmpC β-lactamase worldwide, frequently associated with IncA/C and IncI1 plasmids. Most of the IncI1 plasmids confer only the bla_{CMY}-associated resistance phenotype, whereas IncA/C plasmids confer additional resistance to antibiotics such as chloramphenicol and tetracycline. Recently,

Table 1
Antimicrobial susceptibilities of wild-type, transconjugant and electrotransformant strains.

Strain	MIC (μg/mL)											
	AMP	CTX	CAZ	FOX	CTX/CLA	CAZ/CLA	NAL	CIP	TET	KAN	AMK	CHL
Salmonella Typhimurium 10-16	≥256	256	64	512	128	128	2	0.5	4	0.5	2	4
10-16 TC	≥256	256	64	512	32	256	≥64	0.5	≥32	0.5	2	4
Escherichia coli CAG 12177[a]	2	1	1	1	0.5	1	≥64	0.016	≥32	0.25	0.25	4
10-16 EP	≥256	32	64	512	16	256	≥64	0.5	1	0.5	2	4
E. coli DH5α[b]	2	1	1	1	1	1	≥64	0.06	1	0.25	0.5	4

MIC, minimum inhibitory concentration; AMP, ampicillin; CTX, cefotaxime; CAZ, ceftazidime; FOX, cefoxitin; CLA, clavulanic acid; NAL, nalidixic acid; CIP, ciprofloxacin; TET, tetracycline; KAN, kanamycin; AMK, amikacin; CHL, chloramphenicol; TC, transconjugant; EP, electrotransformant.
[a] Transconjugant recipient strain.
[b] Electrotransformant recipient strain.

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IncI1 plasmids carrying bla_{CMY-2} have been identified from isolates of *Escherichia coli* and *S.* Typhimurium in human patients from Argentina and Uruguay [2,4]. In this study, we report the emergence of the bla_{CMY-2} gene in *S.* Typhimurium isolated from an animal source, within a transposon-like element. Upstream of bla_{CMY-2}, insertion sequence IS*Ecp1* was found truncated by IS*1294*. This IS*1294b*–ΔIS*Ecp1*–bla_{CMY-2} segment [5] showed 99% similarity with the bla_{CMY-2} context identified in pTN38148 (GenBank accession no. FM246883.1) and pR7AC (GenBank accession no. KF434766), both IncI1 plasmids isolated from *E. coli*. A similar arrangement was also found in *E. coli* isolated from equine harbouring an IncFII plasmid pEQ011 (GenBank accession no. KF582523). To our knowledge, the arrangement of this work was not previously described in *Salmonella*. The genetic organisation downstream of bla_{CMY-2} (*blc–sugE–ecnR* genes) was in good agreement with previously reported flanking regions [2].

Taking into account that *S.* Typhimurium ranks among the most prevalent causes of human and animal salmonellosis, and that ST19 is the major contributor to *S.* Typhimurium reported, the emergence of transferable AmpC β-lactamase recovered from animals is a public health concern. Altogether, these results suggest that a plasmid carrying bla_{CMY-2} could be disseminating among *Salmonella* and *E. coli* strains. Considering the wide diversity of IncI1 group plasmids and the genetic context associated with bla_{CMY-2}, the spread of this mechanism appears to be related both to the mobilisation of transposable elements and the spread of a specific plasmid or clone. To the best of our knowledge, this is the first report describing *Salmonella* from animals capable of producing transferable AmpC β-lactamase in Argentina, and even in South America.

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Competing interests

None declared.

Ethical approval

Not required.

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