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Short communication

Antimicrobial susceptibility of *Actinobacillus pleuropneumoniae* isolates from clinical outbreaks of porcine respiratory diseases

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ABSTRACT

Limited data regarding the susceptibility of *Actinobacillus pleuropneumoniae* to antimicrobials has been published during recent years. Accordingly, the aim of the present study was to investigate the distribution of MICs for the isolates of *A. pleuropneumoniae* from diseased pigs in the Czech Republic between 2007 and 2009.

A total of 242 isolates were tested for susceptibility to 16 antimicrobial agents by a broth microdilution method. A low degree of resistance was observed for florfenicol (0.8%), amoxicillin and clavulanic acid (0.8%), tilmicosin (1.2%), tiamulin (1.7%) and ampicillin (3.3%), whereas resistance to tetracycline was detected more frequently, 23.9% of isolates. Interestingly, resistance to florfenicol has not yet been reported in any study investigating antimicrobial resistance of *A. pleuropneumoniae*. By PCR the presence of the *flo*R gene was confirmed in all florfenicol resistant isolates.

1. Introduction

Actinobacillus pleuropneumoniae is one of the main etiologic agents of contagious bacterial diseases in swine. It causes pleuropneumonia, a severe contagious pulmonary disease of pigs resulting in high morbidity and mortality worldwide (Sebunya and Saunders, 1983).

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*.

In accordance with the recommendation of Schwarz et al. (2010) on the requirement of application of the same methodology and interpretive criteria (which would allow for re-evaluation of the original data if the interpretive criteria change over time), only a limited number of recent studies were conducted with the use of microdilution method (Gutiérrez-Martin et al., 2006; Matter et al., 2007; Godinho, 2008).

Due to the fact that there is a lack of recent results obtained by *A. pleuropneumoniae* testing for antimicrobial susceptibility which could be compared with results from subsequent years, the purpose of the current study was to assess the distribution of the minimum inhibition concentration (MIC) of 16 antimicrobial agents for *A. pleuropneumoniae* isolates, using the broth microdilution method.

This paper presents the results obtained by evaluation of the distribution of the MICs, clinical resistance rates, and phenotypic drug resistance profiles for 242 isolates of *A. pleuropneumoniae* from diseased pigs in the Czech Republic between 2007 and 2009. Furthermore, presence of the *flo*R gene was confirmed in florfenicol resistant isolates.

2. Materials and methods

2.1. Sampling

All *A. pleuropneumoniae* isolates used in this study were derived from the lungs of growing pigs (body weight from 18 to 50 kg) that died of acute respiratory diseases. The

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isolates were obtained from 127 herds across the Czech Republic in 2007–2009.

Not more than one isolate of *A. pleuropneumoniae* from the same farm per six-month period was included in the study. Isolates from animals that had been treated with antimicrobials during the two weeks prior to sampling were not included in this study.

2.2. Bacterial isolates

Two hundred and forty-two isolates were isolated on Columbia blood agar plates, using a strip of *Staphylococcus aureus* culture to display the positive CAMP reaction. After an additional passage through Columbia chocolate agar, cultures were identified and serotyped using serological and molecular techniques as described previously (Mittal et al., 1983; Gram et al., 2000). All isolates were stored at −80 °C in vials containing 0.25 ml of Foetal Bovine Serum Gold (PAA Laboratories GmbH, Austria) and 0.25 ml of Cation Adjusted Mueller Hinton Broth II (CAMHB) (Becton, Dickinson and Company, USA).

2.3. Antimicrobial susceptibility testing

All *A. pleuropneumoniae* isolates were investigated for their *in vitro* susceptibility by the microdilution broth method using veterinary fastidious medium (VFM) according to the CLSI standard M31-A3 (CLSI, 2008). MIC-determinations were performed using a commercially prepared microtitre plates (Trek Diagnostic Systems, East England and Trios, Czech Republic). The antimicrobial agents were tested and their dilution ranges are shown in Table 1. In categorizing the MIC results, CLSI breakpoints of resistance for swine respiratory disease pathogens were generally used (CLSI, 2008). The interpretive criteria for *A. pleuropneumoniae* taken from a proposal of clinical breakpoints for amoxicillin (Schwarz et al., 2008) were used for ampicillin and amoxicillin/clavulanic acid and breakpoints of *A. pleuropneumoniae* for tulathromycin (Godinho, 2008) were accepted. The breakpoints used are indicated in Table 1. *A. pleuropneumoniae* ATCC 27090 was used as reference strain for quality-control (QC) testing each batch of the plates and lot of VFM. QC testing was also performed simultaneously in each series of investigated isolates. The expected MIC values (CLSI, 2008) for the control strain were established: tetracycline ≤ 0.5 mg/L; gentamicin 8–16 mg/L; trimethoprim/sulfamethoxazole ≤ 0.5/9.5 mg/L; ceftiofur ≤ 0.5 mg/L; cefquinome ≤ 0.25 mg/L; tilmicosin 4–8 mg/L; tiamulin 8–16 mg/L; tulathromycin 16–32 mg/L; florfenicol 0.25–0.5 mg/L.

2.4. Polymerase chain reaction (PCR)

The primers for the detection of the *flo*R gene were 5′ GCG ATA TTC ATT ACT TTG GC 3′ and 5′ TAG GAT GAA GGT GAG GAA TG 3′ (Faldynova et al., 2003). To obtain a template DNA, a loop of bacterial culture was re-suspended in 50 μl of water and boiled for 20 min. The suspension was spun for 1 min and 2 μl of the supernatant was used for the reaction. The PCR was carried out in 20 μl volumes using 10 pmol of each primer and PCR Master Mix (Qiagen, Germany). PCR cycling consisted of 35 cycles of 40 s at 95 °C, 45 s at 55 °C and 1 min at 72 °C. The amplification products were separated by electrophoresis in 1.5% agarose gel, stained with ethidium bromide and visualized under UV light.

2.5. Sequencing of the floR gene

The PCR product obtained by amplification with primers covering the whole sequence of the *flo*R gene (5′ ACC ACC ACA CGC CCC GCG TG 3′ and 5′ GAC GAC TGG CGA

Table 1
MICs for 16 antimicrobial agents of the *Actinobacillus pleuropneumoniae* ($n = 242$) isolates identified in this study.

Antimicrobial agent	Number of isolates with MIC of (mg/L)												MIC_{50}	MIC_{90}	Percentage of resistance
	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256			
Ampicillin			229	5	2	0	1	1	1	0	3		≤0.5	≤0.5	3.3
Amoxicillin/clavulanic acid[a]			238	2	2	0	0	0	0	0			≤0.5	≤0.5	0.8
Ceftiofur			241	0	0	1	0	0	0	0			≤0.5	≤0.5	0.0
Cefquinome		238	3	1	0	0	0	0	0				≤0.25	≤0.25	–
Tulathromycin			0	2	19	51	145	23	2	0			8	16	0.0
Tilmicosin				3	11	107	115	3	1	1	0	1	4	8	1.2
Chloramphenicol		197	23	6	3	4	4	3	2				≤0.25	0.5	–
Florfenicol		88	137	3	8	4	2	0	0				0.5	0.5	0.8
Oxolinic acid			190	14	4	5	9	7	7	3	3		≤0.5	8	–
Flumequine			211	4	4	13	4	0	2	4			≤0.5	2	–
Enrofloxacin	223	11	6	2	0	0	0	0					≤0.12	≤0.12	–
Tetracycline			153	31	10	11	6	8	13	6	4		≤0.5	16	24.0
Tiamulin			3	3	13	63	126	30	3	1			8	16	1.7
Gentamicin			17	34	94	79	13	1	0	1	3		2	4	–
Streptomycin				6	16	90	102	15	2	1	5	5	8	16	–
Trimethoprim/sulfamethoxazole[b]		215	6	9	6	3	1	0	2				≤0.25	0.5	–

[a] Amoxicillin and clavulanic acid in the ratio 2:1; test ranges are expressed as the amoxicillin concentration.
[b] Trimethoprim and sulfamethoxazole in the ratio 1:19; test ranges are expressed as the trimethoprim concentration. The dilution ranges tested are those contained in the white area. Values above this range indicate MIC values higher than the highest concentration within the range. Values corresponding to the lowest concentration tested indicated MIC values lower or equal to the lowest concentration within the range. When available, breakpoints of resistance used are indicated with vertical black lines. Cross: no breakpoint for *A. pleuropneumoniae* is available.