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Antimicrobial resistance of *Pasteurella multocida* strains isolated from pigs between 2010 and 2016

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ABSTRACT

Pasteurella multocida is one of the significant causes of respiratory infection outbreaks in the Korean pig industry. Although antimicrobial treatment is an effective strategy for controlling respiratory diseases, limited information is available regarding the antimicrobial susceptibility of the pathogens infecting Korean pigs. Therefore, in this study, we evaluated the antimicrobial resistance of *P multocida* against widely used antimicrobials in order to enable the selection of appropriate drugs and to evaluate any trends in resistance. A total of 454 isolates of *P multocida* were collected from all provinces in Korea between 2010 and 2016. Antimicrobial susceptibility of all isolates was determined using a broth microdilution method. The most frequently observed resistance was to sulphadimethoxine (76.0 per cent), followed by oxytetracycline (66.5 per cent), chlortetracycline (36.8 per cent) and florfenicol (18.5 per cent). Although no consistent increase or decrease in resistance was observed for most antimicrobials, resistance to fluoroquinolones tended to increase over the study period. A variety of resistance patterns were observed, most frequently for tetracyclines and sulphonamides. These findings could provide information enabling the selection of optimal antimicrobials for efficient treatment of pneumoniae pasteurellosis in pig farms, which would impede the emergence of antimicrobial resistance.

INTRODUCTION

Pasteurella multocida is an important cause of pneumonia and atrophic rhinitis in pigs, which results in significant losses on farms worldwide.[1] Antimicrobial therapy is still the most effective tool for the treatment of infectious diseases caused by *P multocida*. In order to select appropriate therapy, it is necessary to isolate the causative organism and define its in vitro antibiotic sensitivity. However, because of the time-consuming nature of these laboratory procedures, it is common to start antibiotic therapy against the suspected pathogen immediately after observing the clinical signs. Despite this being common practice, these prediagnostic therapies appear to be inefficient, as evidenced by the widespread existence of antimicrobial resistance,[2 3] even when the choice of antimicrobials is based on clinical experience or expert opinions. Moreover, the unjustified use of antimicrobial agents puts considerable selective pressure on genes encoding antibiotic resistance. Treatment failure caused by resistant bacteria leads to an increase in morbidity and mortality in pigs.[4] Thus, long-term surveillance of antimicrobial resistance to pathogens is important for understanding how antimicrobial resistance among these pathogens changes over time, for highlighting significant trends and clusters in resistance and for assessing whether these data could prove valuable to practitioners and surveillance stakeholders.

Along with *Mycoplasma* species, *P multocida* is one of the most common pathogens causing single or complex respiratory disease in Korean pigs.[3 5] However, limited information is available regarding the antimicrobial susceptibility trends of recently isolated *P multocida* strains. Therefore, the purpose of this study was to evaluate changes occurring over a period of seven years (2010–2016) in the resistance of *P multocida* isolates to 18 antimicrobials routinely used to treat pigs.

MATERIALS AND METHODS

Bacterial strains

A total of 454 *P multocida* isolates were collected from all provinces in Korea from 2010 to 2016: 87 from the Animal and Plant Quarantine Agency (48, 30 and 9 in 2011, 2014 and 2015, respectively) and 367 from nine laboratories/centres participating in the Korean Veterinary Antimicrobial Resistance Monitoring System (64, 64, 58, 64, 27, 61 and 29 from 2010 to 2016, respectively). These *P multocida* strains were isolated from nasal swabs and lungs of diseased pigs from 282 farms throughout Korea in 2010 (n=36), 2011 (n=51), 2012 (n=45), 2013 (n=32), 2014 (n=39), 2015 (n=60) and 2016 (n=19). Each isolate was selected to comprise only

one isolate per animal in order to obtain a collection of epidemiologically unrelated strains. For each farm, one to five isolates were retained for antimicrobial susceptibility testing. Bacteria were isolated on Columbia agar with 5 per cent sheep blood, and suspicious colonies were identified by PCR[6] or matrix assisted laser desorption ionisation-time of flight mass spectrometry (bioMerieux, Marcy L'Etoile, France).

Antimicrobial susceptibility testing

Minimum inhibitory concentration (MIC) values for all isolates were determined using a broth microdilution method with 96-well microtitre plates (BOPO6, Sensititre, Trek Diagnostic Systems, East Grinstead, UK) containing a total of 18 antimicrobials according to the Clinical and Laboratory Standard Institute (CLSI)[7] guidelines. *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213 were used as quality control strains. The MIC of each antimicrobial was interpreted on the basis of break points provided by the CLSI,[8] where available. The break point used for trimethoprim/sulfamethoxazole was the one described by the EU veterinary pathogen monitoring programme.[9] The proportion of *P multocida* isolates categorised as resistant could not be evaluated in this study because the break points of clindamycin, danofloxacin, gentamicin, neomycin, tiamulin and tylosin had not been determined according to the CLSI criteria. The MIC_{50} and MIC_{90} were calculated as the MIC that inhibited 50 and 90 per cent of the isolates, respectively. *P multocida* isolates were defined as multidrug resistance (MDR) when they were resistant to three or more different antimicrobial classes.[10]

Statistical analysis

Trends in antimicrobial resistance, MIC_{50} and MIC_{90} of isolates over time were determined by linear regression of the annual data. A P value <0.05 was considered statistically significant. Statistical analyses were performed using the Statistical Package for the Social Science (SPSS V.22.0).

RESULTS

The most frequently observed resistance was to sulphadimethoxine (76.0 per cent), followed by oxytetracycline (66.5 per cent), chlortetracycline (36.8 per cent) and florfenicol (18.5 per cent). As shown in table 1, during the study period, resistance rates of less than 5 per cent were observed for ampicillin (4.8 per cent), spectinomycin (2.9 per cent), enrofloxacin (2.6 per cent), tilmicosin (2.6 per cent), trimethoprim/sulfamethoxazole (1.1 per cent), tulathromycin (0.4 per cent) and ceftiofur (0.2 per cent). No trends of increase or decrease were observed for most antimicrobials over the seven-year period, except for enrofloxacin, which showed an increase in resistance from 0 per cent of isolates in 2010–2011 to 10.3 per cent in 2016 (P<0.05; online supplementary table S1). During the same period, the MIC_{90} values for neomycin, danofloxacin and penicillin tended to increase from 8 to 64 µg/ml, ≤0.125 to **0.25**–2 µg/ml and ≤0.125 to 0.25 µg/ml, respectively (online supplementary table S1). Among the 454 isolates, 415 (91.4 per cent) resistant to one or more antimicrobials showed 45 different resistant patterns. Only 39 (8.6 per cent) isolates were susceptible to the 12 antimicrobials tested in this study (table 2). The most frequently observed resistance patterns were for sulphadimethoxine, chlortetracycline and oxytetracycline (22.5 per cent); sulphadimethoxine and oxytetracycline (18.1 per cent); and sulphadimethoxine (15.4 per cent). These three resistance patterns comprised more than 50 per cent of isolates. MDR was observed in 73 isolates (16.1 per cent).

DISCUSSION

The current investigation is the first large study of the antimicrobial resistance of *P multocida* in clinical samples collected from all nine provinces in Korea between 2010 and 2016. Antimicrobial resistance patterns in this study were similar to those observed in previous studies in Korea[2 3] and other countries.[9 11] However, the resistance rate overall in the present study was higher than the rates reported for the EU,[9] North America[12] and other countries.[4 11] For example, the published resistance rates in the present study, the EU,[9] North America,[12] Australia[11] and the Czech Republic[4] were 66.5, 20.4, 64.7–72.4, 28.0 and 32 per cent, respectively, for tetracycline and 18.5, 0.7, 0, 2.0 and 1.5 per cent, respectively, for florfenicol. The higher resistance rate in Korea might be due to the heavy use of antimicrobials in Korea compared with the EU and Australia, especially in the pig industry.[13 14] Furthermore, the resistance rate was low (<3 per cent) for critically important antimicrobials such as ceftiofur (0.2 per cent), tilmicosin (2.6 per cent), tulathromycin (0.4 per cent) and enrofloxacin (2.0 per cent) in this study; however, no resistance to these antimicrobials was observed in the EU[9] and Australia.[11]

The MIC_{90} values of antimicrobials among the isolates collected between 2010 and 2016 were similar to or lower than those reported in a study conducted with samples collected between 1995 and 1998.[2] However, the MIC_{90} values for tylosin, tetracycline, penicillin, spectinomycin, ceftiofur and danofloxacin in the study by Choi *et al*[2] and our study were 64 v 32 µg/ml, 64 v 16 µg/ml, 1 v 0.25 µg/ml, 64 v 32 µg/ml, 1 v 0.25 µg/ml and 2 v 0.25 µg/ml, respectively. The lower values observed in our study might be related to the decrease in administration of antimicrobials to pigs since the time the study by Choi *et al*[2] was conducted. In early 2000 and 2010–2016, about 900 and 384–581 tons, respectively, of antimicrobials were used annually in pigs in Korea[13]; however, consumption data from before 2000 are unavailable.

Resistance rates and MIC_{50} or MIC_{90} for most antimicrobials varied from year to year during the study period. Furthermore, during the study period, there was no consistent increase in antimicrobial resistance rates. However, resistance rates of fluoroquinolones increased

TABLE 1 Minimum inhibitory concentration distribution of *Pasteurella multocida* (n=454) isolated from pigs during 2010–2016

Antimicrobials	Break points (µg/ml)	Distribution (%) of MICs (µg/ml)*†													MIC_{50}‡	MIC_{90}‡	Resistance (%)
		≤0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	>256			
Ampicillin	≥2		93.8	0.4	0.9	0.2	0.7	0.7	0.9	2.4					0.25	0.25	4.8
Ceftiofur	≥8			99.1	0.2	0.2				0.2		0.2			0.25	0.25	0.2
Clindamycin	ND		0.4			0.7	0.7	4.6	93.6						16	16	–§
Chlortetracycline	≥2			15.6	47.6	29.3	6.4	0.2	0.9						1	2	36.8
Danofloxacin	ND	87.9	4.2	3.1	2.2	2.6									≤0.125	0.25	–
Enrofloxacin	≥1	91.2	4.0	2.2	0.9	1.8									≤0.125	≤0.125	2.6
Florfenicol	≥8		28.0	49.3	2.2	0.7	1.3	18.5							0.5	8	18.5
Gentamicin	ND				11.5	47.4	37.4	0.4	3.3						2	4	–
Neomycin	ND						43.2	46.5	4.4	2.6	3.3				8	16	–
Oxytetracycline	≥2			9.7	23.8	46.0	8.6	1.5	10.4						2	16	66.5
Penicillin	≥1	87.7	6.6	0.2	0.2	0.9	0.4	4.0							≤0.125	0.25	5.5
Spectinomycin	≥128							8.8	65.0	22.5	0.9	2.9			16	32	2.9
Sulphadimethoxine	≥512												24.0	76.0	>256	>256	76.0
Tiamulin	ND			0.4	0.2	0.2	13.2	4.4	42.1	39.4					16	32	–
Tilmicosin	≥32			0.2			77.8	7.7	11.7	2.2	0.4				4	16	2.6
Trimethoprim/sulfamethoxazole	≥4					98.9	1.1								2	2	1.1
Tulathromycin	≥64		89.4	7.9	2.0	0.2				0.4					1	2	0
Tylosin	ND			0.9	0.2		0.2	10.6	56.2	31.9					16	32	–

*The tested ranges are those contained in the white area.
†Years included (number of isolates in parentheses) are 2010 (n=64), 2011 (n=112), 2012 (n=58), 2013 (n=64), 2014 (n=57), 2015 (n=70) and 2016 (n=29).
‡MIC_{50} and MIC_{90} at which 50 and 90 per cent of the isolates were inhibited.
§A dash indicated that no figure could be calculated because no Clinical and Laboratory Standards Institute (CLSI) interpretive criteria are available.
MIC, minimum inhibitory concentration; ND, not determined.

TABLE 2 Antimicrobial resistant patterns detected in 454 *Pasteurella multocida* isolated from pigs

		Number of isolates (%)								
Resistance patterns		**2010 (n=64)**	**2011 (n=112)**	**2012 (n=58)**	**2013 (n=64)**	**2014 (n=57)**	**2015 (n=70)**	**2016 (n=29)**	**Total (n=454)**	**MDR**
No resistance detected		12 (18.8)	6 (5.4)	2 (3.4)	10 (15.6)	2 (3.5)	5 (7.1)	2 (6.9)	39 (8.6)	–
1	CTC		1		1				2 (0.4)	–
	ENO				2				2 (0.4)	–
	FFC	1	2		1	2	1	2	9 (2.0)	–
	OTC	3	9	2		3	1	1	19 (4.2)	–
	SDM	8	15	6	28	3	10		70 (15.4)	–
	TIL				3				3 (0.7)	–
	Subtotal	12 (18.8)	27 (24.1)	8 (13.8)	35 (54.7)	8 (14.0)	12 (17.1)	3 (10.3)	105 (23.1)	–
2	AMP PEN							2	2 (0.4)	–
	CTC OTC	1	1	1	1		8	4	16 (3.5)	–
	ENO FFC					1	1		2 (0.4)	–
	ENO SDM				1	1			2 (0.4)	–
	FFC OTC					2			2 (0.4)	–
	SDM CTC						1	1	2 (0.4)	–
	SDM FFC		2		1	2	1	1	7 (1.5)	–
	SDM OTC	23	36	5	5	12	1		82 (18.1)	–
	TIL OTC				1				1 (0.2)	–
	TIL SDM				3				3 (0.7)	–
	Subtotal	24 (37.5)	39 (34.8)	6 (10.3)	12 (18.8)	18 (31.6)	12 (17.1)	8 (27.6)	119 (26.2)	–
3	AMP PEN FFC						1		1 (0.2)	–
	AMP PEN SDM				1	1			2 (0.4)	–
	AMP PEN SPT	1							1 (0.2)	–
	ENO CTC OTC							2	2 (0.4)	–
	ENO SDM FFC					1			1 (0.2)	1 (0.2)
	ENO SDM OTC					1			1 (0.2)	1 (0.2)
	FFC CTC OTC			7		2			9 (2.0)	–
	SDM CTC OTC	13	17	20	2	14	31	5	102 (22.5)	–
	SDM FFC OTC	1	17	3		5		1	27 (5.9)	27 (5.9)
	SDM SXT OTC			1					1 (0.2)	–
	Subtotal	15 (23.4)	34 (30.4)	31 (53.4)	3 (4.7)	24 (42.1)	32 (45.7)	8 (27.6)	147 (32.4)	29 (6.4)

Continued

TABLE 2 Continued

Resistance patterns		Number of isolates (%)								
		2010 (n=64)	2011 (n=112)	2012 (n=58)	2013 (n=64)	2014 (n=57)	2015 (n=70)	2016 (n=29)	Total (n=454)	MDR
4	AMP PEN SDM FFC					1			1 (0.2)	1 (0.2)
	AMP PEN SDM SPT			1					1 (0.2)	1 (0.2)
	AMP SDM CTC OTC		1						1 (0.2)	1 (0.2)
	AMP XNL PEN OTC	1							1 (0.2)	1 (0.2)
	ENO SDM CTC OTC			1				1	2 (0.4)	2 (0.4)
	PEN SDM CTC OTC			2					2 (0.4)	2 (0.4)
	PEN SDM FFC OTC		1						1 (0.2)	1 (0.2)
	SDM FFC CTC OTC		2	4		4	6	3	19 (4.2)	19 (4.2)
	SDM SXT FFC OTC						1	1	2 (0.4)	2 (0.4)
	Subtotal	1 (1.6)	4 (3.6)	8 (13.8)	0 (0)	5 (8.8)	7 (10.0)	5 (17.2)	30 (6.6)	30 (6.6)
5	AMP PEN CTC OTC SPT							1	1 (0.2)	1 (0.2)
	AMP PEN SDM CTC OTC		1					2	3 (0.7)	3 (0.7)
	AMP PEN SDM CTC SPT						1		1 (0.2)	1 (0.2)
	AMP PEN SDM FFC SPT				1				1 (0.2)	1 (0.2)
	AMP TIL PEN SDM SPT				1				1 (0.2)	1 (0.2)
	SDM SXT CTC OTC SPT			1					1 (0.2)	1 (0.2)
	Subtotal	0 (0)	1 (0.9)	1 (1.7)	2 (3.1)	0 (0)	1 (1.4)	3 (10.3)	8 (1.8)	8 (1.8)
6	AMP PEN SDM CTC OTC SPT			2					2 (0.4)	2 (0.4)
	AMP TIL PEN SDM OTC SPT				2				2 (0.4)	2 (0.4)
	Subtotal	0 (0)	0 (0)	2 (3.4)	2 (3.1)	0 (0)	0 (0)	0	4 (0.9)	4 (0.9)
8	AMP TIL TUL PEN SDM FFC CTC OTC						1		1 (0.2)	1 (0.2)
	Subtotal	0 (0)	0 (0)	2 (3.4)	0 (0)	0 (0)	1 (1.4)	0 (0)	1 (0.2)	1 (0.2)
9	TIL TUL PEN SDM SXT FFC CTC OTC SPT		1						1 (0.2)	1 (0.2)
	Subtotal	0 (0)	1 (0.9)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.2)	1 (0.2)

AMP, ampicillin; CTC, chlortetracycline; ENO, enrofloxacin; FFC, florfenicol; MDR, multidrug resistance; OTC, oxytetracycline; PEN, penicillin; SDM, sulphadimethoxine; SPT, spectinomycin; SXT, trimethoprim/sulfamethoxazole; TIL, tilmicosin; TUL, tulathromycin; XNL, ceftiofur.

significantly (P<0.05), with the resistance rate of enrofloxacin and MIC_{90} of danofloxacin increasing from 0 to 10 per cent and from less than 0.125 to 2 µg/ml, respectively, over the seven-year study period. Enrofloxacin, a fluoroquinolone, is approved for the treatment of respiratory disease in pigs. In Korea, although fluoroquinolone antimicrobials such as enrofloxacin are used mainly for poultry, about 20 per cent of consumed enrofloxacin is used for pigs.[13] Furthermore, enrofloxacin has been widely used for years,[13] which might have contributed to the increased resistance to this drug. In contrast, the use of danofloxacin is very rare in Korea. Despite this, the MIC_{90} of danofloxacin has been increasing recently. This increase might be related to cross-resistance with enrofloxacin[15] because all isolates with a high danofloxacin MIC (2 µg/ml) also showed resistance to enrofloxacin.

The determination of MDR could provide additional useful information, although the break points are unavailable for several antimicrobials; consequently, MDR can be determined for only a limited number of antimicrobials. In this study, the most frequently observed resistance patterns were for sulphadimethoxine and tetracyclines. This resistance might be associated with R plasmids, some of which also mediate resistance to sulphonamide and tetracyclines.[16] In our study, 17.1 per cent (73/454) of isolates were found to be MDR. Similar result was reported by Lizarazo *et al*[17] in Spain (18.1 per cent, 24/132). However, our result was much higher than in Australia at 2.0 per cent (1/51).[11]

Furthermore, only 8.6 per cent of isolates were susceptible to all the antimicrobials tested in this study, compared with 12.9 per cent in Spain[17] and 58.8 per cent (30/51) in Australia.[11]

In conclusion, the findings of the present study suggest that the occurrence of MDR *P multocida* in pigs warrants attention. In Korea, as in many other countries, *P multocida* has frequently been isolated from pigs, and is one of the significant causes of outbreak of respiratory infections. The high prevalence of MDR *P multocida* strains in pigs strongly suggests that antimicrobials should be used more prudently to treat pigs efficiently and to prevent the spread of infections through the food chain. Furthermore, studies such as the present one along with antimicrobial susceptibility monitoring programmes for important veterinary pathogens could provide evidence-based guidance for antimicrobial therapy of bacterial diseases when treating pig pasteurellosis.

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