First detection of CMY-2 plasmid mediated β-lactamase in *Salmonella* Heidelberg in South America

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**Abstract**

*Salmonella enterica* serovar Heidelberg ranks among the most prevalent causes of human salmonellosis in the United States and Canada, although it has been infrequently reported in South American and European countries. Most *Salmonella* infections are self-limiting; however, some invasive infections require antimicrobial therapy. In this work we characterized an oxyimino-cephalosporin resistant *S*. Heidelberg isolate recovered from an inpatient in a Buenos Aires hospital. CMY-2 was responsible for the β-lactam resistance profile. *S*. Heidelberg contained a 97 kb plasmid belonging to the Inc N group harboring *bla*~CMY-2~. IS*Ecp1* was located upstream *bla*~CMY-2~ driving its expression and mobilization. The isolate belonged to sequence type 15 and virotyping revealed the presence of *sopE* gene. In this study we identified the first CMY-2 producing isolate of *S*. Heidelberg in Argentina and even in South America.

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**PALABRAS CLAVE**

*Salmonella* Heidelberg; CMY-2 β-lactamasa; ST15

**Resumen**

*Salmonella enterica* serovar Heidelberg es uno de los principales agentes causantes de salmonelosis en humanos en Estados Unidos y Canadá, sin embargo, resulta infrecuente en los países de Sudamérica y Europa. En este trabajo se caracterizó un aislamiento de *S*. Heidelberg resistente a oximino-cefalosporinas recuperado de un paciente internado...
Salmonella enterica serovar Heidelberg is the causative agent of salmonellosis, a self-limiting gastroenteritis that does not usually require antibiotic therapy. However, severe infections may occur, particularly in children and immunocompromised hosts, leading to invasive diseases that require antimicrobial treatment. Fluoroquinolones and extended-spectrum cephalosporins are frequently used in severe Salmonella infections 10.

Since the late ‘80s Salmonella isolates displaying resistance to extended spectrum cephalosporins have emerged worldwide. Coding genes for TEM-, SHV-, PSE-, OXA-, PER-, CTX-M-, CMY-, ACC-, DHA- extended spectrum β-lactamas (ESBL) and also KPC carbapenemases have been reported in S. enterica isolates 9, 14.

S. enterica serovar Heidelberg ranks among the most prevalent causes of human salmonellosis in the United States and Canada, although it is infrequently reported in South American and European countries 1, 8, 9. During the last decade, extended-spectrum cephalosporin resistance has increased among human and agri-food isolates of this serotype in North American countries. This resistance profile is mainly associated with the spread of blaCMY-2 plasmid encoded AmpC β-lactamase 9. S. Heidelberg is also one of the most common Salmonella serovars isolated from poultry and eggs, whose consumption has led to many foodborne infection outbreaks. Infections caused by person-to-person transmission or direct contact with infected animals have been rarely reported 9.

In Argentina, S. Heidelberg isolates are very infrequent among those submitted to the Centro Nacional de Referencia (Mariana Pichel- Instituto Nacional de Enfermedades Infecciosas-ANLIS “Carlos G. Malbrán”- personal communication).

In this study, we characterized oxyimino-cephalosporin resistance in an S. Heidelberg isolate recovered from a diarrheal stool sample of an HIV adult inpatient, in February 2012, in Buenos Aires. Identification was carried out using conventional culture methods. Serotyping was conducted at the Centro Nacional de Salmonella (CNS) in Montevideo, Uruguay. The CNS, housed in the Departamento de Bacteriología y Virologia, Instituto de Higiene, Universidad de la República, has characterized Salmonella isolates of human, animal, food, feed and environmental origin, voluntarily submitted by several private and public laboratories for the last 60 years in Uruguay.

Minimal Inhibitory Concentrations (MICs) of different antimicrobial agents were determined using broth microdilution testing and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines 5. S. Heidelberg was resistant to ampicillin, cephalothin, cefoxitin, ceftriaxone, ceftazidime, intermediate to tetracycline and susceptible to cefepime, imipenem, aztreonam, kanamycin, gentamicin, ciprofloxacin, levofloxacin and cloramphenicol. Phenotypic screening for β-lactamases was performed by synergy tests using amoxicillin/clavulanic acid (10 µg/10 µg) and phenyl-boronic acid (300 µg)-containing disks. Synergy was observed between phenyl-boronic acid and both ceftazidime and cefotaxime disks, suggesting the presence of an AmpC type β-lactamase. Plasmid DNA was purified according to the Kado and Liu method. A multiplex-PCR assay was conducted to reveal the presence of plasmid-encoded ampC alleles 15, rendering a 462 bp amplicon, which suggested the presence of the encoding gene for a CIT cluster β-lactamase. The following specific primers (5’-3’): 16 were used to achieve the complete blaampC gene: CMY-F: ATGATGAGAAAATTGTTATGCT and CMY-R: TTATTGCCAGTTTTCAGAACGTA. The nucleotide sequence of the 1140 bp amplicon obtained corresponded to blaCMY-2. The genetic context of blaCMY-2 was determined by PCR mapping and sequencing, as shown in Figure 1, using the following primers (5’-3’): TN-F: ACCTAGATTCTACGTCACTGACT, AmpC-R: CCCGTGGTAGATAACGCGCA, Blc-F: CATCTCTGTGGTGTCCGGTG, Sug-E: AGCATGGCGATACTGACGAT, EcnR-R: GGATTGAGAGGGCACGAT. CMY-2 gene is associated with the insertion sequence IS15, rendering a 462 bp amplicon, which was identified downstream (Accession number HG931731). The analyzed blaCMY-2 context agrees completely with the conserved regions reported for Type I, II and III environments described in S. enterica, in which blaCMY-2 gene is associated with the insertion sequence IS15, which could enhance blaCMY-2 expression and mobilization 11.

Replicon type of blaCMY-2 harboring plasmid was determined according to Carattoli et al. 2, corresponding to the IncN plasmid. Plasmid size was estimated in 97 kb by PFGE analysis of S1 nuclease digested DNA. Conjugation assays were carried out using E. coli J53 (sodic acid resistant) as recipient strain and Luria Bertani agar plates supplemented with sodium azide (150 µg/ml) and ceftazidime (10 µg/ml) as selection system. blaCMY-2 plasmid could not be transferred by conjugation in the assayed conditions.

Multilocus sequence typing (MLST) with seven housekeeping genes (aroC, dnaN, hemD, hisD, purE, sucA, thrA) was conducted according to http://mlst.ucc.ie/mlst/dbs/Senterica. The isolate displayed the following allelic profile: 2, 7, 9, 9, 5, 9, 12, which corresponds to ST 15, as well as the majority of the S. Heidelberg isolates deposited in the MLST database. According to the S. enterica MLST database, ST 15 was more often reported in Europe, North America and Asia, however there is only one description in Africa and two in South America http://mlst.ucc.ie/mlst/dbs/Senterica/.
Based on the ST analysis, in 2012, *S. enterica* isolates were grouped together in 138 discrete genetically related clusters called eBURSTGroups (eBGs). Some eBGs exhibit a unique one-to-one relationship with serovars such as eBG26 and *S. Heidelberg* (http://mlst.ucc.ie/mlst/mlst/dbs/Senterica/).

Virotyping was performed by PCR amplification of coding genes for proteins secreted by type III secretion systems (*avrA*, *sopE*), *Salmonella* Typhimurium genomic island C554 (*shdA*) and phage encoded genes (*gogB* and *sb41*); specific primers for *invA* were included as an internal control. Among the virulence-related genes investigated by PCR amplification, only *sopE* was detected. The *sopE* gene encodes for a Rho-GTPase that induces membrane ruffling and elicits a pro-inflammatory response in epithelial cells. The cytosolic localization of SopE in the absence of other bacterial molecules is sufficient for inducing NF-κB activation.

Although there is a national network of laboratories that conducts an exhaustive surveillance of diarrheal episodes, reports of *Salmonella* spp. infections are not mandatory, except for *S. Typhi*. It is estimated that only 5% of salmonellosis infections are registered. According to national reports, *S. Typhimurium* and *S. Enteriditis* constitute the most prevalent serotypes, being *S. Heiderberg* only sporadically reported. There are no reported data about extended-spectrum cephalosporin-resistant strains among human *S. Heidelberg* isolates in Argentina. Here we report the first CMY-2-producing *S. Heiderberg* human isolate in our country, an even in South America.

*bla*<sub>CMY-2</sub>, gene, constitutes the most common marker among extended-spectrum cephalosporin-resistant *Salmonella* in the United States, mainly mediated by the spread of IncI1 plasmid. This replicon type plasmid has also been described in *Salmonella* isolates from children with diarrhea in Uruguay. More recently IncA/C plasmids have been associated with *Salmonella* isolates from Argentina. However, in the studied isolate *bla*<sub>CMY-2</sub> was located in an IncN plasmid, this replicon type has not been previously associated to *Salmonella* spp. Even in previous studies performed in *E. coli* in Argentina, where we reported the association of *bla*<sub>CMY-2</sub> with IncA/C, IncI1, IncFIA/FI, IncK, IncF, IncY and IncBO plasmids, the IncN group was not detected.

Considering the wide diversity of *Inc/bla*<sub>CMY-2</sub> associations, the spread of *bla*<sub>CMY-2</sub> may be related to the presence of a transposable element responsible for its mobilization. Additionally, the co-mobilization of *bla*<sub>CMY-2</sub> and *sugE* increases the possibility of co-selection processes. SugE is a member of the small multidrug resistance (SMR) transporter family, responsible for conferring resistance to antiseptics such as quaternary ammonium compounds and SDS.

The spread of resistance markers among *S. Heidelberg* isolates constitutes a risk for the management of severe salmonellosis in clinical practice. Therefore, a better understanding of the pathogen distribution and its antimicrobial resistance is important for the development of strategies to limit salmonellosis due to multidrug-resistant strains.

**Ethical responsibilities**

**Protection of human and animal subjects.** The authors declare that no experiments were performed on humans or animals for this investigation.

**Confidentiality of data.** The authors declare that no patient data appears in this article.

**Right to privacy and informed consent.** The authors declare that no patient data appears in this article.

**Conflicts of interest**

The authors declare that they have no conflicts of interest.

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