Identification of a cassette-independent trimethoprim resistance *dfrA8* gene on a multi-resistance plasmid of a porcine *Salmonella enterica* serovar Typhimurium

Authors: Lopes, G.V. [1], Michael, G.B. [2], Schwarz, S. [2], Cardoso, M. [1]

Institutions: [1] Departamento de Medicina Veterinária Preventiva, Universidade Federal do Rio Grande do Sul (UFGRS), Av. Bento Gonçalves 9090, 91540-000, Porto Alegre, Brazil; [2] Institute of Farm Animal Genetics, Friedrich-Loeffler-Institut (FLI), Höltystrasse 10, 31535, Neustadt-Mariensee, Germany

Abstract

Resistance to trimethoprim in *Salmonella* is mainly due to the replacement of a trimethoprim-sensitive dihydrofolate reductase by the acquisition of transferable genetic elements encoding alternative trimethoprim-resistant dihydrofolate reductases. There are more than 30 trimethoprim resistance-mediating dihydrofolate reductase (*dfr*) genes identified until now. During a PCR screening of trimethoprim resistance genes among porcine *Salmonella enterica* subsp. *enterica* serovar (*S.*) Typhimurium, one isolate showed negative results for the most common *dfr* genes (*dfrA1*, *dfrA5*, *dfrA7*, *dfrA14-A17* and *dfrB1-3*) and also for class 1 and 2 integrons. The objective of this study was to determine the genetic basis of a trimethoprim resistance in this porcine *S.* Typhimurium isolate. Plasmids of this isolate from a pork carcass in a slaughterhouse in Southern Brazil were purified and electrotransformed into *Escherichia coli* HB101. A transformed plasmid was digested with PstI endonuclease and the fragments were cloned into pBluescript II SK(+) vector. Recombinant plasmids were subsequently transformed into *E. coli* TOP10. Electrotransformants were analysed by susceptibility testing, PCR assays, restriction analysis and sequencing. A 100-kb plasmid mediating resistance to ampicillin, chloramphenicol, streptomycin, sulphonamides, gentamicin and trimethoprim was found. PCR analysis identified the corresponding resistance genes bla_{TEM}, *floR, strA-strB,* and *sul2*. The trimethoprim resistance gene was identified in a 2,005-bp PstI-fragment of this 100-kb plasmid which belonged to the incompatibility group IncI1. Sequence analysis of this PstI-fragment showed the presence of a trimethoprim resistance gene (*dfrA8*) and an unknown open reading frame (orf1) being bracketed by IS*26* elements located in the same orientation. The 14-bp perfect terminal inverted repeats (TTTGCAACAGTGCC), characteristic of IS*26*, and partial sequences of IS*26* were identified in this PstI-fragment (accession number KJ174469). The *dfrA8* gene encodes a 169-amino acid dihydrofolate reductase enzyme (DfrA8). To the best of our knowledge, this is the first description of a plasmid carrying the *dfrA8* gene in *S.* Typhimurium. The detection of the cassette-independent trimethoprim resistance gene *dfrA8* on a multi-resistant *S.* Typhimurium isolated from a pork carcass underline the potential risk of such isolates to human health when they enter in the food chain.

Key words: mobile genetic elements, antimicrobial resistance, food chain.

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