Antimicrobial susceptibility testing (AST) is an important tool in veterinary diagnostics. So far, veterinary-specific approved standards for performance of AST are only available in the CLSI (Clinical and Laboratory Standards Institute) document M31-A3. This document lacks specific information on the testing of *Rhodococcus equi* and there is only limited information present in the CLSI document M24-A2 (Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes; Approved Standard—Second Edition). Literature search revealed that different media and incubation times have been used for AST of *R. equi* in published studies. The present study was performed to compare different combinations of test medium and incubation time in order to give a recommendation towards harmonized and standardized AST of *R. equi*. During this study repeated susceptibility testing was performed by broth microdilution for the reference strain ATCC® 25729 and six epidemiologically unrelated field strains. For the comparison of AST methods, the following four different test conditions were used: growth in either cation-adjusted Mueller-Hinton broth (CAMHB) or in CAMHB with 2% (v/v) lysed horse blood (LHB) and reading of the results after 24h or 48h incubation under aerobic conditions at 35°C (+/- 2°C). The test panel included 30 antimicrobial agents present on custom-made microtitre plates in 10-12 dilution steps. AST was repeated ten times for *R. equi* ATCC® 25729 and six times for each of the six field strains. This resulted in a total of 1380 data points in total, which corresponds to 46 data points per antimicrobial agent tested, as well as 300 data points for the reference strain and 180 data points for each of the six field strains. For every antimicrobial agent and each strain tested, the most frequently measured MIC value was determined. Moreover, variances in the test results were calculated and used for comparative analysis of the different test conditions. Overall, the test results obtained by using CAMHB + 2% LHB as growth medium and reading of the results after 24h showed the lowest variations. Moreover, addition of 2% LHB facilitated the reading of the results by visual inspection. Based on these results, we recommend that AST of *R. equi* should be performed by using CAMHB + 2% LHB under aerobic incubation conditions with reading of the results after 24 h.
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