pISSN 2466-1384 eISSN 2466-1392
大韓獸醫學會誌 (2016) 第 56 卷 第 2 號
Korean J Vet Res(2016) 56(2) : 109~111
http://dx.doi.org/10.14405/kjvr.2016.56.2.109

<Short Communication>

In vitro antibiotic susceptibility of field isolates of *Mycoplasma hyopneumoniae* and *Mycoplasma hyorhinis* from Korea

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(Received: February 4, 2016; Revised: April 8, 2016; Accepted: April 15, 2016)

Abstract : The present study was conducted to determine the antibiotic susceptibilities of local *Mycoplasma hyopneumoniae* (Mhp) and *Mycoplasma hyorhinis* (Mhr) filed isolates. Minimum inhibitory concentrations (MICs) of Mhp and Mhr field isolates (twelve each) obtained from enzootic pneumonia-like lung lesions during 2009–2011 from Korea were determined using the broth microdilution method. Tylvalosin showed the highest activity against Mhp and Mhr field isolates, with MIC_{90} values of 0.06 μg/mL and 0.12 μg/mL, respectively. Therefore, Korean Mhp and Mhr isolates are highly susceptible to tylvalosin.

Keywords : *Mycoplasma hyopneumoniae*, *Mycoplasma hyorhinis*, antimicrobials, minimum inhibitory concentration, porcine enzootic pneumonia

Swine mycoplasma *Mycoplasma hyopneumoniae* (Mhp) and *Mycoplasma hyorhinis* (Mhr) are respiratory pathogens in pigs. They are associated with enzootic pneumonia (EP) and porcine respiratory disease complex (PRDC) causing huge loses to the porcine industry. Currently commercial vaccines are available only against Mhp; however, their protection ability is not complete [7]. Therefore, use of antimicrobials often becomes necessary to limit the disease in the event of outbreak or as an additional measure to prevent mycoplasma disease. Although considerable data have been obtained on the *in vitro* antibiotic susceptibility of Mhp and Mhr around the world [6, 8-11], there are few reports about their susceptibility, and to our knowledge, there is no recent information on antibiotic susceptibility of these mycoplasmas from Korea. In the present study, minimum inhibitory concentrations (MICs) of Korea Mhp and Mhr isolates to tylvalosin and other commonly used antibiotics (tiamulin, lincomycin, tilmicosin and chlortetracycline) was determined.

The reference strains used in this study were Mhp (ATCC 25934) and Mhr (ATCC 27717) (American Type Culture Collection, USA). Field isolates (twelve each) of Mhp and Mhr which were obtained from EP-like lung lesions during 2009–2011 were used in this study [1, 2]. Each of these isolates was identified by multiplex polymerase chain reaction (mPCR) [3] and passaged 6 times before using in the antimicrobial assay. Both *Mycoplasma* species were propagated in Friis broth [4] and used at final concentration of approximately 1×10^5 CCU/mL. The antimicrobials chlortetracycline, tiamulin and tilmicosin were obtained from Sigma (USA). The lincomycin and tylvalosin were obtained from Dong Bang (Korea) and ECO Animal Health (UK), respectively. The antimicrobials were prepared by binary micro dilution method, in 96-well micro plate (ranging between 64 and 0.0038 μg/mL; SPL Life Sciences, Korea). MICs-were determined using the broth microdilution method as described by Hannan *et al.* [5]. Readings were taken after incubation of 7 days at 37°C, and the lowest concentration of antimicrobial inhibiting color change from red to yellow/orange of the medium was defined as MIC of the drug.

The results of the *in vitro* antimicrobial testing for mycoplasma field isolates and type strains are presented in Table 1 as MIC ranges, MICs at which 50% and 90% of the isolates were inhibited (MIC_{50} and MIC_{90}). The values for three replicate testing of type strains (both Mhp and Mhr) were equal or differed from each other by only one doubling dilution, indicating good reproducibility of the test. For the Mhp field isolates, tylvalosin showed the lowest MICs values with MIC_{50} and MIC_{90} of 0.03 μg/mL and 0.06 μg/mL, respectively. Next to tylvalosin and tiamulin showed high activity and its MIC_{90} value was 0.12 μg/mL. High potency was also

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Table 1. Minimal inhibitory concentrations (MIC) ranges for 5 antimicrobials against *Mycoplasma* (*M.*) *hyopneumoniae* and *M. hyorhinis* isolates in Korea

Mycoplasma species and antibiotic*	Field isolates			
	Type strain†	Range	MIC_{50}	MIC_{90}
M. hyopneumoniae				
Tylvalosin	≤ 0.03	0.0075–0.06	0.03	0.06
Lincomycin	0.5	0.12–0.5	0.25	0.5
Chlortetracycline	4	4–64	16	64
Tiamulin	0.12	0.06–0.25	0.12	0.12
Tilmicosin	≤ 4	0.5–4	1	4
M. hyorhinis				
Tylvalosin	0.06	0.06–0.12	0.06	0.12
Lincomycin	0.25	0.25–1	1	1
Chlortetracycline	2	2–64	64	64
Tiamulin	0.06	0.12–0.25	0.12	0.25
Tilmicosin	≤ 1	0.12–4	2	4

*All data are given in μg/mL. †The type strains used in this study were *M. hyopneumoniae* ATCC 25934 and *M. hyorhinis* ATCC 27717. Values indicate results of three replicate testing of type strains.

observed for antimicrobials lincomycin and tilmicosin with MIC_{90} of 0.5 μg/mL and 4 μg/mL, respectively. The antimicrobial chlortetracycline, with MIC range from 4 to 64 μg/mL and MIC_{90} of 64 μg/mL, displayed low activity against the Mhp field isolates.

Similar findings were observed for the Mhr field isolates. Tylvalosin had an MIC_{90} of 0.12 μg/mL and was the most active antimicrobial against all Mhr isolates. It was followed by tiamulin, lincomycin, and tilmicosin with MIC_{90} values of 0.25 μg/mL, 1 μg/mL and 4 μg/mL, respectively. As with the Mhp isolates, chlortetracycline was also less active against the Mhr field isolates with MIC range from 4 to 64 μg/mL and MIC_{90} of 64 μg/mL.

For the Mhp field isolates, MIC_{90} values of tylvalosin, tiamulin and chlortetracycline were consistent with the previous studies [8-10]. Lincomycin had an MIC_{90} value almost same as the isolates from Thailand [9], but it was four times of the corresponding MIC_{90} values for the isolates from Spain [8] and Belgium [10]. Likewise, tilmicosin had MIC_{90} value four and eight times of the values for isolates from Spain [8] and Belgium [10], respectively. In case of Mhr isolates, the MIC_{90}s of lincomycin, tilmicosin and tiamulin are in agreement with the previously published reports [6, 11]. Tylvalosin, which had an *in vitro* activity up to thousand times higher compared to other antibiotics, showed highest efficacy against both mycoplasma species.

To conclude, our findings of *in vitro* susceptibility must be taken into consideration while treating these swine mycoplasma in Korea and will help for a choice among several antibiotics. The *in vitro* sensitivity testing of field isolates of Mhp and Mhr from Korea shows that tylvalosin was the most effective antimicrobial against these *mycoplasma* followed by tiamulin, lincomycin, and tilmicosin.

Acknowledgments

This research was supported by the Technology Development Program for Agriculture and Forestry, Ministry for Food, Agriculture, Forestry and Fisheries, and was technically supported by the Institute of Veterinary Science, Kangwon National University, Korea.

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