Rev Argent Microbiol. 2014;**46(1)**:30-33

REVISTA ARGENTINA DE MICROBIOLOGÍA

www.elsevier.es/ram

BRIEF REPORT

First detection of CMY-2 Plasmid Mediated ß-lactamase in *Salmonella* Heidelberg in South America

Daniela Cejas[a], Rafael Vignoli[b], Mirta Quinteros[c], Ricardo Marino[c], Raquel Callejo[c], Laura Betancor[b], Gabriel O. Gutkind[a], and Marcela A. Radice[a,*]

[a] *Cátedra de Microbiología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina*
[b] *Departamento de Bacteriología y Virología, Instituto de Higiene, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay.*
[c] *Hospital de Infecciosas "F. Muñiz", Ciudad Autónoma de Buenos Aires, Argentina*

Received 4 December 2013; accepted 11 February 2014

KEYWORDS
Salmonella Heidelberg;
CMY-2 β-lactamase;
ST15

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

Primera Detección de CMY-2 en *Salmonella* Heidelberg en Sudamérica

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

* Corresponding author.
E-mail address: mradice@ffyb.uba.ar (M.A. Radice).

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en un hospital de la Ciudad de Buenos Aires. Se evidenció la presencia de un plásmido de 97 kb perteneciente al grupo de incompatibilidad IncN, portador del gen bla_{CMY-2}. IS*Ecp1* fue localizado corriente arriba de bla_{CMY-2}, promoviendo su expresión y movilización. El aislamiento de *S.* Heidelberg correspondió al secuenciotipo 15 y en la virotipificación se detectó el gen *sopE*. En este trabajo describimos por primera vez la producción de CMY-2 en una cepa de *S.* Heidelberg en nuestro país y América Latina.
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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-lactamases (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 bla_{CMY-2} plasmid encoded AmpC β-lactamase[10]. *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[7].

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 Virología, 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 a coding gene for a CIT cluster β-lactamase. The following specific primers (5´- 3´) were used to achieve the complete bla_{CMY} gene: CMY-F: ATGATGAAAAATCGTTATGCT and CMY-R: TTATTGCAGCTTTTCAAGAATGCG. The nucleotide sequence of the 1140 bp amplicon obtained corresponded to bla_{CMY-2}. The genetic context of bla_{CMY-2} was determined by PCR mapping and sequencing, as shown in Figure 1, using the following primers (5´- 3´): TN-F: ACCTAGATTCTACGTCAGTACT, AmpC-R: CCCTGGTAGATAACGGCA, Blc-F: CATTCCTGGTTGTCGCGTGT, SugE-F: AGCATGGCGATACTGACGAT, SugE-R: GCCTGATATGTCCTGGATCGT, EcnR-R: GGATTGAGAGGGCACGAT. IS*Ecp1* was located upstream bla_{CMY-2}, and *blc*, *sugE* and *ecnR* were identified downstream (Accession number HG931731). The analyzed bla_{CMY-2} context agrees completely with the conserved regions reported for Type I, II and III environments described in *S. enterica,* in which bla_{CMY-2} gene is associated with the insertion sequence IS*Ecp1,* which could enhance bla_{CMY-2} expression and mobilization[13].

Replicon type of bla_{CMY-2} harboring plasmid was determined according to Carattoli et al.[2], corresponding to the IncN group. Plasmid size was estimated in 97 kb by PFGE analysis of S1 nuclease digested DNA[9]. Conjugation assays were carried out using *E. coli* J53 (sodic azid resistant) as recipient strain and Luria Bertani agar plates supplemented with sodium azide (150 μg/ml) and ceftadizime (10 μg/ml) as selection system. bla_{CMY-2} plasmid could not be transferred by conjugation in the assayed conditions.

Multilocus sequence typing (MLST) with seven housekeeping genes (*aro*C, *dna*N, *hem*D, *his*D, *pur*E, *suc*A, *thr*A) 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/mlst/dbs/Senterica/.

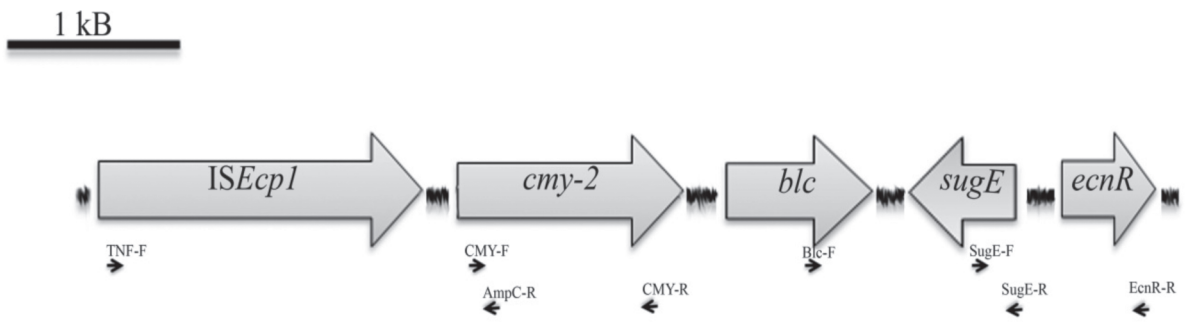

Figure 1 Genetic context of bla_{CMY-2}. IS*Ecp1*: Insertion sequence *Ecp1, blc*: gene encoding for outer membrane lipoprotein, (lipocalin); *sugE*: gene encoding for small multidrug resistance protein; *ecnR*: coding gene for a transcriptional regulatory protein, entericidinR.

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 (*avr*A, *sop*E), *Salmonella* Typhimurium genomic island CS54 (*shd*A) and phage encoded genes (*gog*B and *sb*41); specific primers for *inv*A were included as an internal control[6]. Among the virulence-related genes investigated by PCR amplification, only *sop*E 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[11].

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 resistance 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_{CMY-2} gene, constitutes the most common marker among extended-spectrum cephalosporin-resistant *Salmonella* in the United States, mainly mediated by the spread of IncI1 bla_{CMY-2} plasmid[1]. This replicon type plasmid has also been described in bla_{CMY-2} producing *S.* Typhimurium isolated from children with diarrhea in Uruguay[6]. More recently IncA/C plasmids have been associated with bla_{CMY-2} bovine isolates of *S.* Heidelberg[10]. However, in the studied isolate bla_{CMY-2} was located in an IncN plasmid, this replicon type has not been previously associated to bla_{CMY-2} in *Salmonella* spp. Even in previous studies performed in *E. coli* in Argentina, where we reported the association of bla_{CMY-2} with IncA/C, IncI1, IncFIA/FI, IncK, IncF, IncY and IncBO plasmids, the IncN group was not detected[3,4,6].

Considering the wide diversity of Inc/bla_{CMY-2} associations, the spread of bla_{CMY-2} may be related to the presence of a transposable element responsible for its mobilization. Additionally, the co-mobilization of bla_{CMY-2} 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[12].

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.

Acknowledgements

This work was partially supported by Grants from UBACyT and ANPCyT to M. Radice and G. Gutkind. G. Gutkind and M. Radice are members of Carrera del Investigador Científico (CONICET). D. Cejas was recipient of a doctoral fellowship from CONICET and is now recipient of a postdoctoral fellowship from Fundación Bunge y Born.

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