Phenotypic Characterization and Random Amplified Polymorphic DNA (RAPD) analysis of *Pasteurella multocida* isolated from Korean Pigs

Ki-Eun LEE¹⁾, Hye-Young JEOUNG¹⁾, Ji-Youn LEE¹⁾, Myoung-Heon LEE¹⁾, Hwan-Won CHOI²⁾, Kyung-Soo CHANG³⁾, Young-Hee OH⁴⁾ and Dong-Jun AN^{1)*}

¹⁾ Animal, Plant and Fisheries Quarantine and Inspection Agency, Anyang, Gyeonggi-do, 430–824, South Korea

²⁾ ChoongAng Vaccine Laboratories, Daejeon, 305–348, South Korea

³⁾ Catholic University of Pusan, Busan, 609–757, South Korea

⁴⁾ Seoul metropolitan Government Research Institute of Public Health and Environment, Gwacheon, Gyeonggi-do, 427–070, South Korea

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ABSTRACT. *Pasteurella multocida* causes various respiratory disease symptoms in pigs, including atrophic rhinitis and pneumonia. In the present study, 69 strains of *P. multocida* were isolated from 443 pigs with respiratory clinical symptoms at 182 farms located throughout South Korea from 2009 to 2010. A multiplex capsular PCR typing assay revealed that 69 strains of *P. multocida* isolated in this study had the biosynthetic locus of the capsules of either serogroup A (47 strains, 68.1%) or serogroup D (22 strains, 31.9%). The 22 strains positive for serogroup D-specific primers were divided into four clusters and the 47 strains positive for serogroup A-specific primers were divided into 12 clusters according to the results of Random Amplified Polymorphic DNA (RAPD) analysis. *P. multocida* strains in the present study were susceptible to most of the antimicrobial agents used. An analysis of antimicrobial resistance and virulence gene pattern combined with RAPD indicated that a certain *P. multocida* strain appeared to be genetically identical, implying the persistence of the strain within a single farm.

KEY WORDS: antimicrobial, *Pasteurella multocida*, RAPD, serogroup, swine.

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Pasteurella multocida is a pathogen that induces respiratory disease with symptoms such as cough, bronchial pneumonia, and severe breathing difficulty in pigs. Chronic respiratory *P. multocida* infection results in a characteristic dry cough and delayed growth, whereas acute infection can bring about death in 5 to 10 days [9]. *P. multocida* is divided into five serogroups (A, B, D, E, and F) based on their capsular polysaccharide. Serogroups A and D lead to major pig lung disease, and if they are combined with other pathogens, such as *Mycoplasma hyopneumoniae* or pseudorabies virus, they can increase the severity of pneumonia lesions [2, 4, 7, 10]. *P. multocida* infections are transmitted by droplet or contact.

In 2003, *P. multocida* was isolated from lung lesions in 10.3% of pigs with pneumonia in South Korea. The strains isolated were sensitive to most antimicrobial agents, including ampicillin, ceftiofur and enrofloxacin, but had low-level resistance to neomycin [9, 11, 18].

Other studies of *P. multocida* have investigated pathogenic factors such as capsular polysaccharide, virulence factors, lipopolysaccharide, and outer membrane protein [6, 14, 16]. These characteristics have also been analyzed by ribotyping, single-primer PCR, and Random Amplified

Polymorphic DNA (RAPD) PCR [5, 12, 15]. The merit of the RAPD-PCR assay is that it is simple and requires less time to process than other methods of restriction endonuclease analysis [12, 15]. RAPD-PCR analysis for characterization of *P. multocida* has yielded reproducible and reliable results in comparison with other analytical methods such as whole cell protein, outer membrane protein, serotyping, and restriction endonuclease analyses [15]. An ornithological study showed that 66 strains of *P. multocida* isolated from birds could be easily and quickly categorized into 18 groups through RAPD-PCR [12]. *P. multocida* has been isolated from many pigs with respiratory disease, but no major studies characterizing the strains, nor any major epidemiological studies, have been undertaken for isolates from South Korea.

The purpose of this study was to investigate the distribution of serogroups and virulence genes, and determine the antimicrobial susceptibility of *P. multocida* isolated from pigs with respiratory disease in South Korea from 2009 to 2010. In addition, RAPD-PCR was performed to clarify and compare the genetic characteristics of *P. multocida*.

MATERIALS AND METHODS

Isolation and identification of *P. multocida*: Lung samples were collected from pigs with respiratory diseases, such as pneumonia and breathing difficulties by Choon-Ang Vaccine Laboratories of the animal appraisal organization. A total of 443 pig lung samples were obtained from 182 pig farms in nine provinces (Gyeonggi, Gang-

*CORRESPONDENCE TO: AN, D.-J., Research Planning Division, Animal, Plant and Fisheries Quarantine and Inspection Agency, Anyang, Gyeonggi-do 430-824, South Korea.
e-mail: andj67@korea.kr

won, Chungbuk, Chungnam, Jeonbuk, Jeonnam, Gyeongbuk, Gyeongnam, and Jeju) throughout South Korea from January 2009 to November 2010. Among the farms where lung samples were collected, pigs with respiratory clinical symptoms were again detected after several months. All lung samples were collected under aseptic conditions and were inoculated on to brain heart infusion (BHI) agar (Difco, Beckton, Dickinson and Co., Sparks, MD, U.S.A.) containing 5% sheep blood and incubated for 24 hr at 37°C. The suspected colonies (mucoid, non-hemolytic colonies) were tested with MacConkey agar (Difco, Beckton, Dickinson and Co.), gram stain, and biochemistry (oxidase, indole, and urease).

Preparation of *P. multocida* genomic DNA: The isolated *P. multocida* were inoculated into BHI broth (Difco, Beckton, Dickinson and Co.) and cultured for 18 hr. *P. multocida* genomic DNA was extracted using the Genomic DNA Extraction Kit (INTRON, Gyeonggi, South Korea) and was stored at -20°C until use.

Detection of *P. multocida* by PCR: The KMT1 gene (common gene of *P. multocida* capsule) of *P. multocida* was amplified with a primer set (5'-ATCCGCTATTACCCAGTGG-3' and 5'-GCTGTAAACGAACCTCGC-CAC-3') as previously described [16]. The PCR conditions consisted of 5 min at 95°C, followed by 30 cycles of 30 sec at 95°C, 30 sec at 55°C, and 30 sec at 72°C. A DNA fragment of 460 bp was expected.

Genotyping of capsular polysaccharide gene by multiplex-PCR: To distinguish capsular polysaccharide, multiplex-PCR was performed as previously described [16]. The multiplex-PCR was performed by 5 min at 95°C, 30 cycles of 30 sec at 95°C, 30 sec at 55°C, and 30 sec at 72°C. The amplification fragments, anticipated to be 1,044 bp (serogroup A), 760 bp (serogroup B), 657 bp (serogroup D), 511 bp (serogroup E), and 851 bp (serogroup F) in length, were visualized on a 1% agarose gel.

Detection of virulence genes by PCR: The detection of virulence genes (*hsf-I*, *pflA*, *hgbB*, *toxA*, and *nanB*) was carried out using a PCR assay as previously described [3]. Three of the genes, *pflA*, *hgbB* and *toxA*, were amplified with 35 cycles of 45 sec at 94°C, 50 sec at 54°C, and 50 sec at 72°C. The two other genes, *nanB* and *hsf-I*, were amplified with 30 cycles of 30 sec at 94°C, 30 sec at 56°C, and 30 sec at 72°C. The amplification fragments, anticipated to be 846 bp (*toxA*), 654 bp (*hsf-I*), 550 bp (*nanB*), 499 bp (*hgbB*) and 275 bp (*pflA*) in length, were visualized using gel electrophoresis on a 2% agarose gel.

Antimicrobial susceptibility testing: Minimal inhibitory concentrations (MICs) were determined using the standard broth dilution methods according to Clinical Laboratory Standards Institute (CLSI) guideline (M31-A3) for ampicillin, penicillin, ceftiofur, danofloxacin, enrofloxacin, spectinomycin, sulphadimethoxine, gentamicin, neomycin, tilmicin, tulathromycin, florfenicol, tiamulin, chlortetracycline, and oxytetracycline. As a control strain, *Escherichia coli* (ATCC 25922) was used.

Random amplified polymorphic DNA (RAPD) PCR: The primer used for RAPD-PCR was MM13 (5'-GAGGGTG-

GNGGNTCT-3'), as previously described [15]. The PCR conditions consisted of 30 cycles of 30 sec at 95°C, 30 sec at 42°C, and 30 sec at 72°C. The products were subjected to electrophoresis at 100 volts in 2% agarose gel, and DNA bands in the gel were stained with ethidium bromide. DNA banding patterns were analyzed using BioNumerics software (Applied Maths) to calculate Dice coefficients of correlation and to generate a dendrogram using the unweighted pair group method of arithmetic averages (UPGMA) clustering.

RESULTS

Isolation of *P. multocida*: *P. multocida* was isolated from 64 of the 182 farms from which pig specimens were collected. The total number of positive farms from which *P. multocida* was isolated in each region during the study period was as follows: Gyeongbuk (n=30), Chungnam (n=10), Gyeongnam (n=8), Gyeonggi (n=6), Jeonbuk (n=5) and other regions (n=5) (Table 1). Because pigs with respiratory symptoms were detected after 4 months in 4 farms (farms A and D in Gyeongbuk, farm B in Jeonnam, and farm C in Gyeongnam), the animals were sampled again to isolate *P. multocida* (Table 4). *P. multocida* was isolated from 19.4% (38/196) of the pigs examined in 2009 and 12.6% (31/247) of the pigs examined in 2010 (Table 1).

Genotyping of the capsular polysaccharide gene by multiplex-PCR: Of the 38 strains isolated in 2009, 24 strains (63.2%) carried the biosynthetic locus of the capsule of serogroup A and 14 (36.8%) carried that of serogroup D. Among the 31 strains isolated in 2010, the biosynthetic locus of the capsules of serogroups A and D were detected in 23 (74.2%) and 8 strains (25.8%), respectively (Table 1).

Distribution of virulence genes: The distributions of five virulence genes (*hsf-I*, *pflA*, *hgbB*, *toxA*, and *nanB*) among *P. multocida* strains isolated in this study are shown in Table 2. The *hsf-I*, *hgbB* (adhesins) and *nanB* (sialidase) virulence genes were each present in >80% of *P. multocida* strains having the locus of the capsule of serogroup D and the *toxA* gene was found in only two of the strains. The virulence genes in *P. multocida* isolated from the same farm at intervals of several months are presented in Table 4.

Antimicrobial susceptibility: Antimicrobial susceptibility testing of *P. multocida* showed that there was no strain resistant to the five antimicrobials tested (ceftiofur, tilmicin, tulathromycin, chlortetracycline, and oxytetracycline), although against the other antimicrobials a low level of resistance was shown (Table 3). The MIC ranges for isolated strains between 2009 and 2010 were somewhat different. Comparing MIC₉₀ (μg/ml), that of danofloxacin decreased from >1 μg/ml in 2009 to ≤0.12 μg/ml in 2010, and that of enrofloxacin decreased from 1 μg/ml in 2009 to 0.25 μg/ml in 2010. On the other hand, the resistance rate for tiamulin increased from 42.10% in 2009 to 74.19% in 2010. The antimicrobial resistance patterns for *P. multocida* isolated from the same farm at intervals of several months are presented in Table 4.

RAPD analysis: The DNA banding pattern for the *P. mul-*

Table 1. Number of farms and number of pigs from which *P. multocida* was isolated during 2009–2010

Province	2009				2010			
	No. of farm tested (No. of positive farm)	No. of pig tested (No. of positive pigs)	No. of strains with serogroup*		No. of farm tested (No. of positive farm)	No. of pig tested (No. of positive pigs)	No. of strains with serogroup*	
			A	D			A	D
Gyeonggi	7 (4)	12 (4)	4	0	11 (2)	13 (2)	2	0
Gangwon	0 (0)	0 (0)	0	0	1 (0)	3 (0)	0	0
Chungbuk	1 (0)	2 (0)	0	0	3 (1)	13 (1)	1	0
Chungnam	8 (4)	17 (4)	2	2	33 (6)	60 (6)	4	2
Jeonbuk	6 (2)	23 (2)	1	1	6 (3)	17 (3)	1	2
Jeonnam	3 (2)	9 (3)	3	0	10 (1)	23 (1)	0	1
Gyeongbuk	32 (18)	103 (18)	10	8	30 (12)	80 (13)	11	2
Gyeongnam	11 (3)	29 (6)	4	2	19 (5)	38 (5)	4	1
Jeju	1 (1)	1 (1)	0	1	0 (0)	0 (0)	0	0
Total	69 (34)	196 (38)	24	14	113 (30)	247 (31)	23	8

*The biosynthetic locus of the capsule of serogroups A and D were detected by a multiplex PCR analysis.

Table 2. Virulence gene distribution in *P. multocida* strains

Gene function	Gene	Description	No. of strains with serogroup*		Total (n=69)
			A	D	
Adhesins	<i>hsf-1</i>	Autotransporter adhesion	1 (2.1%)	20 (90.9%)	21 (30.4%)
Adhesins	<i>pfh-A</i>	Filamentous hemagglutinin	13 (27.6%)	0 (0.0%)	13 (18.8%)
Iron acquisition	<i>hgbB</i>	Hemoglobin-binding protein	33 (70.2%)	21 (95.4%)	54 (78.3%)
Toxins	<i>tox A</i>	Dermonecrotic toxin	0 (0.0%)	2 (9.1%)	2 (2.9%)
Sialidase	<i>nanB</i>	Outer membrane associated protein, an autotransporter protein	18 (38.3%)	18 (81.8%)	36 (52.2%)

*The biosynthetic locus of the capsule of serogroups A and D were detected by a multiplex PCR analysis.

Table 3. Minimal Inhibitory Concentration (MIC) and resistance rates of *P. multocida* isolated from pig during 2009–2010

Antimicrobial agents	Breakpoint	2009 (n=38)			2010 (n=31)		
		MIC range ($\mu\text{g/ml}$)	MIC ₉₀ ($\mu\text{g/ml}$)	Resistance (%)	MIC range ($\mu\text{g/ml}$)	MIC ₉₀ ($\mu\text{g/ml}$)	Resistance (%)
Ampicillin	—*	$\leq 0.25 - 16$	4	—	$\leq 0.25 - > 16$	≤ 0.25	—
Penicillin	—*	$\leq 0.12 - > 8$	> 8	—	$\leq 0.12 - > 8$	2	—
Ceftiofur	≥ 8	$\leq 0.25 - 1$	≤ 0.25	0.0	≤ 0.25	≤ 0.25	0.0
Danofloxacin	≥ 0.25	$\leq 0.12 - > 1$	> 1	26.3	$\leq 0.12 - > 1$	≤ 0.12	3.2
Enrofloxacin	≥ 2	$\leq 0.12 - 2$	1	7.9	$\leq 0.12 - > 2$	0.25	3.2
Spectinomycin	—*	$\leq 8 - > 64$	> 64	—	$16 - > 64$	32	—
Sulphadimethoxine	—*	$256 - > 256$	> 256	—	$256 - > 256$	> 256	—
Gentamicin	≥ 16	$\leq 1 - > 16$	> 16	18.4	$\leq 1 - > 16$	8	6.5
Neomycin	—*	$\leq 4 - > 32$	> 32	—	$\leq 4 - 16$	8	—
Tilmicosin	≥ 32	$\leq 4 - 16$	8	0.0	$\leq 4 - 16$	8	0.0
Tulathromycin	≥ 64	$\leq 1 - 8$	4	0.0	$\leq 1 - 4$	2	0.0
Florfenicol	≥ 8	$\leq 0.25 - > 8$	8	10.5	$\leq 0.25 - > 8$	8	16.5
Tiamulin	≥ 32	$\leq 4 - > 32$	32	42.1	$8 - > 32$	> 32	74.2
Chlortetracycline	≥ 16	$\leq 0.5 - 8$	2	0.0	$\leq 0.5 - 8$	4	0.0
Oxytetracycline	≥ 16	$\leq 0.5 - 8$	8	0.0	$\leq 0.5 - 8$	8	0.0

* There is no antimicrobial breakpoint in the Clinical Laboratory Standard Institute (CLSI) guideline.

multocida strains amplified using the MMI3 primer is indicated in Figs. 1 and 2. The 22 strains with the biosynthetic locus of the capsule of serogroup D strains were classified into four clusters based on 80% similarity in the RAPD banding pattern (Fig. 1). Three of the strains, KVCC-BA1100179, KVCC-BA1100188, and KVCC-BA1100213, were isolated

from farm A in Gyeongbuk province in April, August, and December, 2009, respectively. The remaining 19 strains were isolated from different farms. The 47 strains with the locus of the capsule of serogroup A strains were divided into 12 clusters (Fig. 2). Following strains with the locus of the capsule of serogroup A isolated from the same farm

Table 4. Random Amplified Polymorphic DNA (RAPD), antimicrobial resistance and virulence gene pattern for *P. multocida* isolates from pigs of four farms

Province	Date	Strain No.	Serogroup*	RADP pattern	Antimicrobial resistance pattern**	Virulence gene				
						<i>hsf-1</i>	<i>pfhA</i>	<i>hgbB</i>	<i>toxA</i>	<i>nanB</i>
Farm A (Gyeongbuk)	Apr 2009	KVCC-BA1100179	D	Cluster I	DAN-TIA	+	-	+	-	+
	Aug 2009	KVCC-BA1100188	D	Cluster I	DAN-TIA	+	-	+	-	+
	Dec 2009	KVCC-BA1100213	D	Cluster III	TIA	+	-	+	-	-
Farm B (Jeonnam)	Oct 2009	KVCC-BA1100194	A	Cluster I	DAN	-	-	+	-	+
	Nov 2009	KVCC-BA1100214	A	Cluster XII	GEN	-	+	+	-	-
Farm C (Gyeongnam)	Apr 2010	KVCC-BA1100221	A	Cluster II	No resistance	-	+	+	-	-
	Sep 2010	KVCC-BA1100229	A	Cluster II	No resistance	-	-	+	-	+
Farm D (Gyeongbuk)	Apr 2009	KVCC-BA1100177	A	Cluster VI	DAN	-	-	-	-	-
	Aug 2009	KVCC-BA1100190	A	Cluster VI	DAN	-	-	-	-	-

*The biosynthetic locus of the capsule of serogroups A and D were detected by a multiplex PCR analysis.

**DAN, danofloxacin; TIA, tiamulin; GEN, gentamicin.

Fig. 1. RAPD profiles of *P. multocida* strains with the biosynthetic locus of the capsule of serogroup D that were analyzed using the BioNumerics program (Applied Maths) to generate a dendrogram based on the Dice coefficient. Twenty two strains were divided into four clusters at similar rate of 80%. ^a Strains isolated from farm A in Gyeongbuk.

at different times were classified into identical clusters: KVCC-BA1100221 (April, 2010) and KVCC-BA1100229 (September, 2010) from farm C in Gyeongnam, and KVCC-BA1100177 (April, 2009) and KVCC-BA1100190 (August, 2009) from farm D in Gyeongbuk. The remaining 43 strains were isolated from different farms.

DISCUSSION

The major serogroups of *P. multocida* that reproduce in

the respiratory tract of pigs are A and D [14]. Of 80 strains of *P. multocida* isolated from slaughtered Korean pigs in 1994, 77 (96.3%) were found to be serogroup A and the other three were serogroup D [1]. Of 91 strains isolated in 2009, 69 (75.8%) were serogroup A and 22 (24.2%) were serogroup D [13]. The majority (68.1%) of the isolates from the present study carried the biosynthetic locus of the capsule of serogroup A, suggesting that serogroup A has been the most common cause of pig respiratory disease up to now. No pigs appeared to be coinfecting with multiple

Fig. 2. RAPD profiles of *P. multocida* strains with the biosynthetic locus of the capsule of serogroup A that were analyzed using the BioNumerics program (Applied Maths) to generate a dendrogram based on the Dice coefficient. Forty seven strains were divided into eleven clusters at similar rate of 80%. ^a Strains isolated from farm B in Jeonnam; ^b strains isolated from farm C in Gyeongnam; ^c strains isolated from farm D in Gyeongbuk.

strains with the locus of the capsule of serogroups A and D in the present study. Serious respiratory symptoms in pig may stem from the more pathogenic virulence factors (*hsf-1* and *nanB*) and the dermonecrotic toxin of serogroup D [8, 14]. Therefore, ongoing observation of the pattern of pig respiratory disease is needed.

Antimicrobials that are used domestically for disease prevention and treatment purposes in livestock are administered by addition to feedstuff, but regulation concerning this type of use of antimicrobials is becoming more complicated. Since 2005, in order to prevent the over-use and misuse of antimicrobials and to increase the safety of livestock products, there has been a step-by-step decrease in the types of antimicrobials that are added to feedstuff. According to the Animal, Plant and Fisheries Quarantine and Inspection Agency (www.qia.go.kr), there were 11 types of antimicrobials that were permitted in feedstuff in South Korea up until recently.

A study of 87 strains of *P. multocida* isolated from Korean pigs between 1996 and 1997 showed resistance rates to ampicillin, enrofloxacin, gentamicin and trimethoprim/sulfamethoxazole of 3.2%, 4.6%, 16.1% and 48.3%, respectively [11]. In another study, 31 strains isolated in South Korea in 2003 also showed resistance to ampicillin, enrofloxacin, gentamicin, and trimethoprim/sulfamethoxazole, with resistance rates of 3.4%, 0%, 28.9%, and 38.7%, respectively, as well as to streptomycin, penicillin, and neomycin, indicating low susceptibility [9]. Antimicrobial susceptibility analysis in the present study yielded resistance rates in the isolated strains for both years (2009/2010), as follows: ceftiofur (0.0%/0.0%), enrofloxacin (15.8%/3.2%), gentamicin (28.9%/19.3%), and chlortetracycline (0%/0%). These results were similar to rates of antimicrobial resistance reported previously [9, 11]. Interestingly, tiamulin exhibited a higher resistance rate in the 2010 isolates than in the 2009 isolates. Of 132 *P. multocida* strains isolated in Germany between 2002 and 2003, only 0.2% were found to be resistant to enrofloxacin [17], while the present study found a resistance rate of 15.8% for enrofloxacin. Another previous study reported that *P. multocida* showed 100% susceptibility to quinolone antimicrobials such as enrofloxacin and norfloxacin [1]; however, data from research done after 1999 have shown resistance levels of 6–8%. The addition of quinolone antimicrobials to feedstuff has been prohibited in South Korea since 2008, due to researchers' concerns.

Another paper found resistance rates in *P. multocida* strains isolated from China in 2009 to be 74.2% (trimethoprim/sulfamethoxazole), 65.2% (chlortetracycline), 80.3% (clindamycin), 28.3% (tilmicosin), and 13.7% (gentamicin) [14]. These resistance rates are far higher than those seen in strains isolated in South Korea and Europe. These high resistance rates seen in China are assumed to be due to the over-use and misuse of antimicrobials in feedstuff. In South Korea, the addition of antimicrobials to feedstuff has been totally prohibited since July 2011. In light of the new regulations, continuous observation for any change in antimicrobial susceptibility in strains isolated from livestock

is warranted.

RAPD analysis results for 69 strains show that they can be divided into 16 different clusters. The five strains (KVCC-BA1100175, -BA1100178, -BA1100179, -BA1100188, and -BA1100189) that carried the locus of the capsule of serogroup D had a similar rate of 100% in the RAPD dendrogram analysis. Among these, KVCC-BA1100179 (April, 2009) and KVCC-BA1100188 (August, 2009) strains were isolated from farm A in Gyeongbuk province and they appeared identical in antimicrobial resistance and virulence gene pattern. Also, KVCC-BA1100177 (April, 2009) and KVCC-BA1100190 (August, 2009), which belonged to cluster VI within the strains with the locus of the capsule of serogroup A, were collected on different dates from farm D in Gyeongbuk. The strains have identical antimicrobial resistance and virulence gene pattern, suggesting that persistence of identical strain within a single farm.

This study indicates that thorough disinfection and sterilization on pig farms will be necessary for the eradication of *P. multocida* in light of the prohibition of antimicrobials throughout South Korea.

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REFERENCES

1. Ahn, B. C., Cho, K. H. and Kim, B. H. 1994. Studies on *Pasteurella multocida* isolated from pneumonic lungs of slaughter pigs. *Korean J. Vet. Res.* **34**: 511–516.
2. Amass, S. F., Clark, L. K., van Alstine, W. G., Bowersock, T. L., Murphy, D. A., Knox, K. E. and Albrechts, S. R. 1994. Interaction of *Mycoplasma hyopneumoniae* and *Pasteurella multocida* infections in swine. *J. Am. Vet. Med. Assoc.* **204**: 102–107. [Medline]
3. Atashpaz, S., Shayegh, J. and Hejazi, M. S. 2009. Rapid virulence typing of *Pasteurella multocida* by multiplex-PCR. *Res. Vet. Sci.* **87**: 355–357. [Medline] [CrossRef]
4. Carter, G. R. 1988. Serological classification of *Pasteurella*. *Vet. Rec.* **122**: 311. [Medline] [CrossRef]
5. Dziva, F., Christensen, H., Van Leengoed, L. A. M. G., Mohan, K. and Olsen, J. E. 2004. Differentiation of *Pasteurella multocida* isolates from cases of atrophic rhinitis in pig from Zimbabwe by RAPD and ribotyping. *Vet. Microbiol.* **102**: 117–122. [Medline] [CrossRef]
6. Ewers, C., Lubke-Becker, A., Bethe, A., Kiebling, S., Filter, M. and Wieler, L. H. 2006. Virulence genotype of *Pasteurella multocida* strains isolated from different hosts with various disease status. *Vet. Microbiol.* **114**: 304–317. [Medline] [CrossRef]
7. Fuentes, M. and Pijoan, C. 1986. Phagocytosis and killing of *Pasteurella multocida* by alveolar macrophages after infection with pseudorabies virus. *Vet. Immunol. Immunopathol.* **13**: 165–172. [Medline] [CrossRef]
8. Harper, M., Boyce, J. D. and Adler, B. 2006. *Pasteurella multocida* pathogenesis: 125 years after Pasteur. *FEM. Microbiol. Lett.* **265**: 1–10. [CrossRef]
9. Lee, W. W., Woo, B. G. and Lee, G. R. 2003. Isolation and antimicrobial susceptibility test of *Pasteurella multocida* from respiratory disorder piglets. *Rep. Busan. Inst. Health Environ*

- 13: 142–150.
10. Lichtensteiger, C. A., Steenbergen, S. M., Steenbergen, R. M., Lee, R. M., Polson, D. D. and Vimr, E. R. 1996. Direct PCR analysis for toxigenic *Pasteurella multocida*. *J. Clin. Microbiol.* **34**: 3035–3039. [[Medline](#)]
 11. Shin, N. A., Park, J. Y., Park, Y. H. and Yoo, H. S. 1999. Characteristics of *Pasteurella multocida* isolated from pneumonic lung lesions of swine: antimicrobial susceptibility, plasmid profile and distribution of toxA. *Korean. J. Vet. Res* **39**: 1091–1098.
 12. Shivachandra, S. B., Kumar, A. A. and Chaudhuri, P. 2007. Differentiation of avian *Pasteurella multocida* strains by single primer PCR. *Vet. Res. Commun.* **31**: 941–949. [[Medline](#)] [[CrossRef](#)]
 13. Sohn, J. H., Choi, S. K. and Cho, G. J. 2009. Capsular serogroups and toxA gene of *Pasteurella multocida* isolated from pneumonic lung lesions of swine. *J. Vet. Clin.* **26**: 457–462.
 14. Tang, X., Zhao, Z., Hu, J., Wu, B., Cai, X., He, Q. and Chen, H. 2009. Isolation, antimicrobial resistance, and virulence genes of *Pasteurella multocida* strains from swine in China. *J. Clin. Microbiol.* **47**: 951–958. [[Medline](#)] [[CrossRef](#)]
 15. Taylor, J. D., Fulton, R. W., Dabo, S. M., Lehenbauer, T. W. and Confer, A. W. 2010. Comparison of genotypic and phenotypic characterization methods for *Pasteurella multocida* isolates from fatal cases of bovine respiratory disease. *J. Vet. Diagn. Invest.* **22**: 366–375. [[Medline](#)] [[CrossRef](#)]
 16. Townsend, K. M., Boyce, J. D., Chung, J. Y., Frost, A. J. and Adler, B. 2001. Genetic organization of *Pasteurella multocida* *cap* loci and development of a multiplex capsular PCR typing system. *J. Clin. Microbiol.* **39**: 924–929. [[Medline](#)] [[CrossRef](#)]
 17. Wallmann, J. 2006. Monitoring of antimicrobial resistance in pathogenic bacteria from livestock animals. *Int. J. Med. Microbiol.* **296**: 81–86. [[Medline](#)] [[CrossRef](#)]
 18. Yoshimura, H., Ishimaru, M., Endoh, Y. S. and Kojima, A. 2001. Antimicrobial susceptibility of *Pasteurella multocida* isolated from cattle and pigs. *J. Vet. Med. B Infect. Dis. Vet. Public Health* **48**: 555–560. [[Medline](#)] [[CrossRef](#)]