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Proposal for agar disk diffusion interpretive criteria for susceptibility testing of bovine mastitis pathogens using cefoperazone 30 μg disks

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ABSTRACT

Cefoperazone is a third generation cephalosporin which is commonly used for bovine mastitis therapy. Bacterial pathogens involved in bovine mastitis are frequently tested for their susceptibility to cefoperazone. So far, the cefoperazone susceptibility testing using 30 μg disks has been hampered by the lack of quality control (QC) ranges as well as the lack of interpretive criteria. In 2014, QC ranges for 30 μg cefoperazone disks have been established for *Staphylococcus aureus* ATCC® 25923 and *Escherichia coli* ATCC® 25922. As a next step, interpretive criteria for the susceptibility testing of bovine mastitis pathogens should be developed. For this, 637 bovine mastitis pathogens (including 112 *S. aureus*, 121 coagulase-negative staphylococci (CoNS), 103 *E. coli*, 101 *Streptococcus agalactiae*, 100 *Streptococcus dysgalactiae* and 100 *Streptococcus uberis*) were investigated by agar disk diffusion according to the document Vet01-A4 of the Clinical and Laboratory Standards Institute (CLSI) using 30 μg cefoperazone disks and the results were compared to the corresponding MIC values as determined by broth microdilution also according to the aforementioned CLSI document. Based on the results obtained and taking into account the achievable milk concentration of cefoperazone after regular dosing, the following interpretive criteria were proposed as a guidance for mastitis diagnostic laboratories: for staphylococci and *E. coli* ≥23 mm (susceptible), 18–22 mm (intermediate) and ≤17 mm (resistant) and for streptococci ≥18 mm (susceptible), and ≤17 mm (non-susceptible). These proposed interpretive criteria shall contribute to a harmonization of cefoperazone susceptibility testing of bovine mastitis pathogens.

1. Introduction

Antimicrobial susceptibility testing (AST) plays an important role in veterinary diagnostics, since it is recommended to test the causative bacteria for their susceptibility before applying an antimicrobial agent for therapeutic interventions. For routine diagnostics, clinical breakpoints should be used for the interpretation of the AST results (Schwarz et al., 2010). The Clinical and Laboratory Standards Institute (CLSI) lists in its current document VET01-S (CLSI, 2015b) veterinary-specific clinical breakpoints for several antimicrobial agents. However, for bovine mastitis therapy, clinical breakpoints are only available for ceftiofur, penicillin-novobiocin and pirlimycin (CLSI, 2015b).

Cefoperazone is a third generation cephalosporin approved for the treatment of bovine mastitis in several EU countries since 1991 (EMEA, 1998; Feßler et al., 2012). So far, the evaluation of AST results for cefoperazone when testing mastitis pathogens has been hampered by the fact that there were only human-specific clinical breakpoints available. As shown in a previous study, these breakpoints were not suitable for bovine mastitis pathogens (Feßler et al., 2012). As a consequence, new clinical breakpoints for cefoperazone were proposed for bovine mastitis pathogens applicable to MIC values obtained from broth microdilution and zone diameters obtained from agar disk diffusion using 75 μg disks (Feßler et al., 2012). Since the use of the 75 μg disk resulted in comparatively large inhibition zones, which were impractical for the use in routine diagnostic laboratories, the use of 30 μg disks has been recommended. As a prerequisite for using cefoperazone 30 μg disks in routine diagnostics, quality control (QC) ranges for these disks had been developed for the quality control strains *Staphylococcus aureus* ATCC® 25923 and *Escherichia coli* ATCC®

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25922 (Feßler et al., 2014). These QC ranges have been approved in the meantime by CLSI and are listed in the document VET01-S (CLSI, 2015b).

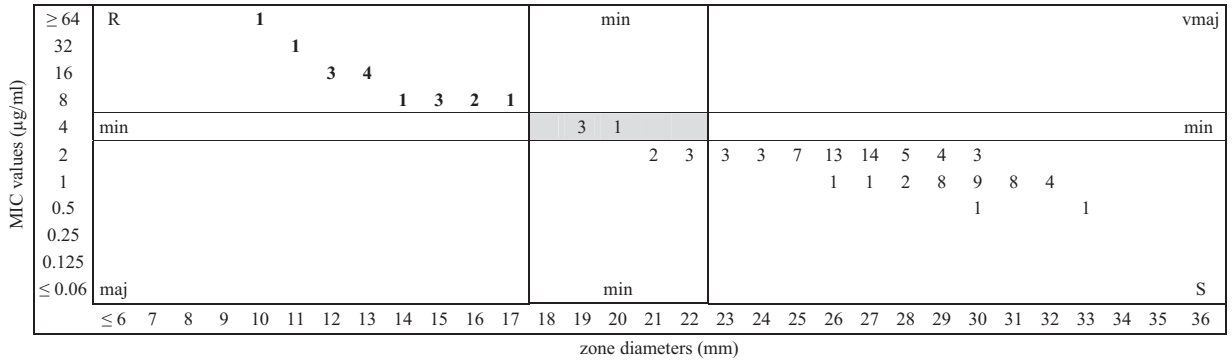
The aim of the present study was to develop interpretive criteria for bovine mastitis pathogens applicable to cefoperazone 30 μg disks. For this, previously published data on the pharmacokinetics-pharmacodynamics of cefoperazone (Hamann et al., 1986; Wilson and Gilbert, 1986) and the results of clinical outcome studies (Weiskopf, 1987) were combined with the AST results obtained from a total of 637 isolates of bovine mastitis pathogens.

2. Material and methods

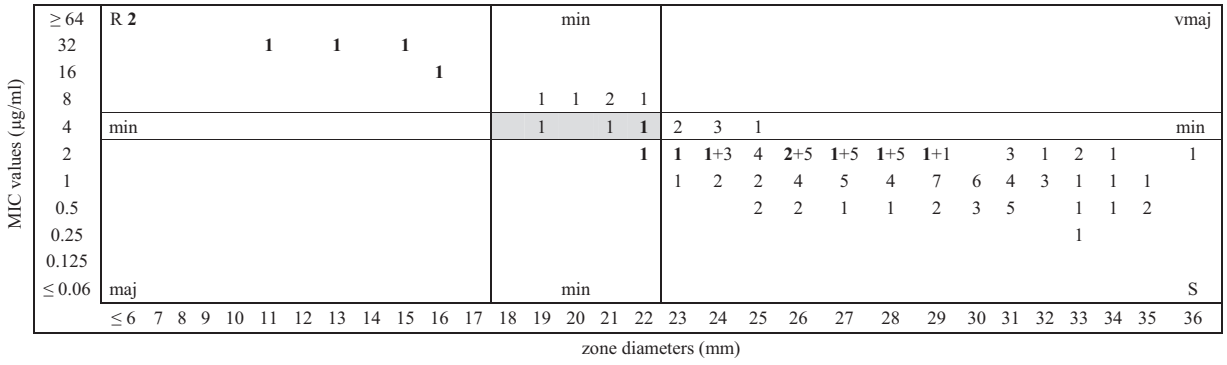
2.1. Susceptibility testing of target pathogens

Susceptibility testing was performed by agar disk diffusion using a cefoperazone 30 μg disk (Oxoid, Wesel, Germany) following the recommendations given in the CLSI documents VET01-A4, VET01-S and M100-S25 (CLSI, 2013, 2015a,b). Staphylococci and *E. coli* were tested on Mueller-Hinton (Oxoid, Wesel, Germany) agar plates and for the streptococci the Mueller-Hinton

a) *S. aureus* (n=112)



b) CoNS (n=121)



c) *E. coli* (n=103)

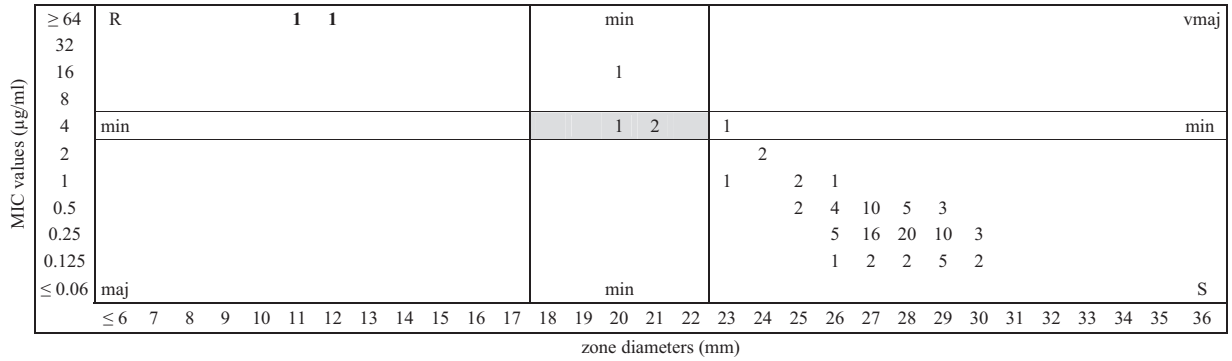


Fig. 1. Scattergrams of cefoperazone MICs versus zone diameters around a 30 μg cefoperazone disk for 112 *S. aureus* (a), 121 CoNS (b), 103 *E. coli* (c), 101 *S. agalactiae* (d), 100 *S. dysgalactiae* (e), and 100 *S. uberis* (f). The suggested interpretive criteria are applied and the categories are classified as R (resistant), S (susceptible), N-S (non-susceptible), min (minor error), maj (major error), and vmaj (very major error). The gray box symbolizes the intermediate category. The numbers in the scattergrams indicate the numbers of isolates that showed a certain MIC/zone diameter combination. Staphylococci carrying the *mecA* gene and ESBL-producing *E. coli* isolates are displayed in bold.

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