MICs and MBCs of Tiamulin against *Actinobacillus pleuropneumoniae (APP)* in broth and 100% Serum

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ABSTRACT

Attempts to integrate the pharmacokinetics of tiamulin (Denagard® – Novartis) in plasma with tiamulin MICs against *Actinobacillus pleuropneumoniae (App)* have proven ineffective, despite good clinical and microbiological responses to treatment. The purpose of this study was to determine MICs and MBCs of tiamulin against *App* and whether these would be consistently reduced by culturing the organism in 100% swine serum.

Tiamulin activity was measured against 19 UK field isolates of *App* (collected 2003-2009) and type strain (ATCC 27090). Broth microdilution MIC/MBC tests were performed in accordance with CLSI guideline M31-A3, using 'Veterinary Fastidious Medium' [supplemented Mueller-Hinton (MH) broth, pH 7.3] without serum or in 100% swine serum.

For precision, a modified overlapping doubling dilution series was used (tiamulin concentration range 0.3 to 72 µg/ml). MIC/MBC plates were incubated in 5% CO_2 for 24-48h. MBC was reported as the lowest concentration producing a 99.9% (1000 fold) reduction in bacterial density in the sub-cultured well contents, relative to the positive control well.

The mean MBC/MIC ratio for tiamulin against *App* was only 1.74: 1, although tiamulin is classed as a bacteriostatic drug. Only 3 of the isolates and the reference strain grew in 100% swine serum and their MICs were not lower than those determined in MH broth.

INTRODUCTION

Attempts to integrate the pharmacokinetics (PK) of tiamulin (Denagard® - Novartis Animal Health Inc.) concentrations in plasma with the pharmacodynamics (PD) measured as Minimum Inhibitory Concentration (MIC) against *Actinobacillus pleuropneumoniae (App)*, using standardized MIC determinations, have been unsuccessful and led to considerations that lung [Ref.1] or leucocyte concentrations[Ref.2] might play a significant role, as estimated intracellular concentrations were 8.5µg/g but only 0.47µg/ml in plasma (ratio 18:1).

This is considered unjustifiable by some authorities [Ref.3] because the infectious agent resides outside the cell in the extracellular compartment. Moreover, serum/plasma concentrations are better established for pharmacokinetic integration for most antibiotics.

However, in an artificial infection study, an *App* isolate with a tiamulin MIC of 4µg/ml was effectively treated, both clinically and bacteriologically, with 180 ppm tiamulin in the drinking water. Studies using 2.5% serum in the culture media [Ref.4] and 100% serum [Ref.5] reduced the MIC values for tulathromycin against a variety of bacterial respiratory pathogens but in particular *Pasteurella multocida* by eight and fifty times, respectively and against *App* [Ref.4] by thirty-two times.

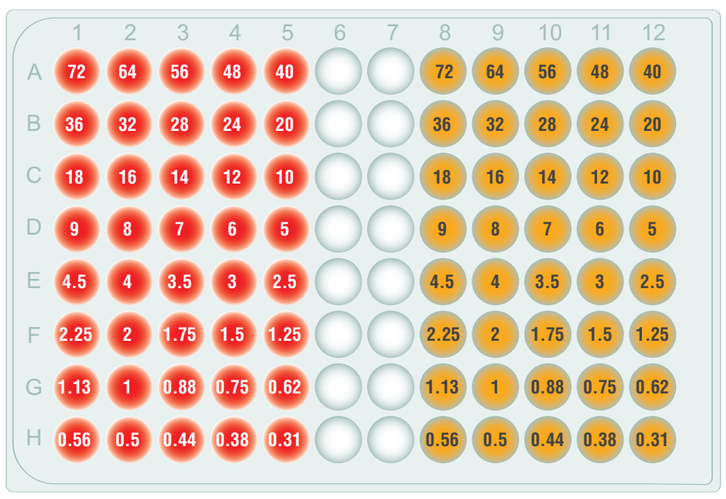
A preliminary study [Ref.6] with serum 50% : MHB 50% indicated that there was a reduction in geometric mean MICs of seven *App* isolates by 6.6 fold and 2 isolates' MICs were reduced by 32 fold in the presence of serum.

It was the purpose of this study to determine whether the MIC and Minimum Bactericidal Concentration (MBC) of tiamulin against *App* would be consistently reduced by culturing the organism in 100% swine serum in comparison with those achieved in serum-free culture medium.

MATERIALS AND METHODS

Tiamulin activity was measured against 19 field isolates (collected 2003 – 2009) and one type strain (ATCC 27090) of *App.*

Broth microdilution MIC tests were performed using an overlapping double-dilution series of tests with tiamulin (range 0.3 to 72 µg/ml) in accordance with CLSI guideline M31-A3, using Veterinary Fastidious Medium (VFM) - MHB, 5% lysed horse blood, yeast extract & yeast concentrate supplement, without serum (exact CLSI method) and in 100% swine serum. MIC plates were incubated in 5% CO_2 for 24-48h.

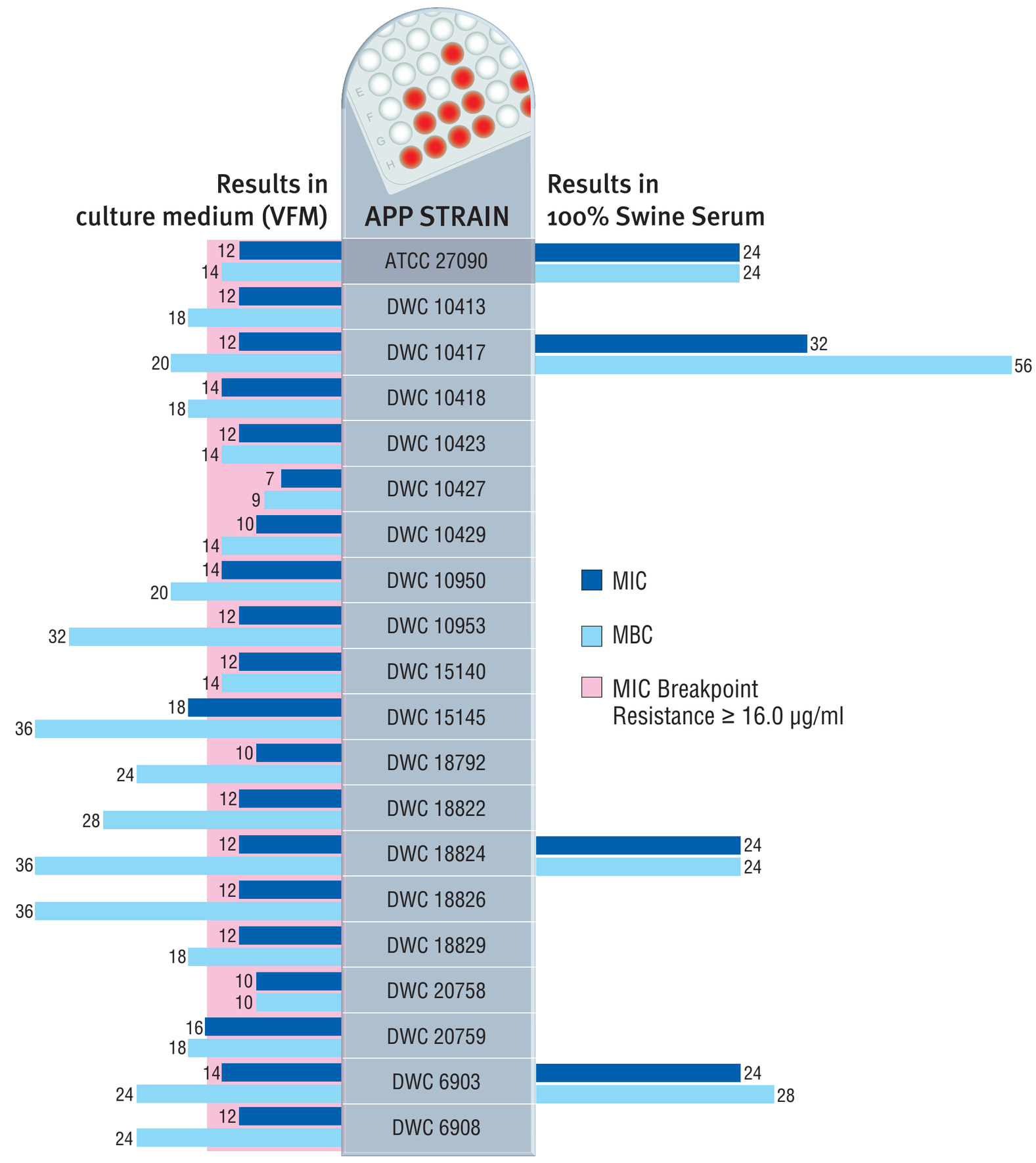


Overlapping dilution series employed for MIC/MBC determination.

The MBC of tiamulin against each isolate was reported as the lowest concentration producing a 99.9% (1000 fold) reduction in bacterial density in the sub-cultured well contents relative to the positive control well.

RESULTS

The MIC and MBC results for 19 field isolates and the reference strain were determined following growth in VFM. Only 3 field isolates and the reference strain grew in 100% serum and there was no reduction in MIC.



MIC (µg/ml)	Results	MBC (µg/ml)
12	MIC/MBC 50%	18
14	MIC/MBC 90%	36
7-18	Range	9-36
12.3	MIC/MBC mean	21.4
1	MIC/MBC ratio	1.74

MIC and MBC results of tiamulin against App grown in VFM.

DISCUSSION

Growth of *App* isolates was poor in 100% serum but the MICs were not reduced as previously observed with 50% serum. The MICs results of the isolates grown in VFM were in accordance with previous surveys. The mean MBC was only 1.74 times the MIC for tiamulin against *App*, which is surprising as it is considered primarily a bacteriostatic antibiotic. This is in accordance with its clinical response [Ref.1] but does not clarify the relationship between plasma concentrations and MIC/MBC.

References:

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