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**Título y Autores**

**AMPICILLIN RESISTANCE IN *VIBRIO CHOLERAE* NON-O1, NON-O139 RECOVERED IN CORDOBA, ARGENTINA, IS LARGELY MEDIATED BY CARB-LIKE BETA-LACTAMASES.**

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**Datos Secundarios**

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**Texto Libre**

*V. cholerae*, a natural inhabitant of the aquatic ecosystem, is the causative agent of cholera. Though only the O1 and O139 serogroups are associated to cholera epidemics, *V. cholerae* non-O1, non-O139 serogroups (VCN) also causes human disease, such as sporadic outbreaks of diarrhea, enterocolitis, soft tissue infections and sepsis. A previous report has identified two formerly unrecognized carbenicillin-hydrolyzing enzymes (CARB-7 and CARB-9) in environmental VCN isolates from Argentina. In a previous study carried out with local VCN recovered from environmental freshwater sources (n=68), contaminated food (n=3) and clinical samples (n=3), we detected 10 ampicillin (Amp) resistant isolates (all from freshwater sources). The aim of this work was to characterize the mechanisms of Amp resistance in those VCN isolates. MICs to several beta-lactams were determined by the agar dilution method (CLSI, 2006). Isoelectric focusing (IEF), conjugative assays, PCR and sequencing were performed by standard methods. Beta-lactams MICs were (range in mg/l): Amp (32-512), ticarcillin (32-512), Amp/sulbactam (1-8), amoxicillin/clavulanate (1-4), ticarcillin/clavulanate (1-8), piperacillin (0.5-4), cephalotin (0.5-4) and cefoxitin (1-2). IEF showed bands with enzymatic activity against Amp at pI of 5.2, 5.4 and 5.7. The Amp-resistant phenotype could not be transferred to *Escherichia coli* by conjugation. PCR detection of *bla*TEM, *bla*SHV and *bla*CARB beta-lactamases genes yielded amplicons of the expected size only for *bla*CARB. Sequence analysis of the PCR products confirmed that the 10 Amp-resistant VCN harbored *bla*CARB genes, and the deduced protein sequences showed the characteristic RSG motif of carbenicillin-hydrolyzing enzymes. Interestingly, the sequence analysis of these CARB enzymes indicated that several strains harbored CARB variants which were distinct from any previously known CARB allele. Since *bla*CARB-7 and *bla*CARB-9 were located in the VCR region of *V. cholerae*, we designed a PCR reaction combining primers directed against VCRs and *bla*CARB genes. All the strains yielded the expected amplicons, indicating that in all the Amp resistant VCN strains the *bla*CARB genes found here were also flanked by VCRs.

**Conclusiones**

Our results support that Amp resistance in local VCN isolates is largely mediated by CARB-like beta-lactamases, comprising several VCR-flanked alleles. This fact reinforces the notion that the *V. cholerae* super-integron may act as a reservoir of resistance genes.