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Contribution towards the establishment of Clinical Breakpoints (CBPs) for veterinarian antimicrobials: Distribution of Minimal Inhibitory Concentrations (MICs) of antimicrobials for Swiss livestock pathogens

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Key words
broth microdilution, VetCAST, wildtype, antimicrobial resistance, antibiotics

Aim of the study
Antimicrobial resistance of bacteria has become a global problem in both veterinary and human medicine. To face antimicrobial resistance, prudent use of antimicrobials has been recommended and is one of the major challenges of the 21th century (WHO, 2017). Antimicrobial Susceptibility Testing (AST) plays a key role as the basis of targeted antimicrobial therapy and Clinical Breakpoints (CBPs) have been set for its interpretation. Several factors have to be considered to determine CBPs including Wildtype (WT) populations of MIC distributions, antimicrobial dosing, pharmacokinetic and pharmacodynamic (PK/PD) parameters and clinical outcome. EUCAST has already established many CBPs of relevant pathogens for humans. In contrast, for veterinary antimicrobial /animal species combinations there is currently few information available on MIC distributions and therefore, the establishment of ECOFFS is not feasible. Consequently, there is a need for a large number of MIC values to be presented as aggregated MIC distributions to set up new ECOFFs. The aim of this study was therefore to determine MIC values of clinically relevant pathogens including Streptococcus (S.) uberis, S. suis, Trueperella (T.) pyogenes, Pasteurella (P.) multocida and Mannheimia (M.) haemolytica and to submit generated MIC distributions to VetCAST as a first step towards definitions of CBPs according to EUCAST.

Material and methods
T A total of 97 S. uberis, 33 S. suis, 78 T. pyogenes, 81 P. multocida and 37 M. haemolytica strains were obtained from clinical samples from different animals at different time points between January 2015 and July 2017. Isolates were recultivated from cryopreservation on Tryptone Soya Agar with 5% sheep blood (TSA-SB) (Thermo Fisher, Pratteln, Switzerland), at 34–37°C for 18–24h. MIC were determined by broth microdilution method using four different customer made Sensititre susceptibility plates (NLVET5, NLVET7, NLVET8 and NLVET9) (TREK Diagnostic Systems Ltd, East Grinstead, United Kingdom). The serial two-fold dilution plates contain ten to twelve individual antimicrobial concentrations per antimicrobial (from ≤0.008µg/ml to ≥512µg/ml). A total of 24 antimicrobials / antimicrobial combinations were tested per isolate.

Results and significance
This study served as a baseline for the determination of CBPs for veterinary pathogens. It gave a first insight into the MIC distributions of different antimicrobials for selected important pathogens like S. uberis, S. suis, T. pyogenes, P. multocida and M. haemolytica. Visual inspection of MIC distributions and histograms, com-bined with calculation of MIC50 and MIC90 values, which effectively allow the estimation of resistance of a given bacterial population if no CBPs are available, were helpful for the categorization of presumptive WT versus non-WT bacterial phenotypes. Isolates representing the WT population typically form unimodal distributions whereas non-WT strains form a second distribution peak as a consequence of increased MICs indicating re-duced susceptibility or acquired resistance. Therefore, populations of WT and non-WT isolates will form bi-modal MIC distributions. Nevertheless, MIC distributions have to be statistically examined to calculate WT MIC ranges, which

enable the setting of reliable ECOFFs. Attribution of ECOFFs is determined by the official committee of EUCAST, which is creating MIC data including those generated during this study.
However, our results may already serve to indicate susceptibility and potential resistance development of certain antimicrobials in Swiss livestock pathogens. Of note, MIC were determined for different classes of antimicrobials including those which are not authorised for a specific indication in veterinary medicine. These include human antimicrobials as well as antimicrobial classes, which are not indicated for a specific type of diseases. Given a broad analysis permits to determine the general situation of MIC distributions and possible acquisition of resistance in veterinary pathogens.

Publications, posters and presentations
A publication in a peer-reviewed journal is in preparation. Moreover, an oral presentation of the main findings is planned to be held for diagnostic laboratories in 2018 (SVVLD annual meeting/Laborleitertagung)

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