

Antimicrobial Susceptibility of Some *Actinobacillus pleuropneumoniae* Strains

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Abstract. A total of 100 *Actinobacillus pleuropneumoniae* strains isolated from Hungary and Romania were tested for *in vitro* susceptibility to 7 antimicrobial agents by using standard disc diffusion method. Results showed that majority of *A. pleuropneumoniae* isolates were found susceptible to most antimicrobial agents tested.

Keywords: *Actinobacillus pleuropneumoniae*, pleuropneumonia, pig, disc diffusion, antimicrobial susceptibility

INTRODUCTION

Actinobacillus pleuropneumoniae is the causative agent of porcine pleuropneumonia, a highly contagious swine disease, causing severe production losses in some countries (Bosse et al., 2002; Zutic et al, 2008). Although proper management, sanitation, nutrition, and vaccination are generally useful in preventing *A. pleuropneumoniae* infections, use of antibiotics for treatment of infected pigs is necessary to avoid economic losses. However, application of antibiotics in the treatment of pig's pleuropneumonia may lead to selective survival of drug resistant strains (Wang et al. 2010). Variations in antimicrobial use for the control of *A. pleuropneumoniae* infections from one country to another and variations in methodology can contribute to evident differences in antimicrobial susceptibility of *A. pleuropneumoniae* (Kucerova et al. 2011).

MATERIALS AND METHODS

A total of 100 *A. pleuropneumoniae* strains, isolated between 2008 and 2011 from the 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, Üllő, Hungary.

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

Antimicrobial susceptibility of *A. pleuropneumoniae* isolates was tested by the disc diffusion test in Petri dishes containing Haemophilus test medium base (HTM) with HTM supplement (OXOID) following the recommendations of Clinical and Laboratory Standards

Institute M31-A3 (CLSI, 2008). HTM is a medium specifically formulated for the susceptibility testing of fastidious organisms containing Mueller Hinton agar and yeast extract - specifically selected for low antagonist levels. The results achieved using HTM have been found to be highly reproducible. The transparency of the medium allows zones of inhibition to be read easily through the bottom of the Petri dish. HTM contains low levels of antimicrobial antagonists, which allows testing of trimethoprim/sulphamethoxazole to be carried out with greater confidence. HTM supplement contains NAD and haematin.

The following seven antimicrobial agents, commonly recommended and administered to treat pleuropneumonia in pigs, were tested: amoxicillin (AX-25 µg), enrofloxacin (ENR-5 µg), tiamulin (TIA-30 µg), trimethoprim/sulphamethoxazole (SXT-25 µg), tilmicosin (TIL-15 µg), florfenicol (FFC-30 µg), doxycycline (DO-30 µg). Tests were done with commercially available discs (Biolab, Hungary; Oxoid, UK).

The density of direct colony suspension was adjusted to 0.5 McFarland standard (1 to 2×10^8 CFU/ml) and plates were inoculated by spilling the suspension over the surface. The appropriate drug-impregnated discs were placed individually on the dry surface of the plates within 15 minutes after inoculation, allowing for any excess surface moisture to be absorbed. Inhibition zones were read after 20–24 hours of incubation at 37°C, results were evaluated according to CLSI M31-A3 (2008).

RESULTS AND DISCUSSION

Table 1 presents disc diffusion susceptibility test results of 100 *A. pleuropneumoniae* isolates to 7 antimicrobial agents.

Tab. 1.

Antimicrobial susceptibility of 100 *A. pleuropneumoniae* strains

Antimicrobial agent	Disk content (µg)	Number of tested isolates		
		S	I	R
Tiamulin	30	100	-	-
Florfenicol	30	98	2	-
Tilmicosin	15	78	17	5
SXT-TMP	25	76	14	10
Enrofloxacin	5	73	23	4
Amoxicillin	25	67	28	5
Doxycycline	30	56	30	14

Generally, our 100 *A. pleuropneumoniae* isolates showed low levels of antimicrobial resistance. Out of the tested compounds, tiamulin proved to be the most effective. All *A. pleuropneumoniae* isolates were susceptible to this antimicrobial, which is in accordance with previous studies (Aarestrup and Jensen, 1999). The percentage of sensitivity to florfenicol was also high, 98% of the tested strains being susceptible and 2% intermediate to this amphenicol antibiotic. No resistance was encountered. Regarding β-lactams, 67% of isolates were susceptible and only 5% resistant to amoxicillin. Similar results regarding florfenicol and amoxicillin were obtained by Pascu (2008), who didn't detect resistance to florfenicol either, 87.5% of the strains being susceptible and 12.5% intermediate to this antibiotic; though the resistance to amoxicillin in her study was higher 12.9%.

A higher percentage of resistance was registered to trimethoprim-sulphamethoxazole and doxycycline, 10% and 14% respectively. Earlier publications reported higher resistance of *A. pleuropneumoniae* to trimethoprim-sulphamethoxazole (Chang et al., 2002; Zutic et al., 2008). With regard to macrolides, a low-level resistance to tilmicosin was exhibited by five isolates. Only 4% of our isolates were resistant to enrofloxacin, 23% were intermediate and 73% were susceptible. Similar level of sensitivity to this quinolone has been reported by Chang et al. (2002), somewhat higher resistance has been reported by Gutiérrez et al. (1995).

CONCLUSIONS

In this study, most isolates of *A. pleuropneumoniae* were found susceptible *in vitro* to most antimicrobial agents tested. According to our results, the most effective antimicrobial agent was tiamulin.

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