Molecular serotyping and antimicrobial resistance profiles of *Actinobacillus pleuropneumoniae* isolated from pigs in South Korea

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**ABSTRACT**

**Background:** *Actinobacillus pleuropneumoniae* (APP) causes porcine pleuropneumonia (PP).

**Objective:** Serotypes and antimicrobial resistance patterns in APP isolates from pigs in Korea were examined.

**Methods:** Sixty-five APP isolates were genetically serotyped using standard and multiplex PCR (polymerase chain reaction). Antimicrobial susceptibilities were tested using the standardized disk-agar method. PCR was used to detect β-lactam, gentamicin and tetracycline-resistance genes. The random amplified polymorphic DNA (RAPD) patterns were determined by PCR.

**Results:** Korean pigs predominantly carried APP serotypes 1 and 5. Among 65 isolates, one isolate was sensitive to all 12 antimicrobials tested in this study. Sixty-two isolates was resistant to tetracycline and 53 isolates carried one or five genes including tet(B), tet(A), tet(H), tet(M)/tet (O), tet(C), tet(G) and/or tet(L)-1 markers. Among 64 strains, 9% and 26.6% were resistance to 10 and three or more antimicrobials, respectively. Thirteen different antimicrobial resistance patterns were observed and RAPD analysis revealed a separation of the isolates into two clusters: cluster II (6 strains resistant to 10 antimicrobials) and cluster I (the other 59 strains).

**Conclusion:** Results show that APP serotypes 1 and 5 are the most common in Korea, and multidrug resistant strains are prevalent. RAPD analysis demonstrated that six isolates resistant to 10 antimicrobials belonged to the same cluster.

1. Introduction

*Actinobacillus pleuropneumoniae* causes porcine pleuropneumonia (PP), a highly contagious endemic disease that results in significant global livestock loss. Symptoms of the disease range broadly from pre-acute to chronic, and clinical signs include fever, dyspnea, anorexia, vomiting, coughing, frothy hemorrhage, diarrhea, and cyanosis (Bossé et al. 2002). In the acute stage, the disease is characterized by hemorrhagic necrotizing pneumonia and fibrous pleuritis, while the chronic stage involves localized lung lesions and adhesive pleuritis (Haesebrouck et al. 2004). This disease affects pigs of all ages and has a major impact on the economics, ecology, and animal welfare in the pig-rearing industry (Bossé et al. 2002). It is essential that an appropriate therapy be applied as accurately and quickly as possible.

*A. pleuropneumoniae* is divided into 15 serotypes based on the antigenic properties of capsular polysaccharides and cell wall lipopolysaccharides (Chiers et al. 2010). Each serotype produces different virulence factors leading to a variety of clinical signs. Vaccines based on killed whole-cell *A. pleuropneumoniae* provide protection only against the serotype present in the vaccine; no *A. pleuropneumoniae* serotype provides a cross-immune response for another serotype (Ramjee et al. 2008). A number of serological assays have been developed for serotyping *A. pleuropneumoniae*. While there are rapid methods such as slide agglutination, specificity is compromised in some cases due to cross-reactivity (Mittal 1990). Other more time-consuming methods such as immunodiffusion and indirect hemagglutination can be used for definitive serotyping (Nielsen & O’Connor 1984). A critical issue with all serological testing is the need for high titers and highly specific anti-sera, which are requirements that often limit the performance of serotyping to central reference laboratories (Turni et al. 2014).

In recent years, PCR (polymerase chain reaction) assays for detecting genes associated with capsular polysaccharides have been developed to identify *A. pleuropneumoniae* serotypes (Jessing et al. 2003; Bossé et al. 2014; Turni et al. 2014). Sequence differences in the middle region of the *A. pleuropneumoniae* outer membrane lipoprotein omlA gene divide the *A. pleuropneumoniae* serotypes into four distinct groups, and this sequence variation has been used for speculating serotypes (Gram et al. 2000; Angen et al. 2008).
Antimicrobial agents are still intensively used in pig husbandry to treat and/or prevent infectious diseases such as those caused by intestinal and respiratory tract pathogens (Arnold et al. 2004). Antibiotic selective pressure is known to affect resistance in commensal Actinobacillus strains that reside in the tonsils without causing disease (Matter et al. 2007). Previous studies have reported that different A. pleuropneumoniae serotypes displayed varying levels of antimicrobial resistance (Asawa et al. 1995; Lee et al. 2015). Such heterogeneous antimicrobial resistance among the different serotypes has caused significant problems for PP treatment. In South Korea, many antibiotics such as tetracycline, neomycin, and colistin have been used in feed additives to increase animal production and prevent disease. The link between the use of antimicrobial drugs in livestock and the emergence of antimicrobial resistance in human pathogenic bacteria has been well established (Ahmed et al. 2009). Therefore, the use of antimicrobials as feed additives to prevent the outbreak of disease in the pig industry has been banned in South Korea since July 2011. However, various antimicrobials are still being used for therapeutic purposes in South Korean pig farms. There is a need for a novel standard therapy according to serotype variation and antimicrobial resistance patterns of A. pleuropneumoniae isolates in South Korea (Yoo et al. 2014; Lee et al. 2015).

The objective of the present study was to examine the prevalence of serotype and antimicrobial resistance patterns in recently isolated A. pleuropneumoniae strains from pigs with PP in South Korea. Random amplified polymorphic DNA (RAPD) patterns using arbitrarily primed PCR (AP-PCR) were also investigated to analyze correlations with the patterns and antimicrobial resistance pattern.

2. Materials and methods

2.1. Bacterial strains

A total of 65 A. pleuropneumoniae clinical isolates, identified by conventional phenotypic methods, were collected from pigs in South Korea with PP between 2010 and 2013. The isolates were confirmed by species-specific PCR as described by Jessing et al. (2003) (Table 1). The 15 A. pleuropneumoniae serotype reference strains were kindly supplied by the National Veterinary Research and Quarantine Service (NVRQS), Anyang, Korea. Serotyping of the 15 reference strains was performed by typical serological methods using antibody agglutination tests.

2.2. Preparation of whole-cell DNA for PCR and RAPD analysis

Genomic DNAs were prepared as described previously (Angen et al. 2008).

2.3. Molecular serotyping of A. pleuropneumoniae by PCR

Primer sequences are listed in Table 1. PCR was performed using an Eppendorf Mastercycler nexus X2 thermal cycler (Eppendorf, Hauppauge, NY, USA). The conditions for PCR were previously described by Jessing et al. (2008) for serotypes 1, 7, and 12, by Jessing et al. (2003) for serotypes 2, 5, and 6, by Bossé et al. (2014) for serotypes 3 and 8, by Turni et al. (2014) for serotype 15, and by Xiao et al. (2009) for omlA amplifications. PCR serotyping of the 65 isolates was performed after PCR specificity was confirmed using the 15 reference strains.

2.4. Antimicrobial resistance testing

Antimicrobial resistance for all A. pleuropneumoniae isolates was tested using the disk-agar method standardized by the Clinical and Laboratory Standards Institute (CLSI 2008). The interpretive standards for the following antimicrobials were as follows: amikacin (AMK; S ≥ 17, R ≤ 14), ampicillin (ABPC; S ≥ 22, R ≤ 18), ceftriaxone (CTF; S ≥ 20, R ≤ 14), colistin (CL; S ≥ 11, R ≤ 8), florfenicol (FF; S ≥ 29, R ≤ 25), gentamicin (GM; S ≥ 15, R ≤ 12), kanamycin (KM; S ≥ 18, R ≤ 13), neomycin (NEO; S ≥ 17, R ≤ 12), penicillin (PCG; S ≥ 15, R ≤ 14), tetracycline (TC; S ≥ 29, R ≤ 25), amoxicillin/clavulanic acid (AMC; S ≥ 20, R ≤ 19), and trimethoprim/sulfamethoxazole (TMP/SMX; S ≥ 16, R ≤ 10) (Becton Dickinson Company, Sparks, MD, USA). A. pleuropneumoniae ATCC 27090\T was used as the control strain for antimicrobial tests.

2.5. Detection of genes associated with antimicrobial resistance

All isolates were screened for the presence of 12 genes encoding resistance to β-lactam, gentamycin and tetracycline antimicrobials. Target genes, nucleotide sequences of PCR primers, and predicted sizes of the amplified products for each PCR are outlined in Table 1.

2.6. AP-PCR

AP-PCR was performed to determine the RAPD pattern as described by Lee and Choi (2006) (Table 1). AP-PCR analyses were performed in duplicate for all isolates. RAPD patterns were analyzed visually and with computer-aided methods. Visual interpretation of banding patterns was performed according to previously reported guidelines (Lee & Choi 2006). Briefly, gels were stained with ethidium bromide and DNA band patterns were analyzed using BioNumerics v. 5.1 software (Applied Maths NV, Sint-Martens-Latem, Belgium) to determine strain relatedness. The extent of variability was determined using the Dice coefficient with a 1.0% band position tolerance. Clustering was based on
the unweighted pair group method using the arithmetic average (UPGMA).

3. Results

3.1. Molecular serotyping by standard PCR and multiplex PCR

Molecular serotyping was determined for the collection of 65 A. pleuropneumoniae isolates (Table 2). A species-specific fragment of approximately 442 bp was amplified from all A. pleuropneumoniae strains tested. Nonspecific PCR products were not observed from any of the strains when the optimized conditions for multiplex PCR assay were used. The main South Korean A. pleuropneumoniae isolate serotypes identified in this study are shown in Table 2. The species-specific fragment of approximately 442 bp was amplified from all A. pleuropneumoniae strains tested.

Table 1. PCR primers used in this study.

| Purpose of PCR and the target gene | Primer name | Sequence | Size (bp) | Reference or source |
|-----------------------------------|-------------|----------|-----------|---------------------|
| Species specific PCR              | omlA gene for serotypes 1 to 15 | HPF: AAG GTT GAT ATG TCC GCA CC | 950 | Jessing et al. 2003 |
|                                   |             | HPR: CAC GTA TGG CTT GCA A |          |                     |
| Serotype specific PCR             | cps-region of serotype 1 | Ap1F: GGG CAA GGC TCT GCT CAA AA | 754 | Jessing et al. 2008 |
|                                   |             | Ap1R: GAA AGA ACC AAG CTC TGG CAA T |          |                     |
|                                   | cps-region of serotype 2 | Ap2F: ACT ATG GCA ATC AGT CGA TCT AT | 500 | Jessing et al. 2003 |
|                                   |             | Ap2R: CCT AAT CGG AAA CGC CAT TCT G |          |                     |
|                                   | cps9D-region of serotype 3 | Ap3F: CTA TCA TGT GTC CAA AGG TCC TAC AC | 520 | Bossé et al. 2014 |
|                                   |             | Ap3R: GTT TTA GAG GGA CAA ATT GAC TGG |          |                     |
|                                   | cps-region of serotype 5 | Ap5A: TTT ATC ACT ATC ACC TGA TGA CTC G | 1100 | Jessing et al. 2003 |
|                                   |             | Ap5B: CAT TGC GGT CTT GTC ACT AA |          |                     |
|                                   | cps-region of serotype 6 | SG1J: ACG CAC TCA TTT ACA TTA G | 720 | Jessing et al. 2003 |
|                                   |             | SG1J5: AAT CGG AAG GGT TTG TGG TCT TG |          |                     |
|                                   | cps-region of serotype 7 | Ap7F: GTG GAC TGG CTT AGG CCA AA | 396 | Jessing et al. 2008 |
|                                   |             | Ap7R: GGG CTG CAG ACT GAT GTA A |          |                     |
|                                   | cps-region of serotype 8 | Ap8F: TTA GTG GGA CAA AGG GCT TTT GAA | 1106 | Bossé et al. 2014 |
|                                   |             | Ap8R: GAT TAA ACT GCG CTA GGC CAA A |          |                     |
|                                   | cps-region of serotype 12 | Ap12F: GGT TCA GGA GAC TCT TCC GAA A | 559 | Jessing et al. 2008 |
|                                   |             | Ap12R: GCT ATT GGA TGA ATC GCA CTC G |          |                     |
|                                   | cpx region of type 15 | Ap15 CF: GGG GAT CGA AAG GCT ATG G | 269 | Turni et al. 2014 |
|                                   |             | Ap15 eR: CTG CCG TAA TCG CTA CCA TTA TCC |          |                     |
|                                   | omlA gene of serotypes 1, 9, 11, and 12 | omal1-L: TTC AGG CAA ATT GTT GGG TTA C | 474 | Xiao et al. 2009 |
|                                   |             | omal1-R: TGC GGT GGA TGA TCA CCC TGC G |          |                     |
|                                   | omlA gene of serotypes 5 and 10 | omal3-L: GCA CTT CCG GAT AAT ACT TCG | 453 | Xiao et al. 2009 |
|                                   |             | omal3-R: ATC AAG TTA CAC AAG ACT CTC G |          |                     |
|                                   | omlA gene of serotypes 3, 4, 6 and 7 | omal4-L: ACA GGG ATT ATT ACT ACG CCA | 509 | Xiao et al. 2009 |
|                                   |             | omal4-R: CCA TCC TTA TCA ACT ACA ACA C |          |                     |

Table 2. Serotypes of 65 Actinobacillus pleuropneumoniae isolates using PCR.

| Serotype | No. of isolates | Rate (%) |
|----------|----------------|----------|
| 1        | 22             | 33.9     |
| 2        | 9              | 13.8     |
| 4        | 2              | 3.1      |
| 5        | 23             | 35.4     |
| 7        | 6              | 9.2      |
| 10       | 1              | 1.5      |
| 12       | 2              | 3.1      |
| Total    | 65             | 100.00   |
study were serotypes 5 (n = 23) and 1 (n = 22). Serotypes 2 (n = 9), 7 (n = 6), and 12 (n = 2) were also observed in this study. Isolates suspected as *A. pleuropneumoniae* serotypes 4 (n = 2) and 10 (n = 1) were detected in this study.

### 3.2. Antimicrobial resistance profiles

Table 3 shows the susceptibility of isolates to commonly used antimicrobials employed in this study. Only one isolate was sensitive to all 12 tested antimicrobials, and each of the remaining 64 strains was resistant to at least one antimicrobial agent tested in this study. No isolates were resistant to AMK, and 62 isolates were resistant to at least one antimicrobial. Thirteen different antimicrobial resistance patterns were observed among the isolates (Table 4).

### 3.3. Antimicrobial resistance genes

The genes associated with antimicrobial resistance were identified by PCR. Of the 62 isolates resistant to TC, 1 contained five genes, tet(A), tet(B), tet(C), tet(G), and tet(L)-1, 1 contained four genes, tet(A), tet(B), tet(C), and tet(G), 1 contained three genes, tet(A), tet(B), and tet(M)/tet(O), 3 contained both tet(A) and tet(B), 15 contained tet(A), 1 contained both tet(B) and tet(M)/tet(O), 3 contained both tet(B) and tet(H), 1 contained both tet(B) and tet(L)-1, 15 contained tet(B), 6 contained tet(H), and 6 contained tet(M)/tet(O). Nine TC resistant strains were negative for tet gene PCR. Among the 14 isolates resistant to both ABPC and PCG, five harbored *bla*~ROB-1~. Nine strains resistant to ABPC and all strains resistant to AMC were negative for the *bla*~ROB-1~ gene. Of the 12 isolates resistant to GM, 11 harbored *aadB* (*ant(2")-Ia*), and one harbored *aac*~C4~ (*aac(3)-Iva*).

### 3.4. RAPD

Molecular similarity of the 65 isolates was examined by generating RAPD patterns via AP-PCR. The RAPD isolates are shown in Figure 1. Genetic relatedness of the isolates ranged from 73.2% to 100%. The 43 distinct RAPD patterns were categorized into two main clusters. All the six antimicrobial resistance type (ART) isolates were resistant to 10 antimicrobials (Table 4) and belonged to cluster II; the other isolates belonged to cluster I.

### 4. Discussion

In this study, the serotype distribution and antimicrobial resistance to 12 antibiotic agents of *A. pleuropneumoniae* isolates collected from pigs suspected of PP in South Korea between 2010 and 2013 were investigated and the genotypes related to resistance were surveyed. The distribution of *A. pleuropneumoniae* serotypes varied by region and with time. For example, in Australia, the most prevalent serotypes are 1, 5, 7, and 15 with a recent increase in serotype 12 (Turni et al. 2014). Serotypes 2 and 9 are the most prevalent in the Czech Republic (Kucerova et al. 2011), and serotypes 2 and 4 are dominant in Spain (Gutiérrez-Martín et al. 2006). Serotypes 1 and 5 are common in North and South America, and serotype 2 is common in Europe (Bossé et al. 2014), whereas these serotypes are absent or rare in the United Kingdom (O’Neill et al. 2010). In South Korea, *A. pleuropneumoniae* serotype 2 was the most common between 1995 and 1998, while serotypes 2 and 5 have dominated since the year 2000, with an increase in the incidence of serotype 1 (Kim et al. 2001; Yoo et al. 2014). Lee et al. (2015) reported that *A. pleuropneumoniae* serotypes 1 and 5 were the most common between 2012 and 2013; similarly, we found that

| Table 4. Profile of antimicrobial resistance in *Actinobacillus pleuropneumoniae* isolates. |
|-----------------------------------------------|
| **Type** | Resistance Pattern | Isolation |
|--------|------------------|-----------|
| 1      | PCG,ABPC,AMC,NEO,GM,TMP/SMX,TC,KM,FF,CL | 6 | 9.2 |
| 2      | PCG,ABPC,AMC,TMP/SMX,TC,FF,CTF,CL | 1 | 1.5 |
| 3      | PCG,ABPC,GM,TC,KM,FF | 3 | 4.6 |
| 4      | PCG,ABPC,TC,FF | 3 | 4.6 |
| 5      | GM,TMP/SMX,TC | 2 | 3.1 |
| 6      | TMP/SMX,KM,CL | 1 | 1.5 |
| 7      | PCG,ABPC,TC | 1 | 1.5 |
| 8      | GM,TC | 1 | 1.5 |
| 9      | TC,FF | 14 | 21.5 |
| 10     | TMP/SMX,TC | 3 | 4.6 |
| 11     | TCCl | 1 | 1.5 |
| 12     | TC | 27 | 41.5 |
| 13     | CTF | 1 | 1.5 |
| Total  | 64 |           |

Notes: ABPC, ampicillin; CTF, ceftiofur; CL, colistin; FF, florfenicol; GM, gentamicin; KM, kanamycin; PCG, penicillin; TC, tetracycline; AMC, amoxicillin/clavulanic acid; TMP/SMX, trimethoprim/sulfamethoxazole.
A. pleuropneumoniae serotypes 1 and 5 were dominant in South Korean pigs between 2010 and 2013. This may be because only vaccines against serotype 2 are available in Korea, which decreased morbidity and mortality, but did not provide cross-immunity against other serotypes. It is also possible that the prevalence of serotype 1 might be due to a recent introduction from North America, where serotype 1 is predominant (Jacques 2004). Therefore, it is necessary to determine the serotype distribution in a given area so that PP can be efficiently controlled by adding newly recognized serotypes into new vaccine construction.

Antimicrobial agents have been extensively used for therapeutic and prophylactic purposes in swine rearing, and the consequent selective pressure has intensified the risk for resistant bacteria (Aarestrup et al. 2008). The movement of swine between herds or between countries is another key factor that contributes to the spread of antimicrobial-resistant isolates in animal populations (McEwen & Fedorka-Cray 2002). In the present study, the frequencies and combinations of A. pleuropneumoniae antimicrobial resistance were determined in strains isolated from pigs. Among the 65 isolates, 64 were resistant to at least one antibiotic. In addition, many (26.2%) of the isolates were resistant to three or more drugs, and six isolates were resistant to 10 of the 12 antimicrobials tested. This result shows that future options for treating PP may be complicated if multi-drug resistant A. pleuropneumoniae strains spread into pig farms of this region. There was a high resistance rate to particular antimicrobials, notably TC, FF, ABPC, PCG, and TMP/SMX in this study. Although previous reports revealed a relatively high degree of activity against A. pleuropneumoniae isolates (Asawa et al. 1995), TC and TMP/SMX are still among the recommended antimicrobials for PP therapy (Burch et al. 2008). Nevertheless, since these antimicrobials have been used extensively for the treatment of numerous swine diseases over the past several decades, increasing resistance rates have been reported in several European countries (Gutiérrez-Martín et al. 2006). Approximately 95.0% of the strains found in our study were resistant to TC. FF, a fluorinated chloramphenicol derivative, is a broad-spectrum antimicrobial agent that has been licensed in Europe since 2000 for the treatment of bacterial respiratory tract infections in pigs (Kehrenberg et al. 2004). The in vitro activity of FF against clinical A. pleuropneumoniae isolates has been studied extensively, and low resistance rates have been found in Germany, Spain, Switzerland, and Japan (Priebe & Schwarz 2003; Gutiérrez-Martín et al. 2006; Matter et al. 2007; Morioka et al. 2008). In contrast, the present study revealed that approximately 45% of the isolates were FF resistant. Yoo et al. (2014) also reported a high prevalence of FF resistance (45%) in Korean strains isolated from 2006 to 2010. To the best of our knowledge, this high prevalence of FF resistance is unique to South Korea and indicates that the prudent use of antimicrobials will be needed to prevent the spread of FF resistance in South Korea. As already reported, β-lactams normally show a high degree of in vitro activity against A. pleuropneumoniae (Matter et al. 2007); however, a relatively large number of resistant

Figure 1. Dendrogram generated by BioNumerics software with 1% optimization and 1% position tolerance showing the results of cluster analysis on the basis of RAPD analysis of 65 Actinobacillus pleuropneumoniae isolates. The percentage of similarity among strains was determined using the Dice coefficient, and clustering was performed using UPGMA. Sero represents serotypes. ART represents antimicrobial resistance type.

A. pleuropneumoniae serotypes 1 and 5 were dominant in South Korean pigs between 2010 and 2013. This may be because only vaccines against serotype 2 are available in Korea, which decreased morbidity and mortality, but did not provide cross-immunity against other serotypes. It is also possible that the prevalence
isolates, mostly against ABPC and PCG, have been reported in recent years in Spain (Gutiérrez-Martín et al. 2006) and Italy (Vanni et al. 2012). An increasing trend of \(\beta\)-lactam resistance was also observed in South Korea. Interestingly, isolates resistant to ABPC were also resistant to PCG and all of the isolates resistant to AMC (7 strains) were also resistant to ABPC and PCG. Acquired resistance to aminoglycosides has been widely observed among \(A.\) pleuropneumoniae strains, mainly due to target modification subsequent to the widespread acquisition of mobile genetic elements (Matter et al. 2007). However, the prevalence of strains resistant to only AMK, NEO, or CTF was found to be less prevalent relative to other antimicrobials tested in this study. These results show that NEO, AMK, and CTF may be effective for the control of PP in this region.

In isolates from 2006 to 2010, resistance rates to GM, KM, NEO, and PCG were 43%, 93%, 73%, and 79%, respectively (Yoo et al. 2014). In this study, these rates were reduced to 19%, 15%, 9%, and 21%, respectively. Antimicrobial use in pig farms had not been well controlled in Korea, especially before 2010. The use of antimicrobials as feed additives to increase animal production and prevent disease has been banned since July 2011 in Korea. Our findings indicate that the control of antibiotic use is required in Korean pig farms, and the surveillance of antimicrobial resistances among \(A.\) pleuropneumoniae strains is needed to ensure proper treatment of PP.

Treatment of PP is achieved mainly through the use of antimicrobials. Some studies have reported that there is no correlation between \(A.\) pleuropneumoniae serotype and antimicrobial resistance (Matter et al. 2007; Kucerova et al. 2011), whereas other studies concluded that different \(A.\) pleuropneumoniae serotypes displayed varying levels of antimicrobial resistance (Asawa et al. 1995; Lee et al. 2015). Such heterogeneous antimicrobial resistance among serotypes has caused significant problems for controlling PP. Our results showed that \(A.\) pleuropneumoniae serotypes 1 and 5 were the most common serotypes in South Korea, and that there was no correlation between serotype and distribution of antimicrobial resistance.

TC resistance is generally acquired by (tet) genes associated with plasmids or transposons transferred between bacterial species (Kehrenberg et al. 2001). The tet(B) gene, which codes an efflux protein reducing the intracellular TC levels, was predominant among other tet genes in Korea and in other countries (Blanco et al. 2006; Matter et al. 2007). In addition, in Spain (Blanco et al. 2006) and Switzerland (Matter et al. 2007), \(A.\) pleuropneumoniae strains carrying tet(H), tet(M)/tet(O) and tet(L) were isolated. In Japan, \(A.\) pleuropneumoniae isolates carrying tet(A), tet(H) and tet(M)/tet(O) were reported by Morioka et al. (2008). Although the tet(A) gene was not found in Korean \(A.\) pleuropneumoniae isolates in the report by Yoo et al. (2014), approximately one-third of TC resistant \(A.\) pleuropneumoniae isolates contained the tet(A) gene in this study. \(A.\) pleuropneumoniae strains carrying tet(H), tet(M)/tet(O) and tet(L) were also isolated in this region. Furthermore, TC-resistant isolates containing the tet(C) and tet(G) genes were found in this study, not in the report by Yoo et al. (2014), revealing that a large variety of tet genes (\(n = 7\)), such as tet(B), tet(A), tet(H), tet(C), tet(G), tet(M)/tet(O), and tet(L)-1, are present in Korean \(A.\) pleuropneumoniae isolates. All strains containing tet genes showed phenotypic resistance to TC as assessed by disk diffusion tests in this study. This result shows that most TC-resistant isolates in Korea can be associated with the tet genes. The production of \(\beta\)-lactamase is the primary cause of resistance to \(\beta\)-lactam antibiotics in Gram-negative bacteria (Rodríguez et al. 2009); however, some ABPC-resistant strains are not associated with the \(\beta\)-lactamase gene (Hasegawa et al. 2003). Yoo et al. (2014) reported that approximately 30% of 22 ABPC resistant \(A.\) pleuropneumoniae strains were resistant to ABPC without the bla\(_{\text{ROB-1}}\) gene. Our study revealed an increase in the \(\beta\)-lactam-resistant isolates that was not associated with the bla\(_{\text{ROB-1}}\) gene since 64% of the ABPC/PCG and all of the AMC resistant isolates were negative for the bla\(_{\text{ROB-1}}\) gene based on PCR assays. The \(a a d B\) [\(\text{ant(2\')-Ia}\)], \(a a c C 2\) [\(\text{aac(3)-IIc}\)] and \(a a c C 4\) [\(\text{aac(3)-Iva}\)] genes are associated with resistance to GM (Matter et al. 2007). Among the 12 isolates resistant to GM, approximately 92% and 8% harbored the \(a a d B\) and \(a a c C 4\) genes, respectively, suggesting that \(A.\) pleuropneumoniae strains resistant to GM are mainly associated with the \(a a d B\) gene.

RAPD pattern analyses for all the isolates in this study generated two major clusters of \(A.\) pleuropneumoniae, and these two were basically grouped according to antimicrobial resistance pattern. All six ART 1 isolates belonged to cluster II and all of them were resistant to NEO. Thus, genetic similarity based on RAPD indicates that isolates with resistance to as many as 10 antimicrobial agents from pigs with PP may be closely related to each other, irrespective of serotype.

In conclusion, among the \(A.\) pleuropneumoniae serotypes recently isolated from pigs in South Korea, serotypes 1 and 5 were the most common. In addition, serotypes 2 and 7 comprised about 10% of the isolates. Sixty-four of 65 \(A.\) pleuropneumoniae strains were resistant to at least one of the 12 tested antimicrobials, and 26.6% of the isolates were resistant to three or more. Almost all isolates were resistant to TC and approximately 40% of the isolates were resistant to FF. The isolates with resistance to as many as 10 antimicrobial agents belonged to RAPD cluster II. The high rate of resistant multi-drug resistant \(A.\) pleuropneumoniae strains identified in our study may be complicated for future options of treating PP.
Disclosure statement

No potential conflict of interest was reported by the authors.

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