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Antimicrobial susceptibility of *Mycoplasma bovis* isolates from veal calves and dairy cattle in the Netherlands

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ABSTRACT

Control of *Mycoplasma bovis* infections depends on good husbandry practices and antibiotic treatment. To allow more prudent use of antimicrobial drugs, there is a need for information on the susceptibility profile of this pathogen. The objective of the present study was to analyse the *in vitro* antimicrobial susceptibility of clinical *M. bovis* isolates in the Netherlands. The collection comprised 95 bovine isolates, originating from lungs (n = 56), mastitis milk (n = 27), and synovial fluid (n = 12), collected between 2008 and 2014. Minimal inhibitory concentrations (MICs) were assessed by broth microdilution, both by using in-house prepared MIC plates and by using commercially available MIC plates. For each antimicrobial agent, the range of MIC results, the MIC_{50}, and MIC_{90} values were calculated. *M. bovis* strains recently isolated in the Netherlands appeared to be characterized by relatively high MIC values for antimicrobial agents that, until now, have been recommended by the Dutch Association of Veterinarians for treating pneumonia caused by *Mycoplasma* species. Fluoroquinolones appeared to be the most efficacious in inhibiting *M. bovis* growth, followed by tulathromycin and oxytetracycline. The highest MIC values were obtained for erythromycin, tilmicosin, and tylosin. Future studies should be done on determining *M. bovis* specific clinical breakpoints, standardization of methods to determine MIC values as well as molecular studies on detection of antimicrobial resistance mechanisms of *M. bovis* isolates to develop PCR assays for determining resistance.

1. Introduction

In cattle, *Mycoplasma bovis* is able to cause respiratory disease, mastitis, arthritis, otitis, and reproductive disorders (Bürki et al., 2015). It is frequently implicated in cases of bovine respiratory disease (BRD) in calves. However, the role of *M. bovis* in the multifactorial BRD complex is not as easily defined; *M. bovis* mostly occurs in co-infection with viruses and/or other bacteria. *M. bovis* has characteristics that enable it to colonize and persist on mucosal surfaces, to invade tissues, and to persist at sites of disease despite an aggressive immune response (Bürki et al., 2015). In addition to economic costs, there are important animal welfare consequences of *M. bovis* infections. There is no effective vaccine, and therefore control of *M. bovis* infections depends on good husbandry practices and antibiotic treatment. However, *M. bovis* responds poorly to antimicrobial agents. Because mycoplasmas lack a cell wall, β-lactams are not effective against these pathogens. Similarly, mycoplasmas do not synthesize folic acid and are therefore intrinsically resistant to sulfonamides. Mycoplasmas as a class are generally susceptible to drugs that interfere with protein (aminoglycosides, lincosamides, macrolides, tetracyclines, and florfenicol) or DNA (fluoroquinolones) synthesis (Giguère, 2013). However, resistance against these drugs has been reported (Ayling et al., 2014; Gautier-Bouchardon et al., 2014; Kong et al., 2016).

To ensure the prudent use of antimicrobial agents, it is important to effectively target antimicrobial treatment. Unsuitable treatment may lead to selection of resistant strains. However, assessing the antimicrobial susceptibility of mycoplasmas is difficult. This is related to their slow growth, small size and complex growth media requirements. Additionally, nutritional requirements, metabolic activities and fitness vary among species. Standard antimicrobial susceptibility procedures such as the disk diffusion method are therefore not suitable for mycoplasmas. Publications on the susceptibility of *M. bovis* to antimicrobial agents were carried out using broth microdilution (Ter Laak et al., 1993; Ayling et al., 2000; Rosenbusch et al., 2005; Gerchman et al., 2009; Soehnlen et al., 2011; Ayling et al., 2014; Kawai et al., 2014; Sulyok et al., 2014; Kong et al., 2016), agar dilution (Hirose et al., 2003; Uemura et al., 2010; Siugzdaite et al., 2012; Gautier-Bouchardon et al., 2014), the E-test (Francoz et al., 2005; Gerchman

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et al., 2009), and also flow cytometry (Soehnlen et al., 2011) to determine minimum inhibitory concentrations (MICs). However, there are currently no MIC testing control standards for veterinary mycoplasmas, making it difficult to compare studies carried out using different methods. Breakpoints have not yet been determined and so MIC results cannot be defined as susceptible, intermediate, or resistant, making it difficult to evaluate the likely *in vivo* therapeutic efficacy from MIC data established *in vitro*.

Being scarce, recent publications on the antimicrobial susceptibility of *M. bovis* show an increase in MIC values (Ayling et al., 2014; Gautier-Bouchardon et al., 2014). There are no recent data on susceptibility patterns of *M. bovis* isolated from clinical samples in

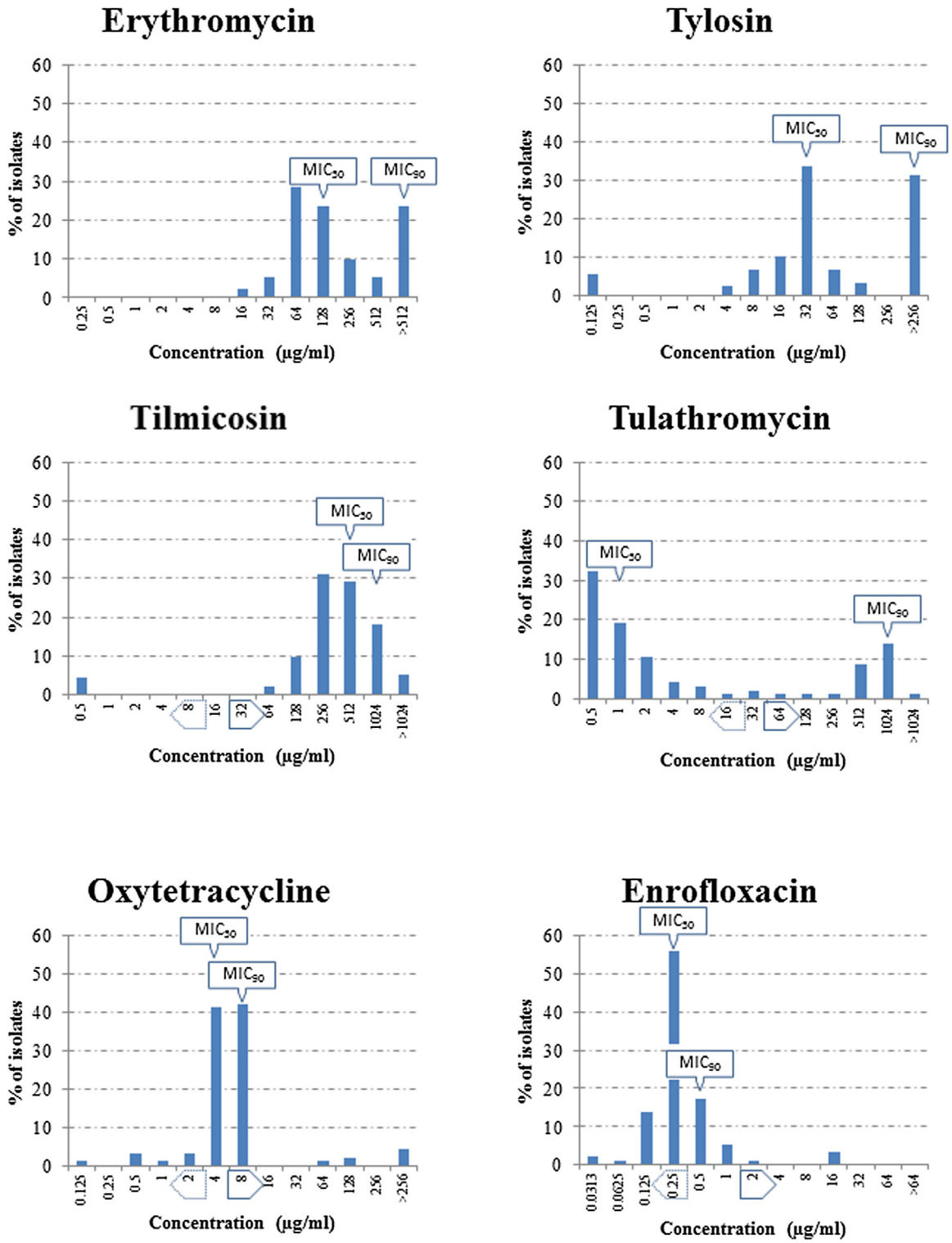

Fig. 1. Distribution (%) of MIC values (µg/ml) of antimicrobial agents tested with in-house prepared MIC plates. The MIC_{50} and MIC_{90} values are marked. Additionally, when available, CLSI breakpoints for other bovine respiratory pathogens are indicated on the x-axis; isolates with MIC values less than or equal to the concentration indicated in the dotted-line arrow pointing to the left are susceptible, isolates with MIC values greater than or equal to the concentration indicated in the full-line arrow pointing to the right are resistant, the remaining isolates being intermediate.