Antimicrobial Susceptibility of Actinobacillus pleuropneumoniae Isolated from Pigs in Korea Using New Standardized Procedures

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ABSTRACT. The in vitro susceptibilities of 76 isolates of Actinobacillus pleuropneumoniae collected from pigs with pleuropneumonia were tested with 12 commonly used antimicrobial drugs by an agar dilution minimal inhibitory concentration procedure according to National Committee for Clinical Laboratory Standards (NCCLS) guidelines. Field isolates had low MICs for ceftiofur, danofloxacin and penicillin. No correlation of antimicrobial resistance was related to serotype.

KEY WORDS: Actinobacillus pleuropneumoniae, antimicrobial susceptibility, NCCLS, swine.

Actinobacillus pleuropneumoniae is the causative agent of pleuropneumonia which is one of the most important respiratory diseases in pigs worldwide. To date, at least twelve A. pleuropneumoniae serotypes (1 to 12) have been described with serotypes 1 and 5 being further subdivided into subtypes A and B [3, 7]. Current bacterins provide only serotype specific protection. Differences in antimicrobial susceptibility of A. pleuropneumoniae isolates from country to country were detected for antimicrobial agents when comparing minimal inhibitory concentration (MIC) data from one country with those from another [1, 2, 5, 10, 11]. For pleuropneumonia treatment to be effective, it is important to know the antimicrobial susceptibility of A. pleuropneumoniae isolates that are isolated in each country. The objective of this study was to determine the antimicrobial susceptibility of A. pleuropneumoniae isolated from pigs in Korea using new standardized procedures for the antimicrobial susceptibility test for animal pathogens issued by the National Committee for Clinical Laboratory Standards (NCCLS) [6].

Between 1995 and 1998, 76 isolates of A. pleuropneumoniae were isolated from 115 growing and finishing pigs with pleuropneumonia submitted from across Korea to the Department of Veterinary Pathology, Seoul National University. All pigs were submitted alive, and immediately upon receipt they were euthanatized for necropsy. The lung specimens were collected immediately for bacteriological culture on 5% sheep blood agar.

The identification of isolates as A. pleuropneumoniae was based on Gram-staining, positive hemolysis on 5% sheep blood agar, a positive Christie-Atkins-Munch-Petersen (CAMP) reaction, a requirement and detection of nicotinamide adenine dinucleotide, urease production, and xylose- and mannose-fermentation. The CAMP reaction was determined on 5% sheep blood agar, with a beta-hemolys producing Staphylococcus intermedius strain. Five isolates of A. pleuropneumoniae were isolated in 1995; 15 were isolated in 1996; 26 were isolated in 1997; and 30 were isolated in 1998. The serotypes of the 76 isolates of A. pleuropneumoniae tested in this study have been published: 46 were serotype 2, 20 were serotype 5, and 10 were serotype 6 [4].

The following antimicrobial agents and combinations of antimicrobial agents were tested: ampicillin, amoxicillin, danofloxacin, trimethoprim-sulfamethoxazole (19:1), tiamulin, tylosin, tetracycline, penicillin, spectinomycin, lincomycin, gentamicin, and ceftiofur. The dilution ranges used for these antimicrobial agents are shown in Table 1. Susceptibility testing was performed in duplicate on each isolate using standardized methods to determine the agar dilution MIC using supplemented Mueller-Hinton agar (1% bovine hemin, 1% IsovitaleX) as described previously by the NCCLS [6]. Preparation of agars, dilution, and inoculation were performed by the same technician for consistency. All plates were set up and read by one technician. The MIC of antimicrobial agents was interpreted and reported according to NCCLS guidelines. Ranges of susceptibility were noted along with the MIC that inhibited 50% (MIC50) and 90% (MIC90) of the isolates [6, 9]. Three control strains (Escherichia coli ATCC 25922, Enterococcus faecalis ATCC 29212, and A. pleuropneumoniae ATCC 27090) were used to test each set of plates.

The cumulative percentages of susceptibility (MIC50 and MIC90) of A. pleuropneumoniae are presented in Table 1. Most of the isolates were susceptible to low concentrations (MIC50) of danofloxacin (2 µg/ml), penicillin (1 µg/ml) and ceftiofur (1 µg/ml). The patterns of antimicrobial resistance among the serotypes are summarized in Table 2. No correlation of antimicrobial resistance was related to serotype. The MIC values of three control strains for the 12 antibiotics tested were within acceptable ranges on the basis of NCCLS guidelines, indicating the accuracy of the agar minimal
inhibitory concentration procedure [6].

MIC determinations are normally regarded as most reliable for susceptibility testing of bacteria although in vitro susceptibility does not guarantee clinical efficiency of the antimicrobial agent tested. Our isolates had low MICs for danofloxacin, penicillin, and ceftiofur. Penicillin had good antimicrobial activity against A. pleuropneumoniae isolates in Denmark [1]. However, in Japan and other countries a relatively high number of penicillin-resistant isolates have been reported [2, 5, 11]. In this study, the in vivo activities of the ceftiofur and danofloxacin were in agreement with those of previous reports [2, 10, 11].

The present and previous results suggest that the different serotypes of A. pleuropneumoniae display various antimicrobial resistances [2, 5]. The wide range of antibiotic resistance among the serotypes causes significant problems for controlling this disease. There is also the possibility of increasing resistance to currently available antimicrobial drugs for the treatment of pleuropneumonia. Serotype 2 strains showed no increase in their antimicrobial resistance when compared with MIC data from other serotypes [12]. Since serotype 2 is predominant in Korea [4], ceftiofur, danofloxacin, and penicillin should be effective in the prevention and treatment of porcine pleuropneumonia in Korea.

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REFERENCES