Minimal Inhibitory Concentrations of Tiamulin against Actinobacillus pleuropneumoniae

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Abstract. The study was conducted in order to determine the antimicrobial susceptibility of a collection of 110 biotype I. Actinobacillus pleuropneumoniae strains to tiamulin using standard broth microdilution method.

Keywords: Actinobacillus pleuropneumoniae, antimicrobial susceptibility, tiamulin, minimal inhibitory concentration, broth microdilution

INTRODUCTION

Actinobacillus pleuropneumoniae (APP) is the causative agent of porcine pleuropneumonia, a severe respiratory disease, which is a serious problem in pig production worldwide. The acute form of the disease is often fatal, resulting in considerable economic losses for pig producers (Gutiérrez-Martin et al, 2006; Zutic et al, 2008).

Tiamulin is a pleuromutilin derivative antimicrobial used in the control and treatment of veterinary Gram-positive and Gram-negative pathogens, with a particular emphasis on infections in swine (Prescott and Walker, 2000).

Tiamulin treatment is capable of preventing mortality, reducing or eliminating clinical signs and reducing or eliminating lesions of experimental pleuropneumonia. It can also eliminate the organism completely from diseased pigs (Taylor D.J., 2008).

MATERIALS AND METHODS

A total of 110 biotype I. A. pleuropneumoniae strains, isolated between 2008 and 2011 from lungs of diseased pigs from herds located in Hungary and Romania, were included in this study. The study was performed at the Diagnostic Laboratory of the Large Animal Clinic, Faculty of Veterinary Science, Szent István University, Úllő, Hungary.

The strains were isolated on blood agar plates, using V factor discs (Difco). Identification of the strains as was based on Gram-stain, hemolysis on 5% sheep blood agar, positive Christie-Atkins-Munch-Petersen (CAMP) reaction, requirement of NAD and urease production. Strains were stored at -80°C and were thawed and striked on blood agar plates prior to use.

All A. pleuropneumoniae isolates were tested for their in vitro susceptibility by broth microdilution method using veterinary fastidious medium (VFM) according to CLSI guidelines M07-A8 (2009) and M31-A3 (2008). VFM is cation-adjusted Mueller-Hinton broth supplemented with lysed horse blood, yeast extract and yeast concentrate supplement C.
MIC determinations were performed using 96 well microtiter plates (Greiner). Fresh, 16-20 hours cultures were used to prepare bacterial suspensions for the MIC study. Concentration of bacterial suspension was adjusted to approximately 0.08-0.13 optical density (OD) at 650 nm, corresponding to 0.5 McFarland standard. Final dilution range of tiamulin (Sigma) was 0.0625-32 µg/ml. After 20 hours of incubation on a microplate shaker at 37°C, optical densities (OD) of wells were determined with an ELISA plate reader (BioTek) at 650 nm wavelength. The percentage reduction was calculated by comparing the OD values of the control wells (C) containing no drug, with the OD of drug containing wells (D) after the OD values from wells containing media but no organisms (B) has been removed: 100-(D-B) x 100/(C-B).

Minimal inhibitory concentration was defined as the first drug concentration where bacterial growth was decreased by at least 85% compared to the growth control well. The interpretive criteria were taken from CLSI standard M31-A3 (CLSI, 2008). The following clinical breakpoints for tiamulin were used for the classification of A. pleuropneumoniae strains: ≤16 µg/ml for “susceptible” and ≥ 32 µg/ml for “resistant”.

RESULTS AND DISCUSSION

Distribution of MIC values is shown on Fig. 1. MICs ranged from 1 to 8 µg/ml (MIC$_{50}$ 2 µg/ml, MIC$_{90}$ 4 µg/ml). All isolates were inhibited by 8 µg/ml and as the susceptibility breakpoint was established at ≤16 µg/ml, these 110 A. pleuropneumoniae isolates were all considered susceptible to tiamulin.

![Fig. 1. Tiamulin MIC distribution of 110 A. pleuropneumoniae strains](image)

Similar in vitro MIC studies have been reported by a number of authors. Early publications did not always use the current CLSI guidelines, and therefore may not be directly comparable with those of today. Aarestrup and Jensen (1999) classified 40 A. pleuropneumoniae isolates from Denmark as susceptible to tiamulin using the agar microdilution method. The breakpoint used for resistance was ≥16 mg/ml. Jones et al. (2002) used the current CLSI guidelines and found that the MIC$_{50}$ for 170 isolates of A.
*A. pleuropneumoniae* was 8 µg/ml, and the MIC$_{90}$ was 16 µg/ml. Matter et al. (2007), describing *in vitro* susceptibility of 83 *A. pleuropneumoniae* isolates collected in Switzerland between 2002 and 2004 to 20 antimicrobial agents, showed that only nine (11%) of their isolates were resistant to tiamulin. They used the same accepted breakpoints. The study reported by Kucerova et al. (2011) on the antimicrobial susceptibility of 242 *A. pleuropneumoniae* isolates collected from diseased pigs in the Czech Republic between 2007 and 2009 to 16 antimicrobial agents also showed low resistance (1.7%) to tiamulin.

**CONCLUSIONS**

Hungarian and Romanian isolates of biotype I. *A. pleuropneumoniae* were highly susceptible to tiamulin. Due to this lack of resistance, tiamulin is a superior choice than many other antimicrobial agents for the prevention and treatment of *A. pleuropneumoniae*.

**REFERENCES**