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Short communication

Changes in antimicrobial susceptibility of *Actinobacillus pleuropneumoniae* isolated from pigs in Spain during the last decade

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Abstract

A total of 229 Spanish *Actinobacillus pleuropneumoniae* isolates recovered from diseased pigs with pleuropneumonia from 1997 to 2004 was tested for their susceptibility to 11 antimicrobials in a broth microdilution method. All the isolates were susceptible to florfenicol and most of them to cephalothin; however, a high rate of resistance was observed to tetracycline. A bimodal or multimodal distribution of isolates over the MIC range were observed for penicillins, tetracycline, trimethoprim, sulfisoxazole and nalidixic acid, suggesting the development of acquired resistance. Eight resistance patterns were established, and 21.1% of the isolates were resistant to at least two antimicrobials. In addition, a considerable increase in the resistance to tetracyclines was observed during the last decade in Spain, when compared with other *A. pleuropneumoniae* strains isolated during 1987–1988 (Gutiérrez, C.B., Píriz, S., Vadillo, S., Rodríguez Ferri, E.F., 1993. In vitro susceptibility of *Actinobacillus pleuropneumoniae* strains to 42 antimicrobial agents. Am. J. Vet. Res. 54, 546–550); this finding was also observed for gentamicin in minor percentage.

Keywords: *Actinobacillus pleuropneumoniae*; Pleuropneumonia; Antimicrobial susceptibility; Clinical isolates; Pig; Spain

1. Introduction

Actinobacillus pleuropneumoniae 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 highly contagious and often fatal, resulting in considerable economic losses for pig producers (Sebunya and Saunders, 1983). Based on NAD requirements, *A. pleuropneumoniae* has been traditionally divided into biovar 1 strains, which are NAD dependent, and biovar 2 strains, which are NAD

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independent; however, an integration of biovars 1 and 2 in a single biovar has been proposed (Nielsen et al., 1997). To date, 15 serotypes of *A. pleuropneumoniae* have been reported (Blackall et al., 2002). Although all serotypes are potentially pathogenic, they vary in virulence and their prevalence is related to the geographic region (Sebunya and Saunders, 1983). In Spain, the most prevalent serotypes are 2, 4 and 7, whereas serotypes 1, 3, 5, 6 and 8–12 have been scarcely isolated (Gutiérrez et al., 1995).

Although vaccination and control programmes have been described, antibiotic therapy continues to be necessary for the control of pleuropneumonia outbreaks. Correct use of antimicrobial agents for treatment of infections with *A. pleuropneumoniae* requires knowledge of the susceptibility of the infecting strain to antimicrobial agents, because differences in the resistance patterns have been observed between different countries, serotypes and over-time (Vaillancourt et al., 1988; Kawahara et al., 1989; Asawa et al., 1995; Chang et al., 2002b; Yoshimura et al., 2002). Thus, the purposes of the present work were to determine the antimicrobial susceptibility of a large collection of *A. pleuropneumoniae* strains isolated in Spain during 1997–2004 and to compare it with that obtained approximately a decade ago (Gutiérrez et al., 1993).

2. Materials and methods

2.1. Strains

A total of 229 *A. pleuropneumoniae* isolates, which were recovered from 1997 to 2004 from the lungs of diseased pigs from herds located in central and northwest Spain, were included in this study. The bacteria were isolated on chocolate agar supplemented with PolyVitex (Biomérieux, France) and biochemically identified according to standard procedures (Kilian and Biberstein, 1985). The isolates were serotyped by indirect haemagglutination as previously described (Mittal et al., 1983).

2.2. Antimicrobial susceptibilities

The antimicrobial susceptibilities of the isolates were determined by a microdilution method using commercially prepared, dehydrated 96-well microtitre MIC panels (VAV5 and CMP1ASPV, Sensititre; Trek Diagnostic Systems Inc., England). The antimicrobial agents used and their respective dilution ranges were as follows: penicillin (PEN), 0.12–64 μg/ml; amoxicillin (AMOX), 0.06–32 μg/ml; cephalothin (CEP), 0.5–32 μg/ml; tetracycline (TET), 0.25–128 μg/ml; streptomycin (STR), 1–128 μg/ml; gentamicin (GEN), 0.06–8 μg/ml; erythromycin (ERY), 0.03–4 μg/ml; trimethoprim (TMP), 1–64 μg/ml; nalidixic acid (NAL), 1–16 μg/ml; sulfisoxazole (FIS), 32–512 μg/ml; florfenicol (FFN), 0.12–128 μg/ml.

Performance and evaluation of the MIC determinations followed the recommendations of the NCCLS (2004). The MIC was considered to correspond to the first dilution at which no bacterial strain growth was detectable. Ranges of susceptibility were recorded along with the MIC that inhibited 50% (MIC_{50}) and 90% (MIC_{90}) of the isolates. The breakpoints used for CEP, TET, GEN, FIS and FFN were those recommended by the NCCLS (2004). For the remaining antimicrobials, the distribution of strains over the MIC range was considered. The following control strains were included: *Escherichia coli* ATCC 25922 and *A. pleuropneumoniae* ATCC 27090.

3. Results and discussion

Serotypes 2 (41.0%) and 4 (40.2%) of *A. pleuropneumoniae* were the most prevalent, followed by serotypes 6 (6.6%) and 7 (5.7%). Serotypes 1, 5, 8 and 11 were isolated in proportions lower than 3%. The results of the susceptibility testing of the 229 clinical isolates as distribution of the MICs values, MIC_{50}, MIC_{90}, and the percentage of resistant strains (when breakpoint is available) are shown in Table 1. The expected MICs values (NCCLS, 2004) for the control strains were observed (*Escherichia coli* ATCC 25922: TET, 0.5 μg/ml; GEN, 0.5 μg/ml; CEP, 4 μg/ml; FIS, <32 μg/ml; FFN, 2 μg/ml; *A. pleuropneumoniae* ATCC 27090: PEN, 0.12 μg/ml; TET, 0.5 μg/ml; GEN, 8 μg/ml; FFN, 0.25 μg/ml).

The two penicillins tested were not interpreted because of the absence of either proposed breakpoints; nevertheless, these antimicrobials showed multimodal distributions indicating a supposed resistance mechanism. In addition, penicillin and amoxicillin had MICs