

# Investigation of the macrolide-resistance in bovine *Mannheimia haemolytica* isolates from Germany

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*Mannheimia haemolytica* is of considerable importance in the development of the multifactorial bovine respiratory disease (BRD). Recently, data from the German national resistance monitoring program GERM-Vet showed that the number of macrolide-resistant bovine *M. haemolytica* has been slowly increasing since 2009. As bovine respiratory tract infections are often treated with macrolides, this trend may result into severely limited therapeutic options for the management of BRD. In this study, 19 macrolide-resistant bovine *M. haemolytica* isolates from GERM-Vet 2008 – 2020 were investigated. Antimicrobial susceptibility testing (AST) was performed via broth microdilution according to CLSI standards (i) to confirm the macrolide resistance and (ii) to define the resistance phenotypes of the isolates. Minimal inhibitory concentration (MIC) values were obtained for the macrolides erythromycin, tilmicosin, tulathromycin, gamithromycin, and tildipirosin as well as for 22 other antimicrobial agents. All isolates either had elevated MICs or were resistant to at least one or more of the macrolides tested. In particular, isolates Mh190176, Mh191452, and Mh192916 showed resistance to tilmicosin, tulathromycin, gamithromycin, and tildipirosin, and elevated MICs for erythromycin. Furthermore, they were resistant to penicillin, ampicillin, florfenicol, and tetracycline, and had elevated MICs for gentamicin, streptomycin, neomycin, and sulfisoxazole. Based on the AST results, these three isolates were selected for whole-genome sequencing to determine the genetic basis of their multidrug-resistance (MDR) phenotypes. The DNA was prepared by phenol-chloroform extraction. Closed genomes were obtained by hybrid assembly of Illumina MiSeq and Oxford Nanopore MinION reads. Sequence analysis revealed the presence of a Tn7406-like integrative and conjugative element (ICE) in all three isolates containing the antimicrobial resistance genes *erm(T)*, *mef(C)*, *mph(G)*, *floR*, *catA3*, *aac(3)-IIa*, *aph(3'')-Ib*, *aph(3')-Ia*-like, *tet(Y)*-like, and *sul2*. Isolate Mh191452 harbored an additional second copy of the *floR* gene within the Tn7406-like ICE. In addition, isolate Mh190176 carried a 9,226 bp plasmid with two copies of the *bla<sub>ROB-1</sub>* gene, whereas the other two isolates each harbored a 4,614 bp plasmid (100% pairwise identity) with one *bla<sub>ROB-1</sub>* gene. The detection of the macrolide resistance genes *erm(T)*, *mef(C)*, and *mph(G)* together with other resistance genes on a MDR-mediating ICE in bovine *M. haemolytica* shows that these isolates are already resistant to phenicols, penicillins, tetracyclines, and macrolides, which are regularly used for treating BRD. Due to the risk of limited therapeutic options, pathogen identification and subsequent AST is essential to ensure the efficacy of the antimicrobial agents applied to control BRD in cattle herds.