TITLE: Novel macrolide-lincosamide-streptogramin B resistance gene *erm*(56) in *Trueperella pyogenes*

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ABSTRACT (300 words):

Suppurative infections caused by *Trueperella pyogenes*, a commensal and opportunistic Gram-positive pathogen of animals, are occasionally treated using macrolides and lincosamides posing the risk of antimicrobial resistance selection. Acquired resistances to macrolide, lincosamide and streptogramin B (MLS_B) antibiotics in *T. pyogenes* have been so far associated with erythromycin ribosome methylase genes, *erm*(B) or *erm*(X), located within mobile genetic elements.

T. pyogenes strain 09KM1269, isolated from a dog abscess, exhibited constitutive resistance to erythromycin and clindamycin. Whole genome sequence analysis identified a novel gene, *erm*(56), that coded for a 23S rRNA methylase and showed the closest relatedness to Erm(X) with only 54% nucleotide and 58% amino acid identity.

Functionality of the new gene was demonstrated by cloning *erm*(56) and its promoter sequences into pJRD215. The resulting *erm*(56)-containing plasmid pJEM1269 was subsequently electrotransformed into susceptible strains of *E. coli* AG100A and *T. pyogenes* 13OD0707. When *erm*(56) was expressed from pJEM1269 in *T. pyogenes* 13OD0707, the MIC increased by more than 256-fold for erythromycin and clindamycin and by 16-fold for pristinamycin IA. Increased MICs of erythromycin (64-fold) and clindamycin (8-fold) were also measured for *E. coli* AG100A containing pJEM1269.

The *erm*(56) gene was integrated into the chromosome between two IS*6100*, situated next to a class 1 integron containing the sulfonamide resistance gene *sul1*. The *erm*(56) gene associated with IS*6100* was also detected in strains from livestock in China, namely in another *T. pyogenes* and a *Rothia nasimurium*. Although a circular conformation containing one copy of IS*6100* was detected by PCR, the *erm*(56) gene could not be transferred by either filter mating or electroporation of genomic DNA into MLS_B-susceptible and plasmid-free *T. pyogenes* strains.

The detection of *erm*(56) in unrelated bacteria from different animal sources and geographical origins suggests that it has been independently acquired and likely selected by the use of antibiotics.