The trials of Daafit® antibacterial potency against clinical isolates of *Brachyspira hyodysenteriae*

1. Introduction

This trial was performed at the request of Daavision to determine the minimum inhibitory concentrations (MICs) of Daafit® product for *Brachyspira hyodysenteriae* isolates from pigs in clinical conditions. MICs were determined under standard anaerobic conditions in solid medium. The thirty-eight bacterial isolates described in this trial were collected from clinical cases (routine diagnosis procedures) in the central-south region of Taiwan.

2. Experimental subjects

2.1 Bacterial strains

A total of 38 bacterial isolates was collected. All isolates were from clinical cases collected from the central-south region of Taiwan. Besides, all isolates were confirmed as *Brachyspira hyodysenteriae* by polymerase chain reaction (PCR).

2.2 Test product

All concentrations given in this study report were expressed as μg of Daafit® base /mL.

2.3 Stock solutions preparation

Medium: Mueller-Hinton agar (Muller-Hinton Ⅱ agar; BBL™, USA) with 5% defibrinated sheep blood was used as solid medium. Each stock solution (commercialized product Daafit®) was prepared with the final concentration of 4000 μg/mL. During entire experiment processing, all 8 working dilution steps were tested from 4000 to 31.25 μg/mL.

3. Experimental Method

1.1 Incubate test cultures

According to McFarland standard, bacterial suspension was diluted to McFarland 0.5 contains 1.5×10^8 CFU/mL.

1.2 Antimicrobial susceptibility testing: MIC

Bacterial susceptibility to antimicrobials was performed quantitatively by agar micro-dilution with cation-adjusted Mueller-Hinton agar (Muller-Hinton Ⅱ agar; BBL™, USA) with 5% defibrinated sheep blood according to the guidelines of the

Clinical and Laboratory Standards Institute [CLSI, 2014]. The product mentioned above (ranges expressed as mg/ mL) was tested by means of two-fold dilutions. The following part of the experiments, the minimum inhibitory concentration (MIC) was determined after 96 hours of incubation at 37°C. *B. hyodysenteriae* ATCC 27164^{T} was used as quality control for antimicrobial susceptibility in accordance with the CLSI recommendations. The MICs of the quality control strains were within the CLSI quality control ranges.

2. Results

2.1 Commercialized product

It showed that the isolates were confirmed, with MIC_{50} and MIC_{90} around 250 µg/mL shown as Fig 1.

3. Discussion

In order to identify the optimal dosage of Daafit® that could inhibit bacterial growth effectively, the MIC was determined following the standard protocol provided by CLSI, using a agar mico-dilution assay [CLSI, 2014]. The results demonstrated that all wild isolates were inhibited by commercialized Daafit® concentrations: 250 µg/mL shown as Fig 1. However, the MIC test this time was determined as *in vitro* test, paromomycin should be evaluated by other experiments to confirm its actual effectiveness.

4. References

Clothier KA, Kinyon JM, Frana TS, Naberhaus N, Bower L, Strait EL, Schwartz K. Species characterization and minimum inhibitory concentration patterns of *Brachyspira* species isolates from swine with clinical disease. J Vet Diagn Invest 23: 1140-1145, 2011.

Clinical and Laboratory Standards Institute/NCCLS. Performance standards for antimicrobial disk and dilution susceptibility tests for bacterial isolated from animals approved standard M31-A2. NCCLS, Wayne, PA. USA, 2014.

Fig.1 The MIC values distribution of commercial product Daafit®.