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<Short Communication>

Pasteurella multocida isolation from pigs with respiratory disease in Korea

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Abstract : A total of 131 *Pasteurella* (*P.*) *multocida* strains were isolated from the lungs of 1,064 pigs with respiratory clinical signs nationwide during 2009–2010 and 2013–2014. The strains of *P. multocida* comprised 77.1% serotype A and 22.9% serotype D. Analysis of a recent *P. multocida* outbreak in Korean pigs showed that the isolation rate of serotype D decreased annually. The incidence of antimicrobial resistance, as measured using minimal inhibitory concentration values, has decreased recently. Overall, further studies to characterize *P. multocida* isolated from pigs in Korea are needed to prevent *P. multocida* infection in the Korean swine industry

Keywords : *Pasteurella multocida*, antimicrobial resistance, pig, serotype

Pasteurella (*P.*) *multocida* is a pathogen that causes respiratory symptoms such as cough, pneumonia, atopic rhinitis, and severe breathing in pigs, alone or in co-infection with other pathogens, such as *Bordetella bronchiseptica*, *Mycoplasma hyopneumoniae*, and Pseudorabies virus [2, 8].

P. multocida isolates have been classified into five serogroups, A, B, D, E, and F, based on capsular polysaccharide. Both A and D are the major *P. multocida* serogroups detected in the respiratory tract of pigs worldwide. Serogroup A plays a more important role in the pathogenesis of pneumonia than serogroup D, while serogroup D produces several virulence factors (*hsf-1* and *nanB*) and a dermonecrotic toxin. Therefore, infections with serogroup D can cause serious symptoms [6, 7, 10, 13, 15].

To reduce economic loss in the swine industry, various *Pasteurella* vaccines and antimicrobial agents have been used in Korea [5, 11, 12, 14]. According to the Korea Animal Health Products Association, the use of antimicrobial agents in livestock decreased steadily beginning in July 2011, until it was finally banned in March 2013 to avoid the overuse of antimicrobial agents in livestock and maintain public health through better food safety management. Therefore, use of *Pasteurella* vaccines in the animal industry has increased steadily and the several vaccine types are available commercially: 1) killed *P. multocida* cells; 2) whole *P. multocida* cells; 3) formalin-inactivated toxoids produced from authentic *P. multocida* toxin; 4) recombinant subunit *P. multocida* toxin derivatives; and 5) DNA vaccines comprising a modified *P. multocida* toxin gene [1, 4, 9].

The purpose of the present study was to isolate *P. multocida* from pigs with pneumonia in Korea and analyze the serogroup distribution from 2009–2010 and 2013–2014. We also assessed virulence factors and conducted antimicrobial resistance test of the *P. multocida* isolates.

A total of 1,064 lungs were collected from pigs showing respiratory disease from nine provinces (Gyeonggi, Gangwon, Chungbuk, Chungnam, Jeonbuk, Jeonnam, Gyeongbuk, Gyeongnam, and Jeju) nationwide between 2009–2010 and 2013–2014 by ChoonAng Vaccine Laboratories. All lung samples were collected under aseptic conditions and inoculated in brain-heart infusion (BHI) medium (Becton, Dickson and Company, USA.) containing 5% sheep's blood and incubated for 24 h at 37°C. The suspected colonies (mucoid, non-hemolytic colonies) were tested on MacConkey agar, and Gram staining and oxidase, indole, and urease tests were performed. The *P. multocida* isolates were deposited in the Korea Veterinary Culture Collection until further use.

P. multocida was inoculated into BHI broth (BD Difco, USA), cultured for 18 h, and subjected to DNA extraction using the boiling method. The *KMT1* gene of *P. multocida* was amplified using specific primers (forward, 5'-ATCCGCTATTTACCCAGTGG-3'; and reverse, 5'-GCTGTAAACGAACTCGCCAC-3') as described previously [11]. To distinguish capsular polysaccharide types, multiplex PCR was performed as described previously. The amplified fragments were of sizes 1044 bp (*capA*), 760 bp (*capB*), 657 bp (*capD*), 511 bp (*capE*), and 851 bp (*capF*) after resolution in a 1.5% agarose gel. Multiplex PCR was performed to detect the *hsf-*

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Table 1. Number of *Pasteurella multocida* isolated between 2009–2010 and 2013–2014

Province	2009				2010				2013				2014			
	Number of pig		Number of strains with serogroup		Number of pig		Number of strains with serogroup		Number of pig		Number of strains with serogroup		Number of pig		Number of strains with serogroup	
	Tested	Positive	A	D	Tested	Positive	A	D	Tested	Positive	A	D	Tested	Positive	A	D
Gyeonggi	12	(4)	4	0	13	(2)	2	0	24	(5)	4	1	24	(2)	2	0
Gangwon	0	(0)	0	0	3	(0)	0	0	4	(0)	0	0	1	(0)	0	0
Chungbuk	2	(0)	0	0	13	(1)	1	0	29	(0)	0	0	17	(1)	1	0
Chungnam	17	(4)	2	2	60	(6)	4	2	124	(19)	17	2	114	(5)	5	0
Jeonbuk	23	(2)	1	1	17	(3)	1	2	6	(2)	1	1	17	(0)	0	0
Jeonnam	9	(3)	3	0	23	(1)	0	1	2	(0)	0	0	4	(0)	0	0
Gyeongbuk	103	(18)	10	8	80	(13)	11	2	117	(18)	16	2	78	(5)	5	0
Gyeongnam	29	(6)	4	2	38	(5)	4	1	23	(3)	1	2	11	(2)	2	0
Jeju	1	(1)	0	1	0	(0)	0	0	2	(0)	0	0	0	(0)	0	0
Unknown	0	(0)	0	0	0	(0)	0	0	20	(0)	0	0	4	(0)	0	0
Total	196	(38)	24	14	247	(31)	23	8	351	(47)	39	8	270	(15)	15	0

Table 2. Virulence gene distribution of *Pasteurella multocida* isolated between 2009–2010 and 2013–2014

Gene	2009 (n = 38)			2010 (n = 31)			2013 (n = 47)			2014 (n = 15)		
	Number of positive Strains (% of positive strains)	Number of positive strains with serogroup		Number of positive Strains (% of positive strains)	Number of positive strains with serogroup		Number of positive Strains (% of positive strains)	Number of positive strains with serogroup		Number of positive Strains (% of positive strains)	Number of positive strains with serogroup	
		A	D		A	D		A	D		A	D
hsf-1	14 (36.8)	1	13	7 (22.5)	0	7	8 (17.0)	0	8	0 (0.0)	0	0
pfhA	7 (18.4)	7	0	6 (19.3)	6	0	8 (17.0)	8	0	5 (33.3)	5	0
hgbB	29 (76.3)	16	13	25 (80.6)	17	8	37 (78.7)	29	8	10 (66.6)	10	0
toxA	1 (2.6)	0	1	1 (3.2)	0	1	1 (2.1)	1	0	0 (0.0)	0	0
nanB	22 (57.9)	12	10	14 (45.1)	6	8	25 (53.1)	25	0	11 (73.3)	11	0

1, pfhA, hgbB, *toxA,* and *nanB* virulence genes, as described previously [3].

Minimal inhibitory concentrations (MICs) were determined using the standard broth dilution methods described in the Clinical and Laboratory Standard Institute guidelines (M31-A3) for veterinary microorganisms. The antimicrobial agents tested were: ampicillin, penicillin, ceftiofur, danofloxacin, enrofloxacin, gentamicin, neomycin, spetinomycin, tiamulin, tilmicosin, tulathromycin, florfenicol, chlortetracycline, sulphadimethoxacine. *Escherichia coli* ATCC25922 was used as the control strain.

In total, 131 *P. multocida* isolates were obtained from 1,064 pigs with respiratory clinical signs. *P. multocida* was isolated from 19.4% (38/196) of the pigs examined in 2009, 12.6% (31/247) in 2010, 13.4% (47/351) in 2013, and 5.6% (15/270) of the pigs examined in 2014 (Table 1). The regional distribution of isolates was as follows: Gyeonggi (n = 13), Chungbuk (n = 2), Chungnam (n = 34), Jeonbuk (n = 7), Jeonnam (n = 4), Gyeongbuk (n = 54), Gyeongnam (n = 16), and Jeju (n = 1). The seasonal incidence of *P. multocida* ranged from 5% to 13% (January to March), 3% to 16% (from April to June), 5% to 8% (July to September), and 5% to 16% (October to December).

The *P. multocida* isolates comprised 77.10% serotype A (n = 101) and 22.90% serotype D (n = 30). Of the 38 strains isolated in 2009, 24 (63%) were serotype A and 14 (37%) were serotype D. Of the 31 strains isolated in 2010, 23 (74%) were serotype A and 8 (26%) were serotype D. Of the 47 strains isolated in 2013, 39 (83%) were serotype A and 8 (17%) were serotype D. However, all of the 15 strains (n = 15) isolated in 2014 were serotype A (Table 1). Analysis of a recent *P. multocida* outbreak in pigs showed that the isolation rate of serotype D has decreased annually. According to the Korea Animal Health Products Association, the vaccine amount including *P. multocia* type D producing toxoid and toxiod has been increased. For this reason, it is estimated that type D was reduced.

The distribution of *hsf-1, pfhA, hgbB, toxA,* and *nanB* virulence genes among *P. multocida* isolates was presented in Table 2. The *hsf-1* (autotransporter adhesion) and *hgbB*

Table 3. Minimal inhibitory concentration (MIC) range and MIC_{90} of *Pasteurella multocida* isolated between 2009–2010 and 2013–2014

Antimicrobial agents	2009 (n = 38)		2010 (n = 31)		2013 (n = 47)		2014 (n = 15)	
	MIC range (μg/mL)	MIC_{90} (μg/mL)	MIC range (μg/mL)	MIC_{90} (μg/mL)	MIC range (μg/mL)	MIC_{90} (μg/mL)	MIC range (μg/mL)	MIC_{90} (μg/mL)
Ampicillin	≤ 0.25–16	4	≤ 0.25 – ≥ 16	≤ 0.25	≤ 0.25 – ≥ 16	≤ 0.25	≤ 0.25	≤ 0.25
Penicillin	≤ 0.12 – ≥ 8	≥ 8	≤ 0.12 – ≥ 8	2	≤ 0.12 – ≥ 8	≤ 0.12	≤ 0.12	≤ 0.12
Ceftiofur	≤ 0.25–1	≤ 0.25	≤ 0.25	≤ 0.25	≤ 0.25–0.5	≤ 0.25	≤ 0.25	≤ 0.25
Danofloxacin	≤ 0.12 – ≥ 1	≥ 1	≤ 0.12 – ≥ 1	≤ 0.12	≤ 0.12 – ≥ 1	0.25	≤ 0.12 – ≥ 1	0.5
Enrofloxacin	≤ 0.12–2	1	≤ 0.12 – ≥ 2	0.25	≤ 0.12–2	≤ 0.12	≤ 0.12–2	0.25
Spectinomycin	≤ 8 – ≥ 64	≥ 64	16 – ≥ 64	32	≤ 8 – ≥ 64	16	≤ 8–16	16
Sulphadimethoxine	256 – ≥ 256	≥ 256	256 – ≥ 256	≥ 256	256 – ≥ 256	≥ 256	256 – ≥ 256	≥ 256
Gentamicin	≤ 1 – ≥ 16	≥ 16	≤ 1 – ≥ 16	8	≤ 1 – ≥ 16	2	≤ 1–8	4
Neomycin	≤ 4 – ≥ 32	≥ 32	≤ 4–16	8	≤ 4 – ≥ 32	≤ 4	≤ 4–16	≤ 4
Tilmicosin	≤ 4–16	8	≤ 4–16	8	≤ 4 – ≥ 64	≤ 4	≤ 4–8	≤ 4
Tulathromycin	≤ 1–8	4	≤ 1–4	2	≤ 1–8	2	≤ 1	≤ 1
Florfenicol	≤ 0.25 – ≥ 8	8	≤ 0.25 – ≥ 8	8	≤ 0.25 – ≥ 8	8	≤ 0.25–8	8
Tiamulin	≤ 4 – ≥ 32	32	8 – ≥ 32	≥ 32	4 – ≥ 32	32	8–32	32
Chlortetracycline	≤ 0.5–8	2	≤ 0.5–8	4	≤ 0.5 – ≥ 8	4	≤ 0.5–2	2

(hemoglobin-binding protein) genes were present in > 90% of capsular serotype D isolates. The annual rate of detection of the *hsf-1* gene in *P. multocida* decreased gradually while that of *nanB* increased.

The MIC ranges and MIC_{90} of the 131 *P. multocida* isolates against various antimicrobial agents were shown in Table 3. The MIC ranges for isolated strains were somewhat different and MIC_{90}s of Ampicillin, Penicillin, Spectinomycin, Gentamicin, Neomycin, Tilmicosin, and Tulathromycin for isolated strains have been decreasing in 2014 than 2009.

We concluded that the annual rate of detection of *P. multocida* serotype D has decreased recently and that the antimicrobial resistance rate of *P. multocida* isolates has decreased annually. The aim of the present study is a common characteristic study of the porcine *P. multocida* in Korea. The results can be provided as the basis data of current vaccines and antimicrobial agents to porcine respiratory disease and also, ongoing monitoring of *P. multocida* antimicrobial agents to porcine respiratory disease in Korea.

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