JOURNAL OF PURE AND APPLIED MICROBIOLOGY, June 2015. Vol. **9**(2), p. 899-911

The Isolation and Identification of *Actinobacillus pleuropneumoniae* and Antimicrobial Susceptibility From Pigs in China

Yongjun Dong, Lirong Wang*, Xingyou Liu and Kun Zhao

College of Animal Science, Henan Institute of Science and Technology, Xinxiang, Henan, 453003, China.

(Received: 25 December 2014; accepted: 01 February 2015)

Seven strains of bacteria were isolated from clinical cases of swine disease in lungs and trachea. The isolated bacteria was identified as *Actinobacillus pleuropneumoniae* (APP) serotypes 1 through bacterial cultivated, culture characteristic observation, morphology, biochemical, growth of satellite phenomenon, hemolysis and serotype identification. The antimicrobial susceptibility showed that the isolated *A. pleuropneumoniae* resistance to penicillins, semisynthetic antibiotics, β-lactam antibiotics, polypeptide antibiotic, polyene antibiotic, coumarin antibiotic, amphenicols antibiotics, β-lactam/β-lactam inhibitor compounds, cephalosporins, aminoglycosides, tetracyclines, fluoroquinolones, macrolides, sulfa drugs, sugar peptide drugs, furan drugs, ethlhydrocupreine; medium sensitive to cephalothin (CF), cefizime (CEX), ciprofloxacin (CIP), norfloxacin (NOR), ofloxacin (OFL); high sensitive to polymyxin B (PB), fosfomycin (FOS), ceftizoxime (CTZ), cefmetazole (CMZ), cefaclor (CEC), cefoperazone/sulbactam (CFP/SU), cefetamet (CTM), cefotaxime (CTX), ceftazidime (CAZ), cefodizine (CDZ), cefurosimc sodium (CXM), cefotaxime/clayulanic acid (CTX/CA), ceftazidime/clavulanic acid (CAZ/CA), cefoxitin (FOX), cefepime (FEP), ceftriazone (CRO),aztreonam (AZT), minocycline. This result showed that *A. pleuropneumoniae* clinical strains had multi-drug resistance and resistance characteristics, which means that these isolated bacteria was multiple drug resistant strains.

Key words: *Actinobacillus pleuropneumoniae;* Isolation; Identification; Serotype; Antimicrobial resistance; antimicrobial susceptibiligy; Multidrug resistance.

Actinobacillus pleuropneumoniae (APP), the etiological agent of porcine pleuropneumonia, is a worldwide disease and has resulted in significant economic losses in swine production(Garcia-Cuellar *et al*., 2000; Shin *et al*., 2013). The *A. pleuropneumoniae* virulence factors includelipopolysacchared (LPS), capsular polysaccharide (CPS) and proteases(Dubreuil *et al*., 2000; Garcia Gonzalez *et al*., 2004; Negrete-Abascal *et al*., 1998; Negrete-Abascal *et al*., 1994). At present, 15 different serotypes and 2 biotypes have been described based on nicotinamide adenosine dinucleotide (NAD) requirements: exogenous β-NAD dependent (Biovar 1) and β-NAD independent (Biovar 2)(Angen *et al*., 2008; Blackall *et al*., 2002; Fodor *et al*., 1989; Gottschalk, 2000; Nielsen, 1990).Biovar 1 includes 13 serotypes and biovar 2 consists of two serotypes based on the capsular antigen, and tow subtypes (1b and 5b) based on the LPS and a lipoprotein(Blackall *et al*., 2002; Fodor *et al*., 1989; Jolie *et al*., 1995; Serrano-Rubio *et al*., 2008).

The different serotypes vary in virulence, the presence and prevalence of serotypes vary among countries and show different predominance in different geographic regions (Bosse *et al*., 2002; Fedorka-Cray *et al*., 1993; Jessing *et al*., 2003;

* To whom all correspondence should be addressed. Tel: 13653735098; Fax number: 0373-3040718; E-mail: wlr20@126.com

Rosendal *et al*., 1985; Sebunya and Saunders, 1983). In Mexico, serotypes 1a, 3, 5a, 5b and 7 of biovar 1 are generally found, whereas serotypes 2, 5 and 6 account for approximately 94% of the strains isolated from swine with clinical disease(Jessing *et al*., 2003; Serrano-Rubio *et al*., 2008). Serotypes 2 and 9 are the most commonly isolated in many European countries and serotypes 1 and 5 are the mostly common found in North America(Sebunya and Saunders, 1983). In Canada and USA, serotypes 1, 5 and 7 are the most whereas in Germany serotypes 2, 7 and 9 are the most prevalent(Dubreuil *et al*., 2000; Zhou *et al*., 2008). *A. pleuropneumoniae* isolates from England and Wales including serovars 2, 3, 6-8 and 12(O'Neill *et al*., 2010).In the UK serotypes 3 and 8 were the commonly found in 1994 (McDowell and Ball, 1994). In a survey of 100 isolates similar frequencies of serotype 3 (50%) and serotype 8 (26%) were found together with serotypes 2, 6, 7 and 12(Zhou *et al*., 2008).

Pigs of all ages are infection, usually with 2~5 months, weight for 30~60 kg, mainly spread through the respiratory tract. Porcine contagious pleuropneumonia causes swine dead rapidly and with obvious seasonal in April to May and September to November.Sick pigs, resistance pigs and carrier pigs are the potential epidemics source for the spread of the disease. The pigs no clinical symptoms have pathological or no clinical symptoms without pathological in the negative pathogen pig is very common, which is difficult for purification for disease control and eradication.Infection with *A. pleuropneumoniae* is often subclinical, but sometimes results in severe clinical signs and high mortality, causing substantial economic losses (Costa *et al*., 2011; Tobias *et al*., 2012). The typical characteristics of bronchopneumonia caused by *A. pleuropneumoniae* are thrombosis, oedema, fibrin and mucus deposition, and neutrophil and mononuclear cell infiltration into the lung parenchyma (Oh *et al*., 2013).Histopathological changes in the acute stage are characterized by coagulative necrosis, hemorrhage, vascular thrombosis, edema, fibrin and mucin deposition, and neutrophil and mononuclear cell infiltration of the lung parenchyma (Kim *et al*., 2012). The disease mainly caused the death of pigs, pig growth stagnation and rise in the cost of drug treatment.Along with the increase of drug, strain resistance increases, which have caused great economic losses to the pig industry and is the mainly respiratory disease that affects the healthy development of modern pig industry throughout the world.

In September 2013, a pig farm (Henan province, China) 2 months to 5 months breeding pigs showed different degrees of respiratory system disease symptoms with cough, asthma, increased body temperature, difficulty breathing, reduce appetite, spirit depressed, don't want to walk, like lying, angular, slow growing, some symptoms such as a dog sit sample pant. Autopsy visible peritoneal effusion, pericardial with chest wall adhesion, pericardial cavity with a large number of yellowish-white chyle turbid liquid, a purple, pulmonary interstitial lung are full of blood jelly sample liquid, chest with fibrinous exudate. The liver was congestion, dark red.Shallow inguinal lymph nodes and mesenteric lymph node was enlargement, hyperemia and purple. Lung surface with a layer of yellow fibrinous exudate and adhesion with pleural.

The objectives of this study were (1) isolatedbacteria and identified from clinical suspected pathological pig lungs and trachea by cultivating observation, biochemical characteristic test and serotype identification. The isolated strain was identified as *A. pleuropneumoniae*, (2) determine the antimicrobial resistance profiles, by determining the antimicrobial agents for 7 *A. pleuropneumoniae* cultured from China pigs used for bacterial respiratory pathogens. The study provide theoretical basis for the clinical diagnosis and treatment of the disease and guidance. This study presents information and is the first describe on the prevalence of multi-drug antibiotic resistance in China *A. pleuropneumoniae* clinical isolates.

MATERIALS AND METHODS

NAD (Nicotinamide adenine dinucleotide) was purchased from Takara biotechnology (Dalian, China). Standard strains of *Staphylococcus aureus* and *A. pleuropneumoniae* 1~12 positive serum purchased from China institute of veterinary drug control. 95% ethanol, anhydrous ethanol and other reagents are pure homebred analysis. The common

nutrient agar, normal serum broth, Mac Conkey agar, LB agar, tryptone soy agar (TSA), chocolate agar, brain heart infusion agar (containing 10% calf serum and 100 µg/ml NAD) purchased from Beijing land bridge technology co. LTD. Drug sensitive piece of paper was bought from the temple of heaven in Beijing pharmaceutical biotechnology development co. LTD. Gram stain solution was purchased from Nanjing Jiancheng science and technology co. LTD.

Culture and isolation

Take lung diseased tissue and tracheal samples, vaccination in brain heart infusion agar (containing 10% calf serum and 100µg/ml NAD) plate, placed in the volume fraction of 10% CO_2 incubator in 24 h, extraction of single colony purification cultivation.

Observe bacterial culture characteristics

Take pure bacteria cultures for gram staining and observe in the microscope.

Different culture medium growth

Taking pure bacteria cultures were inoculated on different culture medium plate, observe the growth of bacteria. At the same time, conduct the CAMP experiment and observe the hemolysis phenomenon of isolated bacteria.

Bacterial biochemical test evaluation

Take pure bacteria cultures put in medium containing 1% NAD various kinds of bacteria biochemical culture tube, at 10% of the CO_2 incubator in 24 h, observation result of bacteria biochemical reactions.

Bacterial serotype identification

Take the pure culture of bacteria and prepare the appropriate concentration of bacteria bacterium suspension, with different serotype *A. pleuropneumoniae* standard positive serum plate agglutination test, observe the agglutinate phenomenon, determine the serotype of isolated bacteria.

Bacterial susceptibility testing

K-B susceptibility was adopted to test common antimicrobial agents, such as kanamycin, spectinomycin, amoxicillin and other 83 kinds of commonly used of the sensitivity of determination of bacteriostatic ring diameter and reference drug sensitive test standard (WS-T125-1999) for determining susceptibility and determine the drug susceptibility characteristics of isolated bacteria.

RESULTS

Bacteria separation

Petri dishes placed in the volume fraction of 10% CO_2 incubator in 24 h in brain heart infusion agar (containing 10% calf serum and 100µg/ml NAD) plate. There were suspected bacterial growth of 4 sick pig head of lung and 3 head sick pig tracheal tumors. The colony was edge neatly, round, smooth and moist, transparent and the size of the tip colony. Total 7 strains bacteria were isolated, named APHN1, APHN2, APHN3, APHN4, APHN5, APHN6, APHN7, respectively. And then transfer the 7 strains of bacteria in sheep blood agar (containing 10% calf serum and 100µg/ml NAD) plate, can produce stable beta hemolytic (Fig. 1: A, B, C, D, E, F, G).

The above seven strains of bacteria for gram staining, microscopy. The results showed that the bacteria dyeing poles, spherical, rods, or ball rod polymorphism (Fig. 2, A, B, C, D, E, F).

Bacterial culture characteristics

Petri dishes placed in the volume fraction of 10% CO_2 incubator in 24 h, the above 7 strains of bacteria does not grow on the common nutrient agar, normal serum broth, Mac Conkey agar, LB agar, tryptone soy agar (TSA), chocolate agar. But, these isolated bacteria grew well on the brain heart infusion agar (containing 10% calf serum and 100 µg/ml NAD). Isolated bacteria culture together with the staphylococcus aureus (CAMP test) found that close to staphylococcus aureus growth lines isolated bacteria growth momentum is good and with a beta hemolytic phenomena around the growth lines.

Bacterial biochemical characteristics

Take the above 7 strains of bacteria inoculated to biochemical culture tube, cultivated in the condition of 10% CO_2 incubator for 24 h. The result showed that the seven strains of bacteria can ferment glucose, maltose, sucrose and mannose, not ferment lactose, mannitol, arabinose and aesculin, do not produce hydrogen sulfide and indole, urease test and catalase test positive (Table 1).

Bacterial serotype

The pure culture of 7 strains of bacteria was detected by plate agglutination test with type 1~12 *A. pleuropneumoniae* standard positive

serum. The results showed that seven strains of bacteria was appeared obvious agglutinative phenomenon with standard type '! positive serum, but no agglutinative appeared with other standard positive serum agglutination and physiological saline controls. Agglutination test results showed that 7 isolated strains of bacteria were *A. pleuropneumoniae*'! serum type.

Bacterial susceptibility features

The 7 strains of bacteria drug sensitivity was test by K-B susceptibility agar diffusion. The drug sensitive showed that isolated bacteria resistance to penicillins: penicillin G (P), piperacillin (PIP), amoxicillin (AMX), ampicillin (Am), carbenicillin (CB), oxacillin (OX), mezlocillin (MEZ); resistance to semisynthetic antibiotics: rifampicin resistant (RA); resistance to ²-lactam antibiotics: meropenem (MPN); resistance to polypeptide antibiotic: bacitracin (B); resistance to polyene antibiotic: teicoplanin (TCL); resistance to coumarin antibiotic: novobiocin (NB); resistance to amphenicols antibiotics: chloramphenicol (C); resistance to β-lactam/β-lactam inhibitor compounds: ampicillin/sulbactam (AM/SU), ticarcillin/Clavulanic (TIC/CA), amoxicillini/ clavulanic acid (AMX/CA), piperacillin/tazohactam (PIP/TA), amoxicillin/sulbactam (AMX/SU), mezlocillin/sulbactam (MEZ/SU) and azlocillin (AZL); resistance to cephalosporins: cefazolin (CZ), cephalexin (CX), cefradine (CED), cefpiramide (CPM), cefoperazone (CFP), cefadroxil (CDX), cefprozil (CPZ); resistance to aminoglycosides: kanamycin (K), gentamycin (GM), amikacin (AN), netilmicin (NET), tobramycin (TM), streptomycin (S), spectinomycin (SPT), neomyucin (N), micronomicin (MIC), etimicin (EIT); resistance to tetracyclines: tetracycline (TE), doxycycline (DO); resistance to fluoroquinolones: levofloxacin (LVF), nalidixic acid (NAL), fleroxacin (FLE), gatifloxacin (GTF), pefloxacin (PEF), lomefloxacin (LME), enoxacin (ENO), sparfloxacin (SPF); resistance to macrolides: azithrornycin (AZI), clindamycin (CM), erythromycin (E), clarithromycin (CLA), roxithromycin (ROX), midecamycin (MDM), josamycin (JOS); resistance to sulfa drugs: sulfamethoxazole/trim ethoprim (SXT), sulfamethoxazole (SMZ); resistance to sugar peptide drugsÿvancomycin (VA), norvancomycin (NVA); resistance to furan drugs: furazolidone (FR), nitrofurantoin (FT) and resistance to ethlhydrocupreine; medium sensitive to cephalothin (CF), cefizime (CEX) and medium sensitive to ciprofloxacin (CIP), norfloxacin (NOR), ofloxacin (OFL); high sensitive to polymyxin B (PB); high sensitive to more phosphorus antibiotic: fosfomycin (FOS); high sensitive to ceftizoxime (CTZ), cefmetazole (CMZ), cefaclor (CEC), cefoperazone/sulbactam (CFP/SU), cefetamet (CTM), cefotaxime (CTX), ceftazidime (CAZ), cefodizine (CDZ), cefurosimc sodium (CXM), cefotaxime/clayulanic acid (CTX/CA), ceftazidime/ clavulanic acid (CAZ/CA), cefoxitin (FOX), cefepime (FEP), ceftriazone (CRO); high sensitive to single amide rhzomorph: aztreonam (AZT); high sensitive to minocycline (Table 2).

DISCUSSION

Porcine contagious pleuropneumonia was very popular in countries around the world, cause serious economic losses to the pig industry (Hodgetts *et al*., 2004; Jacobsen and Nielsen, 1995; Perry *et al*., 2012; Schuchert *et al*., 2004). Transmission of *A. pleuropneumoniae* is primarily

Table 1. Results of biochemical identification of isolated strains

Strains	Glucose	Lactose	Maltose	Manicol	Sucrose	L-pectin sugar	Aesculin	Urease	H2S	Mannose	Catalase	Indole
AP1	+	-	+	-	+	-	-	+	-	+	+	-
AP2	+	-	+	-	+	-	-	+	-	+	+	-
AP3	+	-	+	-	+	-	-	+	-	+	+	-
AP4	+	-	+	-	+	-	-	+	-	+	+	-
AP5	+	-	+	-	+	-	-	+	-	+	+	-
AP6	+	-	+	-	+	-	-	+	-	+	+	-
AP7	+	-	+	-	+	-	-	+	-	+	+	-

Notes: "+"shows positeve reaction; "-"shows negative reaction.

Table 2. Sensitivity of isolated strains to antibacterial agents

Antibacterial agent (Abbreviation)	Drug concentration (ìg/piece)	AP_1	AP_2	AP_3	AP_4	AP_5	AP_6	AP_7	Sensitivity
		Diameter of inhibition zone/mm							
P	10IU	5	6	6	6	5	6	5	R
PIP	100	5	5	5	6	6	5	6	R
AMX	10	4	4	5	5	4	5	5	R
Am	10	4	4	5	5	5	5	4	R
CB	100	4	4	5	4	5	5	5	R
OX	1	5	5	5	5	4	4	4	R
MEZ	75	6	6	6	6	6	6	6	R
MPN	10	6	6	6	7	7	7	7	R
PB	300IU	15	15	16	16	16	15	16	H
B	0.041IU	6	6	6	6	6	6	6	R
C	30	7	7	7	7	7	7	7	R
TCL	30	6	6	6	6	6	6	6	R
NB	5	6	6	6	6	6	6	6	R
FOS	200	25	25	25	25	25	25	25	H
RA	5	9	9	9	9	9	9	9	R
AM/SU	10/10	7	7	7	7	7	7	7	R
TIC/CA	75/10	15	15	15	15	15	15	15	M
AMX/CA	20/10	12	12	12	12	12	12	12	R
PIP/TA	100/10	20	20	20	20	20	20	20	M
AMX/SU	10/10	6	6	6	6	6	6	6	R
AZL	75	7	7	7	7	7	7	7	R
MEZ/SU	75	14	14	14	14	14	14	14	R
CZ	30	6	6	6	6	6	6	6	R
CF	30	14	14	14	14	14	14	14	R
CX	30	5	5	5	5	5	5	5	R
CTZ	30	32	32	32	32	32	32	32	H
CED	30	12	12	12	12	12	12	12	R
CMZ	30	28	28	28	28	28	28	28	H
CEC	30	16	16	16	16	16	16	16	R
CFP/SU	75/30	20	20	20	20	20	20	20	R
CTM	10	21	21	21	21	21	21	21	R
CTX	30	27	27	27	27	27	27	27	H
CAZ	30	26	26	26	26	26	26	26	H
CDZ	30	22	22	22	22	22	22	22	R
CPM	75	13	13	13	13	13	13	13	R
CXM	30	18	18	18	18	18	18	18	R
CFP	75	6	6	6	6	6	6	6	R
CEX	5	17	17	17	17	17	17	17	M
CDX	30	8	8	8	8	8	8	8	R
CPZ	30	9	9	9	9	9	9	9	R
CTX/CA	30/10	25	25	25	25	25	25	25	H
CAZ/CA	30/10	20	20	20	20	20	20	20	H
FOX	30	19	19	19	19	19	19	19	H
FEP	30	29	29	29	29	29	29	29	H
CRO	30	31	31	31	31	31	31	31	H
AZT	30	29	29	29	29	29	29	29	H
K	30	6	6	6	6	6	6	6	R
GM	120	5	5	5	5	5	5	5	R
AN	30	6	6	6	6	6	6	6	R

NET	30	5	5	5	5	5	5	5	R
TM	10	11	11	11	11	11	11	11	R
S	300	5	5	5	5	5	5	5	R
SPT	100	5	5	5	5	5	5	5	R
N	30	13	13	13	13	13	13	13	R
MIC	10	11	11	11	11	11	11	11	R
EIT	30	5	5	5	5	5	5	5	R
TE	30	6	6	6	6	6	6	6	R
NMO	30	19	19	19	19	19	19	19	R
DO	30	10	10	10	10	10	10	10	R
CIP	5	19	19	19	19	19	19	19	M
LVF	5	11	11	11	11	11	11	11	R
NOR	10	12	12	12	12	12	12	12	R
OFL	5	12	12	12	12	12	12	12	R
NAL	30	6	6	6	6	6	6	6	R
FLE	5	14	14	14	14	14	14	14	R
GTF	5	15	15	15	15	15	15	15	R
PEF	10	13	13	13	13	13	13	13	R
LME	10	13	13	13	13	13	13	13	R
ENO	10	13	13	13	13	13	13	13	R
SPF	5	15	15	15	15	15	15	15	R
AZI	15	12	12	12	12	12	12	12	R
CM	20	6	6	6	6	6	6	6	R
E	15	6	6	6	6	6	6	6	R
CLA	15	8	8	8	8	8	8	8	R
ROX	15	5	5	5	5	5	5	5	R
MDM	15	5	5	5	5	5	5	5	R
JOS	15	6	6	6	6	6	6	6	R
SXT	23.75/1.25	6	6	6	6	6	6	6	R
SMX	300	6	6	6	6	6	6	6	R
VA	30	6	6	6	6	6	6	6	R
NVA	30	6	6	6	6	6	6	6	R
FR	300	11	11	11	11	11	11	11	R
FT	300	18	18	18	18	18	18	18	R
EPR	5	6	6	6	6	6	6	6	R

Notes:"H"shows high sensitive;"M"shows medium sensitive;"R"shows drug resistance.

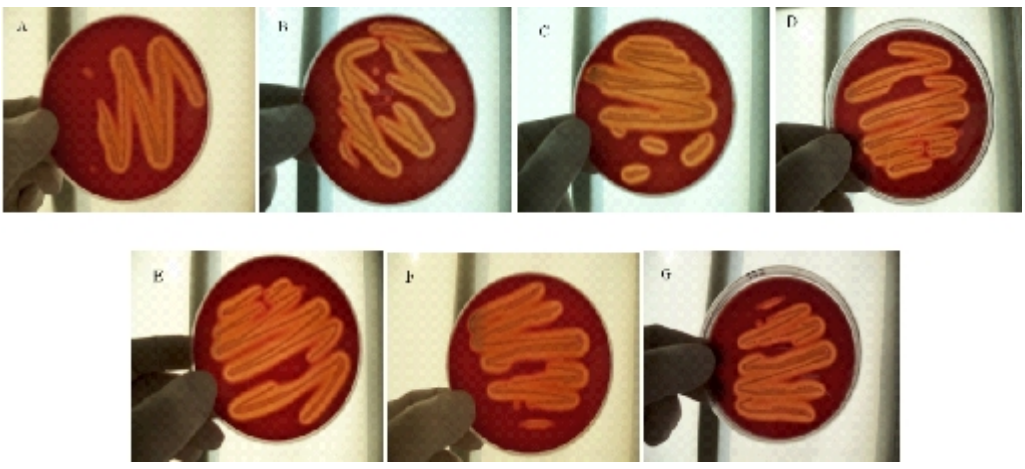

Fig. 1. A, B, C, D, E, F, G

thought to be via direct transfer of mucus from pig to pig, which is via nasal secretion and aerosol (Assavacheep and Rycroft, 2013). The respiratory tract is the primary site for bacterial infection, it has been suggested that bacterial exclusion in the respiratory tract through mucosal immune induction is the most effective disease prevention strategy (Grasteau *et al*., 2011; Lu *et al*., 2011; Seo *et al*., 2013). But, *A. pleuropneumoniae* causes porcine pleuropneumonia, is a highly contagious for which there is no effective vaccine (Bosse *et al*., 2002). There is no vaccines that can protect against all serotypes and prevent colonization (Li *et al*., 2013). As a consequence, the use of antimicrobials continues to be the most effective measure for the control of pig pleuropneumonia outbreaks (Vanni *et al*., 2012).

A. pleuropneumoniae serotype has many serotype, popular serotype is different in different countries and regions (Lo *et al*., 1998). The different serotypes vary in virulence, the presence and prevalence of serotypes vary among countries and show different predominance in different geographic regions. This study was isolated bacteria and identified from clinical cases with standard positive serum using plate agglutination of 7 strains isolated bacteria. The 7 strains of bacteria wereidentified as serum '! type. And drug susceptibility test of this 7 strains. This was provides an important basis for porcine contagious pleuropneumonia control of prevention and immune prevention. Porcine contagious pleuropneumonia is one of the major respiratory diseases in large-scale pig farms. At the same time, the cause of respiratory symptoms has mycoplasmal pneumonia of swine (MPS), swine infectious atrophic rhinitis (AR) and *Haemophilus parasuis*. In this study, for the nasal bone anatomy of the pig disease did not find the septum bends, turbinate atrophy. The tissue samples from pigs were also using PPLO broth PPLO agar to cultivate, but did not isolated *mycoplasma hyopneumoniae*. Therefore, mycoplasmal pneumonia of swine (MPS), swine infectious atrophic rhinitis (AR) and *Haemophilus parasuis* can be exclude.

A rise in frequencies of resistance to multiple antibiotics has been reported in several countries to date (de Jong *et al*., 2014; Gutierrez-Martin *et al*., 2006; Hendriksen *et al*., 2008; Wang *et al*., 2010). There several publications have reported data concerning the *in vitro* activity of amphenicols, cephalosporins, amoxicillin/clavulanic acid and fluoroquinolones against *A. pleuropneumoniae*(Chang *et al*., 2002; Intorre *et al*., 2007; Kucerova *et al*., 2011; Matter *et al*., 2007; Suzuki *et al*., 1989). Previous reports indicated that fluoroquinolone (FQ) resistant *A. pleuropneumoniae* isolates were found in Taiwan, Denmark, Poland and England (Aarestrup and Jensen, 1999; Chang *et al*., 2002; Hendriksen *et al*., 2008). Another reports from Tainwan also indicated that the prevalence of enrofloxacin (ER) resistant *A. pleuropneumoniae* was high (Chang *et al*., 2002). ER resistance of *A. pleuropneumoniae*

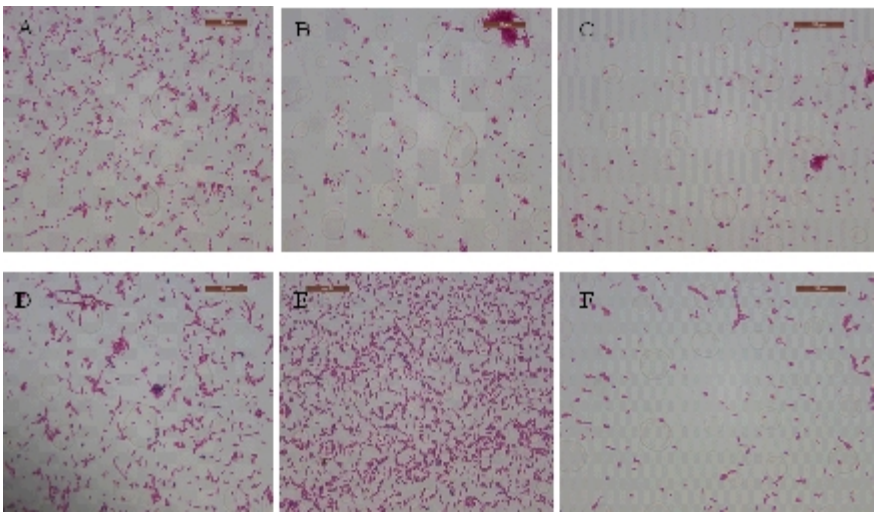

Fig. 2. A, B, C, D, E, F

appears to be linked to multiple target gene mutations at codon positions 75, 83, and 87 of gyrA, codon position 83, 85 and 89 of parC, and codon position 440, 459, 461 and 479 of parE, as well as being linked to active efflux (Wang *et al*., 2010). Nevertheless, increasing levels of acquired resistance to ampicillin, trimethoprim/sulfonamide and tetracycline has been reported worldwide (Asawa *et al*., 1995; Morioka *et al*., 2008). The majority of *A. pleuropneumoniae* isolates were resistance to erythromycin (89%), tetracycline (75%), ampicillin (8.5%), penicillin (8.5%) and tilmicosin (25%) in Australia (Dayao *et al*., 2014). In Switzerland, 83 *A. pleuropneumoniae* strains collected from slaughtered pig resistance to sulfamethoxazole, the combination sulfamethoxazole-trimethoprim, tiamulin, tilmicosin, tetracycline, penicillin and ampicillin (Matter *et al*., 2007). The garlic volatile allyl methyl sulfide (AMS) was shown to exhibit an antibacterial effect against the pig pathogen *A. pleuropneumoniae* serotype 9 (Becker *et al*., 2012), it may be an alternative approaches to control the disease.

According to serotype and drug susceptibility results of isolated bacteria, choose containing serum '! type of porcine contagious pleuropneumonia trivalent inactivated vaccine immunization against sow and health piglets. Pigs by injection with the drug, such as polymyxin B (PB)0fosfomycin (FOS)0ceftizoxime (CTZ)0cefmetazole (CMZ)0cefotaxime (CTX)0ceftazidime (CAZ)0cefotaxime/clayulanic acid (CTX/CA)0ceftazidime/Clavulanic acid (CAZ/CA)0cefoxitin (FOX)0cefepime (FEP)0ceftriazone (CRO)0aztreonam (AZT), effective control and prevention of the porcine contagious pleuropneumonia.

Antimicrobial agents have been extensively used for the treatment of numerous swine diseases during decades in swine production for therapeutic, methaphylactic and prophylactic purposes and the consequent selective pressure has intensified the risk for the emergence of resistant bacteria (Aarestrup and Jensen, 1999; Jensen *et al*., 2006; Vanni *et al*., 2012). The widespread use of antimicrobial agents to treat or prevent diseases in animal has led to increased rates of resistance to various antimicrobial agents (Kang *et al*., 2009; Livrelli *et al*., 1991; Wright *et al*., 1997). A relatively high number of resistant *A. pleuropneumoniae* have been isolated from clinical against penicillin, amoxicillin and cephalexin in the United States and Spain (Gutierrez-Martin *et al*., 2006; Ito *et al*., 2004; Pridmore *et al*., 2011). Previous reports showed that ²-lactams has a high degree of in vitro activity against APP (Aarestrup and Jensen, 1999; Matter *et al*., 2007; Yoshimura *et al*., 2002). In this study, our results confirm this rising trend mostly for penicillins. Another key factor that may contribute to the spread of antimicrobial-resistant isolates in animal populations is the movement of swine between herds or between countries (McEwen and Fedorka-Cray, 2002). There are several reports on the increasing resistance rates during decades in korea, Taiwan, Japan and European countrie(Chang *et al*., 2002; Gutierrez-Martin *et al*., 2006; Hendriksen *et al*., 2008; Kim *et al*., 2001; Yoshimura *et al*., 2002). Similar results of resistance were found in our present study for tetracycline, doxycycline ,sulfamethoxazole/ Trimethoprim, sulfamethoxazole,fluoroquinolones, resistance to levofloxacin (LVF), nalidixic acid (NAL), fleroxacin (FLE), Gatifloxacin (GTF), pefloxacin (PEF), lomefloxacin (LME), enoxacin (ENO), sparfloxacin (SPF), this is agreement with previous reports (Chang *et al*., 2002; Kim *et al*., 2001; Matter *et al*., 2007; Morioka *et al*., 2008; Shin *et al*., 2005; Yoshimura *et al*., 2002). Florfenicol has been studied extensively in vitro activity against of *A. pleuropneumoniae* and showed low resistance rates in Germany, South Korea, Spain and Japan (Gutierrez-Martin *et al*., 2006; Morioka *et al*., 2008; Priebe and Schwarz, 2003; Shin *et al*., 2005).

At present, the antibiotic is the key to the treatment of bacterial disease (Pyorala *et al*., 2014; Sjolund *et al*., 2011; Tobias *et al*., 2014). Antibiotic use in animals correlates with the risk of development of resistance in microorganisms in a multifactorial and complex way (Nedbalcova *et al*., 2014; West *et al*., 1995). The major risk factors associated with antibacterial treatment failure is drug resistance, which continues to increase worldwide problem (Damte *et al*., 2013; Wasteson *et al*., 1996). Because of clinical application of non-standard drug and drug abuse lead to bacterial resistance increase, and lead to damage in animal body can't use drugs to restore, which lead to no obvious effect on antibiotics in the treatment of bacterial disease. There are a lot of multiple drug

resistant strains isolated from clinical. So, it is of great significance for development research on bacterial drug resistance, epidemiology and vaccine.

This study results showed that the separated strains was resistance to multiple drugs, the application of antibiotics is hard to control the occurrence of the disease. Multidrug resistance plasmids were found in *A. porcitonsillarum*, it contain the sulfonamide resistance gene sul2, the ²-lactam resistance gene bla_{ROB-1} and the streptomycin resistance gene *str A*(Matter *et al*., 2008).*A.pleuropneumoniae*serotype is more complex, with local specificity; at the same time between each serotype is difficult to produce strong cross protection. After infected with the *A. pleuropneumoniae* are often prone to secondary infection which increased the difficulty of the prevention and control. By the way, the pigs infected with the *A. pleuropneumoniae* and resistance has become a potential source of infection. Therefore, it has an important guiding significance of timely, accurate of separation and identification of *A. pleuropneumoniae*, drug sensitivity test, new veterinary medicine preparation, and new type of vaccine to control this disease.

In conclusion, this study presented data on the antimicrobial resistance profiles of *A. pleuropneumoniae* isolated from China pigs. The study found that isolates collected in this study resistance to penicillins: penicillin G (P), piperacillin (PIP), amoxicillin (AMX), ampicillin (Am), carbenicillin (CB), oxacillin (OX), mezlocillin (MEZ); resistance to semisynthetic antibiotics: rifampicin resistant (RA); resistance to ²-lactam antibiotics: meropenem (MPN); resistance to polypeptide antibiotic: bacitracin (B); resistance to polyene antibiotic: teicoplanin (TCL); resistance to coumarin antibiotic: novobiocin (NB); resistance to Amphenicols antibiotics: chloramphenicol (C); resistance to ²-lactam/²-lactam inhibitor compounds: ampicillin/sulbactam (AM/SU), ticarcillin/Clavulanic (TIC/CA), amoxicillini/ clavulanic acid (AMX/CA), piperacillin/tazohactam (PIP/TA), amoxicillin/sulbactam (AMX/SU), mezlocillin/sulbactam (MEZ/SU) and azlocillin (AZL); resistance to cephalosporins: cefazolin (CZ), cephalexin (CX), cefradine (CED), cefpiramide (CPM), cefoperazone (CFP), cefadroxil (CDX), cefprozil (CPZ); resistance to aminoglycosides: kanamycin (K), gentamycin (GM), amikacin (AN), netilmicin (NET), tobramycin (TM), streptomycin (S), spectinomycin (SPT), neomyucin (N), micronomicin (MIC), etimicin (EIT); resistance to tetracyclines: tetracycline (TE), doxycycline (DO); resistance to fluoroquinolones: levofloxacin (LVF), nalidixic acid (NAL), fleroxacin (FLE), gatifloxacin (GTF), pefloxacin (PEF), lomefloxacin (LME), enoxacin (ENO), sparfloxacin (SPF); resistance to macrolides: azithrornycin (AZI), clindamycin (CM), erythromycin (E), clarithromycin (CLA), roxithromycin (ROX), midecamycin (MDM), josamycin (JOS); resistance to sulfa drugs: sulfamethoxazole/trim ethoprim (SXT), sulfamethoxazole (SMZ); resistance to sugar peptide drugsÿvancomycin (VA), norvancomycin (NVA); resistance to furan drugs: furazolidone (FR), nitrofurantoin (FT) and resistance to ethlhydrocupreine; medium sensitive to cephalothin (CF), cefizime (CEX) and medium sensitive to ciprofloxacin (CIP), norfloxacin (NOR), ofloxacin (OFL); high sensitive to polymyxin B (PB); high sensitive to more phosphorus antibiotic: fosfomycin(FOS); high sensitive to ceftizoxime (CTZ), cefmetazole (CMZ), cefaclor (CEC), cefoperazone/sulbactam (CFP/SU), cefetamet (CTM), cefotaxime (CTX), ceftazidime (CAZ), cefodizine (CDZ), cefurosimc sodium (CXM), cefotaxime/clayulanic acid (CTX/CA), ceftazidime/ clavulanic acid (CAZ/CA), cefoxitin (FOX), cefepime (FEP), ceftriazone (CRO); high sensitive to single amide rhzomorph: aztreonam (AZT); high sensitive to minocycline. Data from this survey will provide valuable information for veterinary pathogens on resistance epidemiology to provide evidence based guidance for antimicrobial therapy of bacterial diseases, and efficiently contribute to animal health and welfare.

ACKNOWLEDGEMENTS

This work was supported by Xinxiang key scientific and technological project (ZG13009).

REFERENCES

1. Aarestrup, F.M., Jensen, N.E., 1999. Susceptibility testing of Actinobacillus pleuropneumoniae in Denmark. Evaluation of

three different media of MIC-determinations and tablet diffusion tests. *Vet Microbiol* **64**, 299-305.
2. Angen, O., Ahrens, P., Jessing, S.G., Development of a multiplex PCR test for identification of Actinobacillus pleuropneumoniae serovars 1, 7, and 12. *Vet Microbiol*, 2008; **132**; 312-318.
3. Asawa, T., Kobayashi, H., Mitani, K., Ito, N., Morozumi, T., Serotypes and antimicrobial susceptibility of Actinobacillus pleuropneumoniae isolated from piglets with pleuropneumonia. *J Vet Med Sci*, 1995; **57**; 757-759.
4. Assavacheep, P., Rycroft, A.N., Survival of Actinobacillus pleuropneumoniae outside the pig. *Res Vet Sci*, 2013; **94**, 22-26.
5. Becker, P.M., van Wikselaar, P.G., Mul, M.F., Pol, A., Engel, B., Wijdenes, J.W., van der Peet-Schwering, C.M., Wisselink, H.J., Stockhofe-Zurwieden, N., Actinobacillus pleuropneumoniae is impaired by the garlic volatile allyl methyl sulfide (AMS) in vitro and in-feed garlic alleviates pleuropneumonia in a pig model. *Vet Microbiol*, 2012; **154**, 316-324.
6. Blackall, P.J., Klaasen, H.L., van den Bosch, H., Kuhnert, P., Frey, J., Proposal of a new serovar of Actinobacillus pleuropneumoniae: serovar 15. *Vet Microbiol*, 2002; **84**; 47-52.
7. Bosse, J.T., Janson, H., Sheehan, B.J., Beddek, A.J., Rycroft, A.N., Kroll, J.S., Langford, P.R., Actinobacillus pleuropneumoniae: pathobiology and pathogenesis of infection. *Microbes Infect*, 2002; **4**, 225-235.
8. Chang, C.F., Yeh, T.M., Chou, C.C., Chang, Y.F., Chiang, T.S., Antimicrobial susceptibility and plasmid analysis of Actinobacillus pleuropneumoniae isolated in Taiwan. *Vet Microbiol*, 2002; **84**; 169-177.
9. Costa, G., Oliveira, S., Torrison, J., Dee, S., Evaluation of Actinobacillus pleuropneumoniae diagnostic tests using samples derived from experimentally infected pigs. *Vet Microbiol*, 2011; **148**; 246-251.
10. Damte, D., Lee, S.J., Yohannes, S.B., Hossain, M.A., Suh, J.W., Park, S.C., Comparative activities of selected fluoroquinolones against dynamic populations of Actinobacillus pleuropneumoniae in an in vitro model of time-kill continuous culture experiment. *Int J Antimicrob Agents*, 2013; **42**; 544-552.
11. Dayao, D.A., Gibson, J.S., Blackall, P.J., Turni, C., Antimicrobial resistance in bacteria associated with porcine respiratory disease in Australia. *Vet Microbiol*, 2014; **171**: 232-235.
12. de Jong, A., Smet, A., Ludwig, C., Stephan, B., De Graef, E., Vanrobaeys, M., Haesebrouck, F., Antimicrobial susceptibility of Salmonella isolates from healthy pigs and chickens (2008-2011). *Vet Microbiol.*, 2014.
13. Dubreuil, J.D., Jacques, M., Mittal, K.R., Gottschalk, M., Actinobacillus pleuropneumoniae surface polysaccharides: their role in diagnosis and immunogenicity. *Anim Health Res Rev*, 2000; **1**: 73-93.
14. Fedorka-Cray, P.J., Stine, D.L., Greenwald, J.M., Gray, J.T., Huether, M.J., Anderson, G.A., The importance of secreted virulence factors in Actinobacillus pleuropneumoniae bacterin preparation: a comparison. *Vet Microbiol*, 1993; **37**: 85-100.
15. Fodor, L., Varga, J., Molnar, E., Hajtos, I., Biochemical and serological properties of Actinobacillus pleuropneumoniae biotype 2 strains isolated from swine. *Vet Microbiol*, 1989; **20**: 173-180.
16. Garcia-Cuellar, C., Montanez, C., Tenorio, V., Reyes-Esparza, J., Duran, M.J., Negrete, E., Guerrero, A., de la Garza, M., A 24-kDa cloned zinc metalloprotease from Actinobacillus pleuropneumoniae is common to all serotypes and cleaves actin in vitro. *Can J Vet Res*, 2000; **64**: 88-95.
17. Garcia Gonzalez, O., Garcia, R.M., de la Garza, M., Vaca, S., Paniagua, G.L., Mejia, R., Tenorio, V.R., Negrete-Abascal, E., Actinobacillus pleuropneumoniae metalloprotease: cloning and in vivo expression. *FEMS Microbiol Lett*, 2004; **234**: 81-86.
18. Gottschalk, M., Actinobacillus species in animal disease: A topical subject. *Vet J*, 2000; **159**: 5-7.
19. Grasteau, A., Tremblay, Y.D., Labrie, J., Jacques, M., Novel genes associated with biofilm formation of Actinobacillus pleuropneumoniae. *Vet Microbiol*, 2011; **153**: 134-143.
20. Gutierrez-Martin, C.B., del Blanco, N.G., Blanco, M., Navas, J., Rodriguez-Ferri, E.F., Changes in antimicrobial susceptibility of Actinobacillus pleuropneumoniae isolated from pigs in Spain during the last decade. *Vet Microbiol*, 2006; **115**: 218-222.
21. Hendriksen, R.S., Mevius, D.J., Schroeter, A., Teale, C., Jouy, E., Butaye, P., Franco, A., Utinane, A., Amado, A., Moreno, M., Greko, C., Stark, K.D., Berghold, C., Myllyniemi, A.L., Hoszowski, A., Sunde, M., Aarestrup, F.M., Occurrence of antimicrobial resistance among bacterial pathogens and indicator bacteria in pigs in different European countries from year 2002 - 2004: the ARBAO-II study. *Acta Vet Scand*, 2008; **50**: 19.
22. Hodgetts, A., Bosse, J.T., Kroll, J.S., Langford, P.R., Analysis of differential protein expression

in Actinobacillus pleuropneumoniae by Surface Enhanced Laser Desorption Ionisation—ProteinChip (SELDI) technology. *Vet Microbiol,* 2004; **99**: 215-225.

23. Intorre, L., Vanni, M., Di Bello, D., Pretti, C., Meucci, V., Tognetti, R., Soldani, G., Cardini, G., Jousson, O., Antimicrobial susceptibility and mechanism of resistance to fluoroquinolones in Staphylococcus intermedius and Staphylococcus schleiferi. *J Vet Pharmacol Ther,* 2007; **30**: 464-469.
24. Ito, H., Ishii, H., Akiba, M., Analysis of the complete nucleotide sequence of an Actinobacillus pleuropneumoniae streptomycin-sulfonamide resistance plasmid, pMS260. *Plasmid,* 2004; **51**: 41-47.
25. Jacobsen, M.J., Nielsen, J.P., Development and evaluation of a selective and indicative medium for isolation of Actinobacillus pleuropneumoniae from tonsils. *Vet Microbiol,* 1995; **47**: 191-197.
26. Jensen, V.F., Jakobsen, L., Emborg, H.D., Seyfarth, A.M., Hammerum, A.M., Correlation between apramycin and gentamicin use in pigs and an increasing reservoir of gentamicin-resistant Escherichia coli. *J Antimicrob Chemother,* 2006; **58**: 101-107.
27. Jessing, S.G., Angen, O., Inzana, T.J., Evaluation of a multiplex PCR test for simultaneous identification and serotyping of Actinobacillus pleuropneumoniae serotypes 2, 5, and 6. *J Clin Microbiol,* 2003; **41**: 4095-4100.
28. Jolie, R.A., Mulks, M.H., Thacker, B.J., Cross-protection experiments in pigs vaccinated with Actinobacillus pleuropneumoniae subtypes 1A and 1B. *Vet Microbiol,* 1995; **45**: 383-391.
29. Kang, M., Zhou, R., Liu, L., Langford, P.R., Chen, H., Analysis of an Actinobacillus pleuropneumoniae multi-resistance plasmid, pHB0503. *Plasmid,* 2009; **61**: 135-139.
30. Kim, B., Min, K., Choi, C., Cho, W.S., Cheon, D.S., Kwon, D., Kim, J., Chae, C., Antimicrobial susceptibility of Actinobacillus pleuropneumoniae isolated from pigs in Korea using new standardized procedures. *J Vet Med Sci,* 2001; **63**: 341-342.
31. Kim, C.H., Oh, Y., Han, K., Seo, H.W., Kim, D., Kang, I., Park, C., Jang, K.Y., Kim, S.H., Chae, C., Expression of secreted mucins (MUC2, MUC5AC, MUC5B, and MUC6) and membrane-bound mucin (MUC4) in the lungs of pigs experimentally infected with Actinobacillus pleuropneumoniae. *Res Vet Sci,* 2012; **92**: 486-491.
32. Kucerova, Z., Hradecka, H., Nechvatalova, K., Nedbalcova, K., Antimicrobial susceptibility of Actinobacillus pleuropneumoniae isolates from clinical outbreaks of porcine respiratory diseases. *Vet Microbiol,* 2011; **150**: 203-206.
33. Li, L., Sun, C., Yang, F., Yang, S., Feng, X., Gu, J., Han, W., Langford, P.R., Lei, L., Identification of proteins of Propionibacterium acnes for use as vaccine candidates to prevent infection by the pig pathogen Actinobacillus pleuropneumoniae. *Vaccine,* 2013; **31**: 5269-5275.
34. Livrelli, V., Peduzzi, J., Joly, B., Sequence and molecular characterization of the ROB-1 beta-lactamase gene from Pasteurella haemolytica. *Antimicrob Agents Chemother,* 1991: **35**; 242-251.
35. Lo, T.M., Ward, C.K., Inzana, T.J., Detection and identification of Actinobacillus pleuropneumoniae serotype 5 by multiplex PCR. *J Clin Microbiol,* 1998; **36**: 1704-1710.
36. Lu, Y.C., Li, M.C., Chen, Y.M., Chu, C.Y., Lin, S.F., Yang, W.J., DNA vaccine encoding type IV pilin of Actinobacillus pleuropneumoniae induces strong immune response but confers limited protective efficacy against serotype 2 challenge. *Vaccine,* 2011; **29**: 7740-7746.
37. Matter, D., Rossano, A., Limat, S., Vorlet-Fawer, L., Brodard, I., Perreten, V., Antimicrobial resistance profile of Actinobacillus pleuropneumoniae and Actinobacillus porcitonsillarum. *Vet Microbiol,* 2007; **122**: 146-156.
38. Matter, D., Rossano, A., Sieber, S., Perreten, V., Small multidrug resistance plasmids in Actinobacillus porcitonsillarum. *Plasmid,* 2008; **59**: 144-152.
39. McDowell, S.W., Ball, H.J., Serotypes of Actinobacillus pleuropneumoniae isolated in the British Isles. *Vet Rec,* 1994; **134**: 522-523.
40. McEwen, S.A., Fedorka-Cray, P.J., Antimicrobial use and resistance in animals. *Clin Infect Dis,* 2002: **34** Suppl 3, S93-S106.
41. Morioka, A., Asai, T., Nitta, H., Yamamoto, K., Ogikubo, Y., Takahashi, T., Suzuki, S., Recent trends in antimicrobial susceptibility and the presence of the tetracycline resistance gene in Actinobacillus pleuropneumoniae isolates in Japan. *J Vet Med Sci,* 2008; **70**: 1261-1264.
42. Nedbalcova, K., Nechvatalova, K., Pokludova, L., Bures, J., Kucerova, Z., Koutecka, L., Hera, A., Resistance to selected betalactam antibiotics. *Vet Microbiol* 2014.
43. Negrete-Abascal, E., Tenorio, V.R., Guerrero, A.L., Garcia, R.M., Reyes, M.E., de la Garza, M., Purification and characterization of a protease from Actinobacillus pleuropneumoniae serotype 1, an antigen common to all the

serotypes. *Can J Vet Res*, 1998; **62**: 183-190.
44. Negrete-Abascal, E., Tenorio, V.R., Serrano, J.J., Garcia, C., de la Garza, M., Secreted proteases from Actinobacillus pleuropneumoniae serotype 1 degrade porcine gelatin, hemoglobin and immunoglobulin A. *Can J Vet Res*, 1994: **58**: 83-86.
45. Nielsen, R., New diagnostic techniques: a review of the HAP group of bacteria. *Can J Vet Res*,1990; **54:** Suppl, S68-72.
46. O'Neill, C., Jones, S.C., Bosse, J.T., Watson, C.M., Williamson, S.M., Rycroft, A.N., Kroll, J.S., Hartley, H.M., Langford, P.R., Population-based analysis of Actinobacillus pleuropneumoniae ApxIVA for use as a DIVA antigen. *Vaccine*, 2010; **28**: 4871-4874.
47. Oh, Y., Ha, Y., Han, K., Seo, H.W., Kang, I., Park, C., Kim, S.C., Kim, S.H., Chae, C., Expression of leucocyte function-associated antigen-1 and intercellular adhesion molecule-1 in the lungs of pigs infected with Actinobacillus pleuropneumoniae. *J Comp Pathol*, 2013; **148**: 259-265.
48. Perry, M.B., Angen, O., MacLean, L.L., Lacouture, S., Kokotovic, B., Gottschalk, M., n atypical biotype I Actinobacillus pleuropneumoniae serotype 13 is present in North America. *Vet Microbiol*, 2012; **156**: 403-410.
49. Pridmore, A., Burch, D., Lees, P., Determination of minimum inhibitory and minimum bactericidal concentrations of tiamulin against field isolates of Actinobacillus pleuropneumoniae. *Vet Microbiol*, 2011; **151**: 409-412.
50. Priebe, S., Schwarz, S., In vitro activities of florfenicol against bovine and porcine respiratory tract pathogens. *Antimicrob Agents Chemother*, 2003; **47**: 2703-2705.
51. Pyorala, S., Baptiste, K.E., Catry, B., van Duijkeren, E., Greko, C., Moreno, M.A., Pomba, M.C., Rantala, M., Ruzauskas, M., Sanders, P., Threlfall, E.J., Torren-Edo, J., Torneke, K., Macrolides and lincosamides in cattle and pigs: Use and development of antimicrobial resistance. *Vet J*, 2014; **200**: 230-239.
52. Rosendal, S., Boyd, D.A., Gilbride, K.A., Comparative virulence of porcine Haemophilus bacteria. *Can J Comp Med* , 1985: **49**: 68-74.
53. Schuchert, J.A., Inzana, T.J., Angen, O., Jessing, S., Detection and identification of Actinobacillus pleuropneumoniae serotypes 1, 2, and 8 by multiplex PCR. *J Clin Microbiol*, 2004; **42**: 4344-4348.
54. Sebunya, T.N., Saunders, J.R., Haemophilus pleuropneumoniae infection in swine: a review. *J Am Vet Med Assoc*, 1983; **182**: 1331-1337.
55. Seo, K.W., Kim, S.H., Park, J., Son, Y., Yoo, H.S., Lee, K.Y., Jang, Y.S., Nasal immunization with major epitope-containing ApxIIA toxin fragment induces protective immunity against challenge infection with Actinobacillus pleuropneumoniae in a murine model. *Vet Immunol Immunopathol*, 2013; **151**: 102-112.
56. Serrano-Rubio, L.E., Tenorio-Gutierrez, V., Suarez-Guemes, F., Reyes-Cortes, R., Rodriguez-Mendiola, M., Arias-Castro, C., Godinez-Vargas, D., de la Garza, M., Identification of Actinobacillus pleuropneumoniae biovars 1 and 2 in pigs using a PCR assay. *Mol Cell Probes*, 2008; **22**: 305-312.
57. Shin, M.K., Kang, M.L., Jung, M.H., Cha, S.B., Lee, W.J., Kim, J.M., Kim, D.H., Yoo, H.S., Induction of protective immune responses against challenge of Actinobacillus pleuropneumoniae by oral administration with Saccharomyces cerevisiae expressing Apx toxins in pigs. *Vet Immunol Immunopathol*, 2013; **151**: 132-139.
58. Shin, S.J., Kang, S.G., Nabin, R., Kang, M.L., Yoo, H.S., . Evaluation of the antimicrobial activity of florfenicol against bacteria isolated from bovine and porcine respiratory disease. *Vet Microbiol*, 2005; **106**: 73-77.
59. Sjolund, M., Zoric, M., Persson, M., Karlsson, G., Wallgren, P., Disease patterns and immune responses in the offspring to sows with high or low antibody levels to Actinobacillus pleuropneumoniae serotype 2. *Res Vet Sci*, 2011; **91**: 25-31.
60. Suzuki, S., Ohmae, K., Ohishi, K., Muramatsu, M., Takahashi, T., Antimicrobial susceptibility of Actinobacillus (Haemophilus) pleuropneumoniae isolated from pigs with pleuropneumonia. *Nihon Juigaku Zasshi*, 1989; **51**: 450-452.
61. Tobias, T.J., Bouma, A., Klinkenberg, D., Daemen, A.J., Stegeman, J.A., Wagenaar, J.A., Duim, B., Detection of Actinobacillus pleuropneumoniae in pigs by real-time quantitative PCR for the apxIVA gene. *Vet J*, 2012; **193**: 557-560.
62. Tobias, T.J., Klinkenberg, D., Bouma, A., van den Broek, J., Daemen, A.J., Wagenaar, J.A., Stegeman, J.A., A cohort study on Actinobacillus pleuropneumoniae colonisation in suckling piglets. *Prev Vet Med*, 2014; **114**: 223-230.
63. Vanni, M., Merenda, M., Barigazzi, G., Garbarino, C., Luppi, A., Tognetti, R., Intorre, L., Antimicrobial resistance of Actinobacillus pleuropneumoniae isolated from swine. *Vet Microbiol*, 2012; **156**: 172-177.

64. Wang, Y.C., Chan, J.P., Yeh, K.S., Chang, C.C., Hsuan, S.L., Hsieh, Y.M., Chang, Y.C., Lai, T.C., Lin, W.H., Chen, T.H., Molecular characterization of enrofloxacin resistant Actinobacillus pleuropneumoniae isolates. *Vet Microbiol*, 2010; **142**: 309-312.
65. Wasteson, Y., Roe, D.E., Falk, K., Roberts, M.C., Characterization of tetracycline and erythromycin resistance in Actinobacillus pleuropneumoniae. *Vet Microbiol*, 1996; **48**: 41-50.
66. West, S.E., Romero, M.J., Regassa, L.B., Zielinski, N.A., Welch, R.A., Construction of Actinobacillus pleuropneumoniae-Escherichia coli shuttle vectors: expression of antibiotic-resistance genes. *Gene*, 1995; **160**: 81-86.
67. Wright, C.L., Strugnell, R.A., Hodgson, A.L., Characterization of a Pasteurella multocida plasmid and its use to express recombinant proteins in P. multocida. *Plasmid*, 1997; **37**: 65-79.
68. Yoshimura, H., Takagi, M., Ishimura, M., Endoh, Y.S., Comparative in vitro activity of 16 antimicrobial agents against Actinobacillus pleuropneumoniae. *Vet Res Commun*, 2002; **26**: 11-19.
69. Zhou, L., Jones, S.C., Angen, O., Bosse, J.T., Nash, J.H., Frey, J., Zhou, R., Chen, H.C., Kroll, J.S., Rycroft, A.N., Langford, P.R., PCR specific for Actinobacillus pleuropneumoniae serotype 3. *Vet Rec*, 2008; **162**: 648-652.