

## Monitoring of antibiotic resistance in isolates of *Actinobacillus pleuropneumoniae* in the Czech Republic between 2001 and 2003

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**ABSTRACT:** During the period of 2001 and 2003 a total of 238 isolates of *Actinobacillus pleuropneumoniae* obtained from 26 pig herds in the Czech Republic (2001 – 73 isolates, 2002 – 110 isolates, 2003 – 55 isolates) were examined for antibiotic resistance by disc diffusion method. Resistance to erythromycin (95.5% to 98.6%), streptomycin (76.4% to 82.7%) and tiamulin (72.7%) was detected most frequently over the whole monitored period. High increase in percentage of resistant isolates to tetracycline (2001 – 11.0%, 2002 – 36.4%, 2003 – 81.8%), doxycycline (2001 – 9.6%, 2002 – 34.6%, 2003 – 61.8%), nalidixic acid (2001 – 2.7%, 2002 – 14.5%, 2003 – 45.5%), and norfloxacin (2001 – 0%, 2002 – 7.3%, 2003 – 34.6%) was found, while differences in resistance to cephalotine (2001 – 1.4%, 2002 – 7.3%, 2003 – 9.1%), cotrimoxazol (2001 – 4.1%, 2002 – 10.0%, 2003 – 7.3%), amoxicillin (2001 – 12.3%, 2002 – 12.7%, 2003 – 10.9%) and amoxicillin-clavulanic acid (2001 – 9.6%, 2002 – 9.1%, 2003 – 10.9%) were small. Prevalence of isolates resistant to sulfasomidine was detected decreasing (2001 – 43.8%, 2002 – 47.3% and 2003 – 29.1%).

**Keywords:** *Actinobacillus pleuropneumoniae*; porcine pleuropneumonia; serotypes; antibiotics; resistance; pig; Czech Republic

The first cases of porcine pleuropneumonia in the Czech Republic, caused by *Actinobacillus pleuropneumoniae*, were observed by the end of the year 1982, and in 1983 the occurrence of the disease evidently increased (Skollova and Gois, 1987). It was the era of establishment of specialized farms for piglet production and pig fattening. Modified technologies of pig keeping likely resulted in spreading of the disease in the Czech Republic. The incidence of porcine pleuropneumonia increased also in early 1990s, due to liberalization of the market and related transport of animals (Satran and Nedbalcova, 2002).

Increased use of antibiotics, which are broadly administered for both therapy and prophylaxis, is closely associated with relatively frequent occurrence of the disease. The problem of microbial

resistance to antibiotics is particularly important because antibiotic resistance represents a high risk for rapid spreading of the disease. In many cases, rash use of antimicrobials poses a serious hazard for the development of adverse epizootological situation (Wasteson et al., 1996). Therefore, testing of antibiotic resistance of *A. pleuropneumoniae* isolates with standardized methods is necessary for effective treatment of porcine pleuropneumonia (McDermott et al., 2001).

The purpose of the present study was to determine antibiotic resistance of *A. pleuropneumoniae* isolates from pigs in the Czech Republic using standardized methodology described by the National Committee for Clinical Laboratory Standards (NCCLS, 2002).

## MATERIAL AND METHODS

### Isolates

*A. pleuropneumoniae* isolates were obtained from 26 herds in the Czech Republic. A total of 238 isolates (2001 – 73 isolates, 2002 – 110 isolates and 2003 – 55 isolates) originated from lungs, lymph nodes and tonsils from dead or slaughtered pigs. The isolates were grown on blood agar (Blood Agar Base No. 2, HiMedia, India) containing 5% ram blood using a strip of *Staphylococcus aureus* culture as a source of NAD.

### Antisera and serotyping

Twelve reference strains of biovar 1 (strains 4074, S1536, S1421, M62, K17, Femø, WF83, 405, CVJ13261, D13039, 56153, 8329, serotypes 1–12, respectively) were used for the preparation of immune rabbit blood sera according to Mittal et al. (1982). Isolates were serotyped by coagglutination test (Mittal et al., 1983).

### Antibiotic resistance tests

Antibiotic resistance of *A. pleuropneumoniae* isolates was tested by the disk diffusion method in Petri dishes containing *Haemophilus* test me-

dium base (HTM) with HTM supplement (OXOID, England) and Vitox (OXOID, England). The isolates to be tested were first incubated at 37°C overnight and then the cultures were resuspended in PBS (pH 7.2). The density of the suspension was adjusted to 0.5 Mc Farland standard ( $1$  to  $4 \times 10^8$  CFU/ml) and plates were inoculated by spilling the suspension over the surface. The appropriate drug-impregnated discs were placed individually on surface of the plates from three to five minutes, but no longer than 15 minutes, after inoculation for any excess surface moisture to be absorbed. Inhibition zones of individual drugs were read after 20–24 h of incubation at 37°C and results were evaluated according to NCCLS (2002). Strain S 1421 (= ATCC 27090) was used for quality control (NCCLS, 2002).

In 2001, resistance to 11 antimicrobials was tested. Following disks were used: amoxicillin 25 µg, amoxicillin-clavulanic acid 30 µg, cephalotine 30 µg, cotrimoxazol 25 µg, doxycycline hydrochloride 30 µg, nalidixic acid 30 µg, norfloxacin 10 µg, streptomycin 10 µg, sulfasomidine 300 µg, tetracycline 30 µg, erythromycin 15 µg (all from HiMedia, India). In 2002 and 2003 tiamulin 30 µg (Novartis, Switzerland) was also tested.

## RESULTS AND DISCUSSION

Our study confirmed resistance to antibiotics in *A. pleuropneumoniae* isolates. Isolates with resist-

Table 1. Percentage of resistant *A. pleuropneumoniae* isolates between 2001 and 2003

	Percentage of resistant isolates		
	2001	2002	2003
Amoxicillin	12.3	12.7	10.9
Amoxiclave	9.6	9.1	10.9
Cephalotine	1.4	7.3	9.1
Tetracycline	11	36.4	81.8
Doxycycline	9.6	34.6	61.8
Nalidixic acid	2.7	14.5	45.5
Norfloxacin	0	7.3	34.6
Erytromycin	98.6	95.5	96.4
Streptomycin	78.1	82.7	76.4
Tiamulin	0	72.7	72.7
Cotrimoxazol	4.1	10	7.3
Sulfasomidine	43.8	47.3	29.1

ance against one or more commonly used antibiotics were found. The results are shown in Table 1. The highest resistance to antibiotics was observed in erythromycin, streptomycin and tiamulin and, on the other hand, resistance to amoxicillin, amoxicillin-clavulanic acid, cephalotine and cotrimoxazol was the least frequent. Resistance to tetracycline, doxycycline, nalidixic acid and norfloxacin sharply increased in the last year although a relative low resistance was observed in 2001. Interestingly, resistance against sulfasomidine decreased almost to one half in 2003 compared with previous years. To our knowledge, this trend was described only in the Czech Republic so far. It is probably caused by decrease in usage of classic sulfonamides in investigated herds for treatment. Differences between the percentages of strains resistant to amoxicillin, amoxicillin-clavulanic acid, cotrimoxazol, erythromycin, streptomycin and tiamulin detected in particular years are small, although the percentages of strains resistant to amoxicillin, streptomycin and cotrimoxazol slightly decreased in 2003 in comparison with the year 2002 (Figure 1).

Majority of strains were resistant to the group of antibiotics that had been used most frequently in a particular herd for a long time. This is cause of this antibiotics resistance increase, particularly for tetracyclines. They are a broad-spectrum agents, which exhibit their activity against a wide range of bacteria. Therefore, they have been extensively used in the therapy of infections, for prophylactic purposes and for growth promotions in animals.

A various genes are responsible for origin antibiotic resistances in bacterial genera, including strains, belonging to pathogenic as well as nonpathogenic species. Nonpathogenic bacteria could act as reservoir of resistance determinants, which can be disseminated by horizontal transfer into pathogens (Michalova et al., 2004).

In the monitored period all isolates were serotyped by coagglutination test. Two isolates were identified as serotype 1; 52 isolates as serotype 2; one isolate as serotype 7; two isolates as serotype 8; 81 isolates as serotype 9; three isolates as serotype 11; one isolate as serotype 12, and 96 isolates showed cross reaction between serotypes 9 and 11. Moreover, no relationship between resistance to specific antibiotics and serotypes of *A. pleuropneumoniae* was observed (Table 2). Consistently with our results Salmon et al. (1995), Kim et al. (2001) and Fales et al. (1989) did not find relationship between antibiogram and serotypes. However, some authors (Asawa et al., 1995; Nadeau et al., 1988; Yoshimura et al., 2002) detected different numbers of resistant isolates within specific serotypes of *A. pleuropneumoniae*.

Another trend was observed – besides the isolates resistant to individual antibiotics, multidrug resistant isolates were also recorded. We obtained isolates resistant to amoxicillin, amoxiclave, tetracycline, doxycycline, erythromycin, streptomycin, and sulfonamides (two strains in 2001, seven strains in 2002, eight strains in 2003). This indicates increasing trend in prevalence of multi-

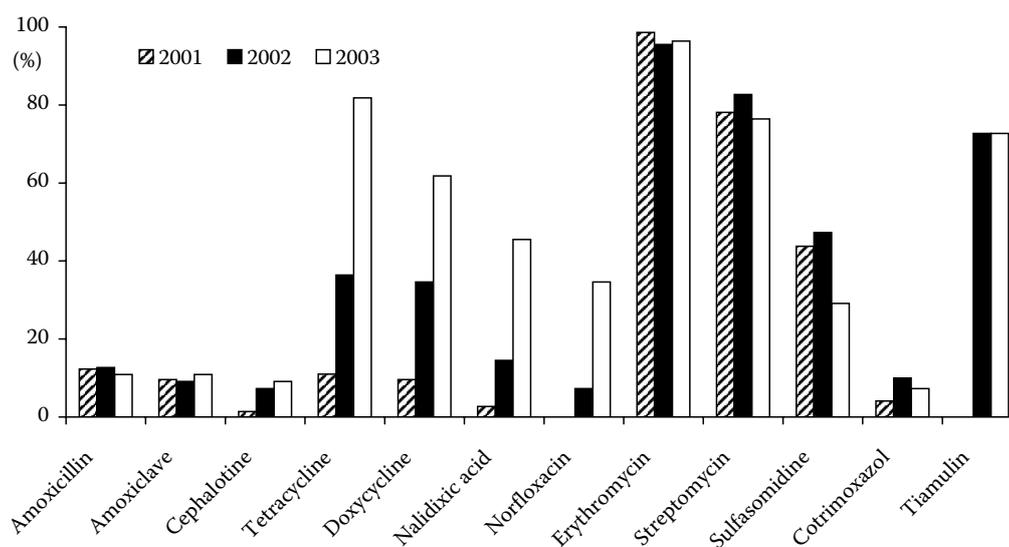


Figure 1. Percentages of resistant strains of *A. pleuropneumoniae* detected between 2001 and 2003

Table 2. Results of serotyping of *A. pleuropneumoniae* isolates between 2001 and 2003

Serotype identification	Isolate No.
2	52
7	1
8	2
9	81
11	3
12	1
9/11*	98

\*isolates showed cross reaction between serotypes 9 and 11

resistance (Chang et al., 2002b; Yoshimura et al., 2002).

When compared with our previous study (Satran and Nedbalcova, 2002), we obtain quite similar results, which generally correspond with results testing of resistances to antibiotics in isolates from Slovakia (Holko et al., 2004). However, comparison of our data with the published results of testing antibiotic resistance of *A. pleuropneumoniae* isolates obtained in many countries of the world showed differences in some cases. It followed from the present study that *A. pleuropneumoniae* isolates were not usually resistant to penicillin-type antibiotics, cephalosporins, quinolones and potentiated sulphonamids; a high number of strains were resistant to macrolides, aminoglycosides and diterpens which is in accord with majority of the published studies (Nadeau et al., 1988; Aarestrup and Jensen, 1999; Kim et al., 2001; Satran and Nedbalcova, 2002; Yoshimura et al., 2002). Moreover, Aarestrup and Jensen (1999) detected that *A. pleuropneumoniae* isolates in Denmark also were not resistant to diterpens, Yoshimura et al. (2002) in Japan registered a low resistance of the isolates to aminoglycosides and diterpens. The results concerning numbers of resistant isolates of *A. pleuropneumoniae* to tetracyclines obtained in many countries of the world showed most differences. A high number of *A. pleuropneumoniae* isolates were not resistant to tetracyclines all the time in Taiwan (Chang et al., 2002a) and Japan (Yoshimura et al., 2002). On the contrary, a high percentage of *A. pleuropneumoniae* isolates resistant to tetracyclines have been already described in previous years in USA, Canada, Denmark (Salmon et al., 1995; Nadeau et al., 1988) and Korea (Kim et al., 2001).

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