

**TITLE:** FIRST REPORT OF THE MULTI-RESISTANCE GENE CLUSTER *lsa(E)/spw/aadE/lnu(B)* IN BRAZIL.

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## **ABSTRACT:**

Enterococci are gut commensal bacteria from human and animal species which can readily acquire and share resistances with other pathogens. Recently, it has been suggested that the new multi-resistance gene cluster comprised of the ABC transporter gene *lsa(E)* (pleuromutilin/lincosamide/streptogramin A resistance), and the *spw* (spectinomycin), *aadE* (streptomycin) and *lnu(B)* (lincosamides) genes may have evolved in enterococci. In this study, we detected the plasmid-borne *lsa(E)/spw/aadE/lnu(B)* cluster in eight *Enterococcus faecalis* (ST591, ST710, ST711) isolated from healthy nursery pigs from unrelated piggeries located in different Brazilian states (DP, PR, SP). We were able to spot that the genetic environment containing the *lsa(E)/spw/aadE/lnu(B)* gene cluster in these porcine *E. faecalis* strains showed high similarity to the sequences identified in other bacteria in Europe and Asia. Minimum inhibitory concentrations (MIC) were determined by broth microdilution testing (CLSI). Genomic DNA of the *E. faecalis* strains was sequenced (Illumina Miseq), assembled (CLC Genomics Workbench 8.0.3) and annotated (Rapid Annotation Server/ RAST). All *E. faecalis* strains exhibited elevated MICs for tiamulin, quinupristin/dalfopristin, streptomycin, and lincomycin. A *lsa(E)/spw/aadE/lnu(B)*-carrying segment of 18.325 bp was detected in all ST591, ST710, ST711 *E. faecalis* tested. Upstream of the multi-resistance gene cluster, a 7.088 bp DNA sequence containing the macrolide/lincosamide/streptogramin B resistance gene *erm(B)*, and the aminoglycoside resistance genes *aac(6')-aph(2'')*, *aadE*, and *aph(3')-III* was detected. Distal to the resistance gene cluster, a disrupted recombinase gene *repUS12* was observed. Two insertion sequences of the IS1380 and ISL3 families were detected downstream of *repUS12*, which are most likely to be involved in interplasmid recombination events. Besides *lsa(E)/spw/aadE/lnu(B)*, all *E. faecalis* strains also carried the oxazolidinone phenicol-transferable resistance gene *optrA*, and two of them co-carried the multiresistance gene *cfr* as well. The use of antibiotics in swine is a common practice in Brazilian farms and it is contributing to the spread of resistances among Gram-positive pathogens.

**Keywords:** *Enterococcus faecalis*, multi-resistance, food-producing animals.

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