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Short communication

Determination of minimum inhibitory and minimum bactericidal concentrations of tiamulin against field isolates of *Actinobacillus pleuropneumoniae*

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ABSTRACT

Tiamulin activity was measured against 19 UK field isolates of *Actinobacillus pleuropneumoniae* collected between 2003 and 2009 and the type strain ATCC 27090 as a control, with the intention of comparing broth with serum as growth media. Broth microdilution MIC/MBC tests were performed in accordance with the Clinical and Laboratory Standards Institute (CLSI) guideline M31-A3, in 'Veterinary Fastidious Medium' (VFM) (supplemented Mueller–Hinton broth at pH 7.3) and in 100% swine serum. For improved precision, a modified, overlapping doubling-dilution series was used (tiamulin concentration range 0.3–72 μg/ml). The MBC was reported as the lowest concentration producing a 99.9% reduction in bacterial density in the sub-cultured well contents, relative to the starting inoculum. The mean MBC/MIC ratio for tiamulin against *A. pleuropneumoniae* in VFM was low (1.74:1), even though tiamulin is classed as a bacteriostatic drug. Only three of the 19 isolates and the reference strain grew in 100% serum and their MICs were higher than those determined in VFM. It is postulated that this difference was due to differences in pH of the matrices or binding of tiamulin to serum proteins or a combination of both factors.

1. Introduction

Serum/plasma concentrations linked to indices such as MIC are well-established surrogates for most antimicrobial drugs. However, correlating the pharmacokinetics (PK) of tiamulin hydrogen fumarate concentrations in plasma with the pharmacodynamics (PD) measured as minimum inhibitory concentration (MIC) against *Actinobacillus pleuropneumoniae* has been unsuccessful. Using standardized Mueller–Hinton broth (MHB), MIC determinations based on the Clinical and Laboratory Standards Institute (CLSI) methods provided a susceptibility breakpoint ≤16 μg/ml against *A. pleuropneumoniae*. This value is much higher than the plasma concentration achieved with therapeutic dose rates of tiamulin, suggesting that either the MIC method of determination in artificial media does not reflect growth conditions in biological fluids such as plasma or circulating concentrations of tiamulin differ significantly from those at the site of infection. High intracellular concentrations of tiamulin in the lung (Anderson et al., 1994; McKellar et al., 2004) and human polymorphonuclear leucocytes (Nielsen and Szancer, 1998) were reported in comparison with plasma concentrations; both tissues: plasma concentration ratios exceeding 18:1. In an artificial *A. pleuropneumoniae* infection study in pigs, an isolate with a tiamulin MIC of 4 μg/ml was effectively treated, both clinically and bacteriologically, with 180 ppm tiamulin in the drinking water (Schultz et

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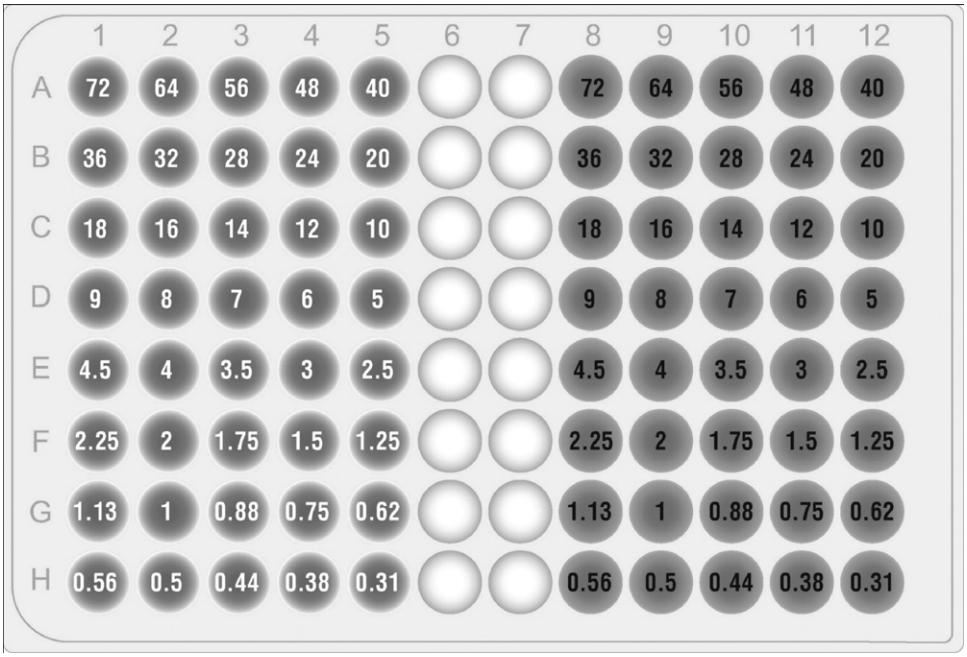

Fig. 1. Overlapping dilution series of tiamulin concentrations (μg/ml) employed for MIC/MBC determinations.

al., 1984; Burch and Klein, 2008). At this dosage, plasma concentrations of approximately 0.47 μg/ml would be obtained, *i.e.* 1/8th of the MIC.

Studies using 2.5% serum in the culture medium (Godinho et al., 2005) and 100% serum (Illambas et al., 2009) reduced the MIC values for tulathromycin against a variety of bacterial respiratory pathogens of bovine origin but in particular *Pasteurella multocida* by 8 and 50 times, respectively, and against *A. pleuropneumoniae* (Godinho et al., 2005) by 32 fold. The aims of this study were: (1) to determine MICs, Minimum Bactericidal Concentrations (MBCs) and MBC:MIC ratios for tiamulin against *A. pleuropneumoniae* using overlapping sets of doubling dilutions to improve accuracy and (2) to determine whether the MIC and MBC of tiamulin against *A. pleuropneumoniae* were reduced substantially by culturing the organism in 100% swine serum in comparison with MHB.

2. Materials and methods

Tiamulin hydrogen fumarate (Denagard® -Novartis Animal Health Inc.) activity was measured against 19 field isolates and one control type strain (ATCC 27090) of *A. pleuropneumoniae*. All field strains were isolated during the period 2003–2009, from pigs exhibiting clinical signs of *pleuropneumonia*, and were identified by veterinary diagnostic laboratories. Following initial isolation, each strain was stored in a cryoprotective suspension at −80 °C and was subjected to not more than three subcultures from the original isolate. Strain identification was confirmed before commencing the current study, on the basis of morphological observations.

Broth microdilution MIC tests were performed using five overlapping sets of double-dilutions of tiamulin (to improve accuracy of the determinations) (range 0.3–72 μg/ml; Fig. 1) in accordance with the CLSI guideline M31-A3, using "Veterinary Fastidious Medium" (VFM) (Mueller–Hinton Broth supplemented with 5% lysed horse blood, yeast extract and yeast concentrate), without added serum (exact CLSI method) and in 100% swine serum (supplied frozen by First Link UK Ltd., Birmingham, UK). The MIC plates were incubated in 5% CO_2 for 24–48 h. The MBC of tiamulin against each isolate was reported as the lowest concentration producing a 99.9% reduction in bacterial viable count in the sub-cultured well contents, relative to the initial inoculum, which was enumerated immediately after inoculation by subculturing the positive control well on to Mueller–Hinton agar plates and counting the colonies.

3. Results

The MIC and MBC data for 19 field isolates and the reference strain were determined following the growth in VFM (Table 1). Only three field isolates and the reference strain grew in 100% serum and there were moderate differences in MIC between VFM and serum for these four strains; MICs were 12/24, 14/24, 12/32 and 12/24 μg/ml (mean 12.5/26 μg/ml) for VFM and serum, respectively.

For the 19 strains grown in VFM the MIC_{50} and MIC_{90} were 12 and 14 μg/ml, respectively, and the MBC_{50} and MBC_{90} were 18 and 38 μg/ml, respectively. The MBC/MIC ratio for all 20 isolates was 1.74:1 (Table 2). The pH of the serum was 7.14 and that of the VFM approximately 7.3 (range 7.29–7.33).