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# Serovar and Antimicrobial Resistance Profiles of *Actinobacillus pleuropneumoniae* Isolated in Japan from 2006 to 2011

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## Abstract

The aim of this study was to provide data on the prevalence of serovars of 48 strains of *Actinobacillus pleuropneumoniae* isolated in Japan from 2006 to 2011, and their antimicrobial resistance profiles (ARPs) by using the disc diffusion method. Of the 48 strains, 10.4% were serovar 1, 60.4% were serovar 2, 14.6% were serovar 5, and 2.1%, 2.1%, 2.1%, and 8.3% were serovars 6, 7, 12, and 15, respectively. We found that 25.0%, 12.5%, 39.6%, 37.5%, 18.8%, and 0% of the strains were resistant to penicillin G, ampicillin, oxytetracycline (OTC), sulfisoxazole, chloramphenicol, and norfloxacin, respectively. The ARPs of each serovar differed, with serovar 1 strains exhibiting multi-antimicrobial resistance to more than three antimicrobials. In contrast, 44.8% of the serovar 2 strains exhibited no resistance, and 34.5% were resistant to only one antimicrobial. All serovar 1, 5, and 15 strains were resistant to OTC, whereas serovar 2 exhibited lower resistance to OTC (6.9%). No significant increase in the prevalence of resistance to these antimicrobials was observed during the last half decade in Japan when compared with published data on the prevalence of resistant strains of *A. pleuropneumoniae* collected from 1989 to 2005. Regarding the prevalence of serovars, there has been a recent increase in the isolated cases of serovar 15.

**Discipline**: Animal health
**Additional key words**: swine, pleuropneumonia

## Introduction

*Actinobacillus pleuropneumoniae* is a causative agent of swine pleuropneumonia, a severe respiratory disease that causes serious economic problems in the pig industry worldwide (Gottschalk 2012). In acute cases, *A. pleuropneumoniae* induces severe and rapidly fatal fibrinohemorrhagic and necrotizing pleuropneumonia, and is one of the most important bacterial pulmonary pathogens frequently found in pigs worldwide including Japan (Gottschalk 2012). To date, 15 serovars of *A. pleuropneumoniae* have been identified (Blackall et al. 2002, Gottschalk 2012), with the most prevalent serovars varying by country (Gottschalk 2012). In Japan, serovar 2 is the most predominant, followed by serovars 1 and 5 (Asawa et al. 1995, Fukuyasu et al. 1996, Morioka et al. 2006, 2008, Yoshimura et al. 2002).

A variety of antimicrobials have been used to treat swine pleuropneumonia, but the appearance of antimicrobial-resistant *A. pleuropneumoniae* has been reported worldwide (Gottschalk 2012). The aim of the present study was to provide data on the prevalence of serovars and their antibiotic resistance profiles (ARPs) of *A. pleuropneumoniae* isolated in Japan from 2006 to 2011, as no nationwide trends of antimicrobial resistance of *A. pleuropneumoniae* strains isolated in Japan after 2006 have been reported since the report by Morioka et al. (2008).

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## Materials and methods

Forty-eight strains of *A. pleuropneumoniae* (isolated from 2006 to 2011 from 47 farms in 11 prefectures in Japan) were sent to our diagnostic laboratory at the National Institute of Animal Health for serotyping. Of the 48 strains, 46 were isolated from different farms. The other two strains of serovar 1 (isolated in 2006 and 2008 at farm A) were subjected to antimicrobial resistance tests to examine ARP changes. *A. pleuropneumoniae* strain S1421 (= strain ATCC27090) was used as a quality control in each test.

*A. pleuropneumoniae* was grown on tryptic soy agar (Difco, Sparks, MD) supplemented with 5% horse blood and 100 µg/mL β-nicotinamide dinucleotide at 37°C with 5% $CO_2$, and after being suspended in phosphate-buffered saline containing 20% glycerol, it was stored at -80°C until use.

Serotyping was carried out with rabbit antisera prepared against serovar reference strains by conducting slide agglutination tests, and when necessary, by immunodiffusion tests (Mittal et al. 1992), along with polymerase chain reaction for genes involved in capsular polysaccharide biosynthesis (Angen et al. 2008, Ito 2010, Zhou et al. 2008).

For the antimicrobial resistance tests, we cultured bacterial strains on chocolate agar at 37°C with 5% $CO_2$ as per the Clinical and Laboratory Standards Institute (CLSI) standards and recommendations (CLSI 2013). The resistance of these strains to the following six antimicrobial agents was tested by using the disc diffusion method (Sensi-Disc, Japan Becton Dickinson, Tokyo), as per the CLSI (2013) standards and recommendations: penicillin G (PCG) 10IU, ampicillin (ABPC) 10 µg, oxytetracycline (OTC) 30IU, sulfonamide (sulfisoxazole (SIX)) 250 µg, chloramphenicol (CP) 30 µg, and norfloxacin (NFLX) 10 µg. Each strain was classified as being resistant, susceptible, or intermediate to the antimicrobial agents tested, in accordance with breakpoints proposed by the Comité de l'Antibiogramme de la Société Française Microbiologie (CASFM 2010) as previously described (Vanni et al. 2012), except for SIX and NFLX, for which the criteria of the National Committee for Clinical Laboratory Standards (NCCLS; later renamed CLSI) were applied (NCCLS 2002).

## Results and discussion

Of the 48 strains, 5 (10.4%) were serovar 1, 29 (60.4%) were serovar 2, 7 (14.6%) were serovar 5, 1 (2.1%) was serovar 6, 1 (2.1%) was serovar 7, 1 (2.1%) was serovar 12, and 4 (8.3%) were serovar 15, showing that the most prevalent serovars were 2, 1 and 5, as found in several previous reports (Asawa et al. 1995, Fukuyasu et al. 1996, Morioka et al. 2006, 2008, Yoshimura et al. 2002). Despite the low number of serovar 15 strains isolated in the present study (n=4), serovar 15 has been the fourth most prevalent serovar of strains sent to our diagnostic laboratory for serotyping (Table 1). This prevalence is particularly noteworthy because serovar 15 was not isolated prior to 2008. The rapid rise in the reported cases of serovar 15 strains suggests that this serovar may

**Table 1. Distribution of antimicrobial resistant strains of *Actinobacillus pleuropneumoniae* isolated in Japan from 2006 to 2011**

| Serovar | No. of strains (%) | PCG | ABPC | OTC | SIX | CP | NFLX |
|---|---|---|---|---|---|---|---|
| 1 | 5 (10.4) | 3 (60) | 3 (60) | 5 (100) | 4 (80) | 4 (80) | 0 (0) |
| 2 | 29 (60.4) | 4 (13.8) | 1 (3.4) | 2 (6.9) | 11 (37.9) | 5 (17.2) | 0 (0) |
| 5 | 7 (14.6) | 3 (42.9) | 0 (0) | 7 (100) | 1 (14.3) | 0 (0) | 0 (0) |
| 6 | 1 (2.1) | 0 (0) | 0 (0) | 1 (100) | 0 (0) | 0 (0) | 0 (0) |
| 7 | 1 (2.1) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| 12 | 1 (2.1) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| 15 | 4 (8.3) | 2 (50) | 2 (50) | 4 (100) | 2 (50) | 0 (0) | 0 (0) |
| Total | 48 (100) | 12 (25) | 6 (12.5) | 19 (39.6) | 18 (37.5) | 9 (18.8) | 0 (0) |

have become important relatively recently in Japan, as it has in Australia (Blackall et al. 2002).

Table 1 lists the prevalence of resistant strains of the 48 *A. pleuropneumoniae* strains tested; Table 2 lists the ARPs of the strains where 31.3% (n=15) showed no resistance to any of the six antimicrobials used, and 33.3% (n=16) were resistant to only one antimicrobial, indicating that almost all of the antimicrobials used are effective for swine pleuropneumonia treatment. All of the tested strains were susceptible to NFLX, one of the fluoroquinolones recommended for use as a last-resort antimicrobial (Burch 2008).

The strains tested in the present study (Table 1) showed a low prevalence of strains resistant to ABPC (12.5%), which is recommended as the first-choice antimicrobial for swine pleuropneumonia treatment (Burch 2008). A similar low prevalence of ABPC-resistant strains (2.0%-12.4%) in *A. pleuropneumoniae* was also observed from 1989 to 2005 (Asawa et al. 1995, Fukuyasu et al. 1996, Morioka et al. 2008).

Of the 48 strains tested, 12 PCG-resistant strains were identified, half of which were ABPC-resistant and the remaining six were ABPC-susceptible. The underlying reason for the difference in resistance to the two β-lactams (PCG and ABPC) remains to be elucidated. A low prevalence of PCG-resistant strains (4.4%-13.6%) in *A. pleuropneumoniae* was observed from 1989 to 2000 (Asawa et al. 1995, Fukuyasu et al. 1996, Morioka et al. 2006, Yoshimura et al. 2002). However, the present study showed a slightly increased prevalence of PCG resistance (25.0%) in strains isolated from 2006 to 2011.

The two *A. pleuropneumoniae* serovar 1 strains isolated in 2006 and 2008 at farm A exhibited different ARPs. Specifically, the strain isolated in 2006 was resistant to OTC-SIX-CP, but the 2008 strain was resistant to PCG-ABPC, as well as to OTC-SIX-CP. The ARPs of three other strains isolated from different pigs at farm A in 2006 were further examined in addition to the 48 strains listed in Tables 1 and 2, in order to rule out the possibility that PCG-ABPC-resistant strains may have been already present at farm A in 2006. Consequently, all three strains were found to be resistant to OTC-SIX-CP, but sensitive to PCG-ABPC (data not included in Tables 1 and 2). These results indicate that PCG-ABPC-susceptible clones may have acquired PCG-ABPC resistance or may have been replaced by PCG-ABPC-resistant clones at farm A, resulting in the ineffectiveness of PCG-ABPC for treatment. This observation demonstrates the chronological

**Table 2. Antimicrobial resistance profiles (ARPs) of 48 *Actinobacillus pleuropneumoniae* strains tested**

| No. of antimicrobials to which the strains showed resistance | ARPs | No. of strains (%) | Serovar | Year isolated | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 |
| 5 | PCG-ABPC-OTC-SIX-CP | 2 (4.2%) | 1 | | | 2 | | | |
| 4 | PCG-ABPC-OTC-SIX | 2 (4.2%) | 15 | | | | 1 | 1 | |
| 3 | OTC-SIX-CP | 4 (8.3%) | 1 | 1 | | | 1 | | |
| | PCG-ABPC-OTC | | 1 | | | | | 1 | |
| | PCG-ABPC-SIX | | 2 | | | | 1 | | |
| 2 | OTC-SIX | 9 (18.8%) | 2 | | | | | 1 | |
| | | | 5 | | | | | | 1 |
| | PCG-OTC | | 5 | | | | | 3 | |
| | PCG-CP | | 2 | | | | 2 | | |
| | SIX-CP | | 2 | | | 1 | 1 | | |
| 1 | SIX | 16 (33.3%) | 2 | | | 3 | 4 | | |
| | OTC | | 5 | 1 | | 2 | | | |
| | | | 15 | | | | | 2 | |
| | | | 2 | | | | | 1 | |
| | | | 6 | | | | 1 | | |
| | PCG | | 2 | | | | | 1 | |
| | CP | | 2 | | 1 | | | | |
| 0 | No anitimicrobial resistance | 15 (31.3%) | 2 | | 3 | 2 | 2 | 6 | |
| | | | 7 | | | | | 1 | |
| | | | 12 | | | | | | 1 |

change in the ARPs of *A. pleuropneumoniae* isolated at the same farm, also highlighting the importance of constantly monitoring the ARPs of organisms isolated at the same farm.

The *A. pleuropneumoniae* strains tested in the present study showed a moderately high prevalence of resistance to OTC (39.6%) and SIX (37.5%) (Table 1). SIX is one of the components of co-trimoxazole (a sulfonamide and trimethoprim combination). Tetracyclines including OTC and co-trimoxazole are recommended as second-choice antimicrobials for swine pleuropneumonia treatment (Burch 2008). A moderately high prevalence of OTC resistance (28%-45%) in *A. pleuropneumoniae* had been observed from 1989 to 2005 (Fukuyasu et al. 1996, Morioka et al. 2006, 2008, Yoshimura et al. 2002). The present study showed a moderately high prevalence of sulfonamide resistance (SIX resistance: 37.5%) in strains isolated from 2006 to 2011. However, a high prevalence of strains resistant to sulfonamides was reported in strains isolated from 1989 to 1995 (Asawa et al. 1995, Fukuyasu et al. 1996). The sulfonamides (sulfamonomethoxine and sulfadimethoxine) were used in these studies instead of SIX, and the prevalence of strains resistant to both sulfonamides was 82.1 and 75.6%, respectively. The underlying reason for the significant reduction of sulfonamide resistance in the recent isolates of *A. pleuropneumoniae* is unknown.

The presence of CP-resistant strains in Japan has been previously reported, although CP use in food animals has been banned in Japan since 1998 (Morioka et al. 2006). A moderately high prevalence of CP resistance (20.6%-43.3%) in *A. pleuropneumoniae* has been observed in strains isolated from 1989 to 2000 (Asawa et al. 1995, Fukuyasu et al. 1996, Morioka et al. 2006, Yoshimura et al. 2002). In the present study, CP-resistant strains were isolated from 2006 to 2009 (9 of 29 strains tested; 31.0%), but no CP-resistant strain was isolated after 2010 (0 of 19 strains tested; 0%) (Table 2). Given the unknown reason for such a drastic decrease in the prevalence of CP-resistant strains after 2010, continuous monitoring for the prevalence of strains resistant to CP is needed.

The present study confirmed that the prevalence of strains resistant to antimicrobials differed by each antimicrobial (Table 1). To elucidate the reason for such differences, the relationship between the prevalence of strains resistant to each antimicrobial and the average sales amount (ASA) of these antimicrobials from 2006 to 2011 (National Veterinary Assay Laboratory 2015) were examined. Although use of the average consumption amount (ACA) of each antimicrobial would have been ideal for this analysis, statistical data on the ACA were unavailable. Instead, the ASA of each antimicrobial that is related to the ACA and available from the National Veterinary Assay Laboratory was used for analysis. The ASAs of tetracyclines, sulfonamides, penicillins, analogues and derivatives of CP, and fluoroquinolones were 253.3, 86.3, 33.1, 14.3, and 2.0 tons, respectively. The prevalence of strains resistant to OTC, SIX, PCG/ABPC, CP, and NFLX were 39.6%, 37.5%, 25.0/12.5%, 18.8%, and 0%, respectively (Table 1). These data show that the ASA (and likely the ACA) of antimicrobials is one of the factors associated with the prevalence of strains resistant to each antimicrobial, although a variety of other factors, such as differing mechanisms of antimicrobial resistance acquisition/transfer (Schwarz 2008) and co-resistance to antimicrobials that were not used (Ozawa et al. 2012), may influence the emergence and spread of strains resistant to antimicrobials. This finding stresses the importance of the prudential use of antimicrobials in swine production.

Different serovars of *A. pleuropneumoniae* have shown varying antimicrobial resistance tendencies (Asawa et al. 1995, Morioka et al. 2006, 2008, Oze et al. 2011, Yoshimura et al. 2002). In the present investigation, serovar 1 strains exhibited multi-antimicrobial resistance, showing resistance to three to five antimicrobials (Table 2). It is interesting to note that the serovar 1 strains showed the highest rates of resistance to antimicrobials other than NLFX (Table 1), whereas 44.8% of the serovar 2 strains did not show resistance to any of the six antimicrobials tested, and 34.5% were resistant to only one antimicrobial.

The prevalence of strains resistant to ABPC was high in the serovar 1 (60.0%) and 15 (50.0%) strains. In contrast, low or no resistance to ABPC was observed in the serovar 2 (3.4%) and serovar 5 (0%) strains (Table 1). The prevalence of strains resistant to OTC was high in the serovar 1 (100%), 5 (100%), and 15 (100%) strains. In contrast, low resistance to OTC was observed in the serovar 2 (6.9%) strains (Table 1). A similar tendency of OTC resistance in serovars 1, 2, and 5 was previously observed (Morioka et al. 2006, 2008, Oze et al. 2011, Yoshimura et al. 2002). The prevalence of strains resistant to SIX was high in serovars 1 (80%) and 15 (50%). Moderately high resistance to SIX was observed in serovar 2 (37.9%), and moderately low resistance to SIX was observed in the serovar 5 (14.3%) strains (Table 1). The prevalence of strains resistant to CP was high in the serovar 1 (80%) stains, whereas low or no resistance to CP was observed in the serovar 2 (17.2%), 5 (0%), and 15 (0%) strains (Table 1).

In conclusion, the 48 *A. pleuropneumoniae* strains collected in Japan from 2006 to 2011 have maintained a relatively low prevalence of resistance to penicillin (except for serovar 1) and no resistance to fluoroquinolones—agents recommended for use as first-choice and last-resort antimicrobials for swine pleuropneumoia treatment, respectively (Burch 2008). No significant increase in resistance to the antimicrobials tested in this study was observed during the last decade in Japan when compared with published data on the prevalence of resistant strains of *A. pleuropneumoniae* collected from 1989 to 2005 (Asawa et al. 1995, Fukuyasu et al. 1996, Morioka et al. 2006, 2008, Yoshimura et al. 2002). However, it is interesting to note that the prevalence of serovar 15 increased in the strains used in the present study.


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