Association of extended spectrum beta-lactamase VEB-5 and 16S rRNA methyltransferase ArmA in *Salmonella enterica* from United Kingdom

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**Running title**: *bla*₅VEB and *armA* in *S. enterica* from the United Kingdom

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Aminoglycosides and beta-lactams are used for the treatment of a wide range of infections due to both Gram-negative and Gram-positive. An emerging aminoglycoside resistance mechanism, methylation of the aminoacyl site of the 16S rRNA, confers high-level resistance to clinically important aminoglycosides such as amikacin, tobramycin and gentamicin. Eight 16S rRNA methyltransferase genes, armA, rmtA, rmtB, rmtC, rmtD, rmtE, rmtF and npmA, have been identified in several species of enterobacteria worldwide (2, 6, 7, 9, 11, 13, 14). Resistance to extended-spectrum β-lactams remains additionally an important clinical problem. Apart from the large TEM, SHV, and CTX-M families, several other extended-spectrum β-lactamases (ESBLs) have been identified, including VEB enzymes, which confer high-level resistance to cephalosporins and monobactams. Although 16S rRNA methyltransferases have been frequently identified associated with different ESBLs, there has been no report of association of a 16S rRNA methyltransferase with a VEB enzyme, except for the identification of rmtC with blaVEB-6 (14).

This study investigates the occurrence of 16S rRNA methyltransferases in a Salmonella enterica serovar Thompson (H093960452) and three S. enterica serovar Worthington isolates (H100680494, H100740257, H095180621) selected from the Health Protection Agency (HPA) Laboratory of Gastrointestinal Pathogens culture collection based on their ability to grow on Isosensitest agar containing 500 mg/L of amikacin (Table 1). PCR screening of the four isolates for the known methyltransferase genes identified armA in all of them. In addition, broth microdilution susceptibility testing revealed that the S. Worthington isolates exhibited high-level resistance to several cephalosporins and aztreonam (Table 1), suggesting the production of an ESBL. A series of multiplex PCRs were used to screen for the presence of genes encoding TEM, SHV, OXA 1/4/30/48, CTX-M-1,3,9,8/25, ACC,
FOX, MOX, DHA, CIT, EBC, GES, PER, VEB, IMP, VIM and KPC-like β-lactamases, and resulted in the identification of \textit{bla}_{VEB} and \textit{bla}_{CMY} alleles (5). Primers for amplification of the entire genes were designed following alignment of the \textit{bla}_{VEB} and \textit{bla}_{CMY} nucleotide sequences deposited in GenBank and used to amplify and sequence the full coding sequences, which were subsequently cloned into the pCR-Blunt II TOPO vector (Invitrogen, Paisley, United Kingdom) and transformed into \textit{E. coli} TOP10 (Invitrogen). The nucleotide sequences were confirmed using vector specific primers and revealed that \textit{bla}_{VEB} gene shared 99% sequence identity with \textit{bla}_{VEB-5} originally identified in \textit{Escherichia coli} in the United States and deposited in GenBank under Acc. No. EF420108. The protein sequence was identical to that of VEB-5 previously identified. The \textit{bla}_{CMY} gene was identified as \textit{bla}_{CMY-2} (Acc. No X91840). Pulsed-Field Gel Electrophoresis (PFGE), performed according to the PulseNet Europe protocol (12) showed that the three \textit{S. Worthington} isolates were identical (data not shown). Transfer of \textit{armA} from the \textit{S. Thompson} and \textit{S. Worthington} isolates by conjugation was assessed using nalidixic acid resistant \textit{E. coli} K802N as recipient and selecting with gentamicin 50 mg/L and nalidixic acid 50 mg/L. Minimal Inhibitory Concentrations (MICs) of different antimicrobial agents were performed following the CLSI Guidelines (4) for the wild-type strains as well as for the transconjugants (Table 1) demonstrating that transconjugants BB1082 and BB1083 showed high-level resistance to aminoglycosides, cefotaxime and aztreonam, respectively. PCRs confirmed presence of \textit{armA}, \textit{bla}_{VEB-5} and \textit{bla}_{CMY-2} in BB1083, whereas only \textit{armA} was amplified from BB1082. The latter also exhibited resistance to tetracycline (Table 1), and PCR and sequencing confirmed the presence of \textit{tetB} gene in this transconjugant. Plasmids from the wild-type strains and the transconjugants were analyzed by S1-PFGE method (1) and replicon typing (3),
showing that armA was located on pB1015, a 245 kb plasmid from IncHI2 family in S. Thompson, whereas in S. Worthington an IncA/C plasmid of 170 kb named pB1016 bore armA, blaVEB-5 and blacMY-2. In order to determine the genetic environment of the armA gene (8), PCR mapping for Tn1548 was performed for S. Thompson and S. Worthington as previously described (10). A genetic structure related to Tn1548 was found in both S. Thompson and S. Worthington to be the mobile element responsible for spread of armA (Figure 1).

Here we describe for the first time the association of ArmA with a VEB β-lactamase. This is also the first report of blaVEB-5 gene in Salmonella enterica. These findings are of a great concern due to the combined presence of resistance to aminoglycosides and all β-lactams except carbapenems. Furthermore, these resistance determinants are located on the same plasmid, raising concern that further spread worldwide is possible. Further surveillance of these resistance genes in bacteria will help to slow down resistance to these clinically relevant antibiotics.

The nucleotide sequence for the blaVEB-5 identified in this study has been deposited in GenBank under accession number JQ815440.

Acknowledgments

This work was supported by grants from the Spanish Ministry of Science and Innovation (BIO 2010-20204, PRI-PIBIN-2011-0915 and BFU2011-14145-E), the EU FP7 Health Project EvoTAR, and the Programa de Vigilancia Sanitaria 2009 AGR/4189 of the Comunidad de Madrid (Spain). LH and BG acknowledge the Comunidad de Madrid and the Universidad Complutense de Madrid for their respective fellowships. We thank Natalia Montero for excellent technical assistance.
References


### Table 1. Strains used in this study and MICs of selected antimicrobial agents

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<th>NEO</th>
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<th>ATM</th>
<th>CTX</th>
<th>STR</th>
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<td>2</td>
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<td>&gt;1024</td>
<td>256</td>
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*MICs are given in mg/L. GEN, gentamicin; AMK, amikacin; TOB, tobramycin; NEO, neomycin; AMP, ampicillin; ATM, aztreonam; CTX, cefotaxime; STR, streptomycin; TMP, trimethoprim; STX, sulfamethoxazole; TET, tetracycline.

bTransconjugant BB1082 and BB1083 were obtained from S. Thompson H093960452 and S. Worthington H100680494, respectively.
FIGURE LEGEND

Scheme showing a Tn1548-like structure as the genetic environment of armA in pB1015 and pB1016, and comparison with the armA genetic environment previously described in human, animal and food isolates. Year of isolation for each isolate is indicated. Arrows of the same colour denote similarity with the same region between the different structures.