한국가축위생학회지 제42권 제4호 (2019)
Korean J Vet Serv, 2019, 42(4), 301-304
ISSN 1225-6552, eISSN 2287-7630
https://doi.org/10.7853/kjvs.2019.42.4.301

Korean Journal of
Veterinary Service
Available online at http://kjves.org

< Short Communication >

Antimicrobial susceptibility pattern of *Lawsonia intracellularis* recently isolated from pig with proliferative hemorrhagic enteropathy in Korea

Byoung-Joo Seo[1], Sang-Eog Koh[2,3], Yeonsu Oh[4]*, Ho-Seong Cho[1]*

[1]*Laboratory of Swine Diseases, College of Veterinary Medicine and Bio-Safety Research Institute, Jeonbuk National University, Iksan 54596, Korea*
[2]*Valad Swine Vet Center, Anseong 17529, Korea*
[3]*College of Veterinary Medicine, Chungbuk National University, Cheongju 28644, Korea*
[4]*Laboratory of Veterinary Pathology, College of Veterinary Medicine and Institute of Veterinary Science, Kangwon National University, Chuncheon 24341, Korea*

(Received 10 December 2019; revised 16 December 2019; accepted 16 December 2019)

Abstract

The objective of this study was to determine the *in vitro* intracellular and extracellular minimum inhibitory concentrations (MICs) of 13 antimicrobials against one recently isolate *Lawsonia intracellularis*, the etiological agent of proliferative enteropathy (PE). The final MICs were assessed by counting the number of heavily infected cells (HICs; > 30 bacteria per cell) using an immunoperoxidase monolayer assay. Enrofloxacin (InMIC; 1～2 μg/mL and ExMIC; 16 μg/mL) still presented the most notable antimicrobial susceptibility, and marbofloxacin (2 μg/mL and 8 μg/mL) was followed. Colistin (0.25 μg/mL and 2 μg/mL) presented a susceptibility followed by tylvalosin (1 μg/mL and 2 μg/mL). Florfenicol and lincomycin had the weakest susceptibility and amoxicillin, penicillin G, chlortetracycline, oxytetracycline, tiamulin, tilmicosin, and tylosin displayed weak susceptibility. Although some antibiotics showed decreased susceptibility patterns, they showed similar patterns to recent antibiotic susceptibility patterns in Korea. In addition, these results could be one of contributions in clinical fields.

Key words : Antimicrobial susceptibility, *Lawsonia intracellularis*, Minimum inhibitory concentration, Pig, Porcine proliferative enteropathy

INTRODUCTION

Porcine proliferative enteropathy (PPE) is one of the most prevalent enteric bacterial diseases in grower and finisher pigs. The etiological agent of this disease is an obligate intracellular, Gram-negative bacterium named *Lawsonia intracellularis*. It has been detected in many other animals worldwide (Lawson and Gebhart, 2000; Hossain et al, 2016; Oh et al, 2017; Park et al, 2015). The subclinical form of Porcine hemorrhagic enteropathy (PHE) has not been easily recognized recently, which can be developed as subacute or chronic at any moment under stressful condition. It is one of the most important diseases in pig industry worldwide (Lawson and Gebhart, 2000).

Up to now, antimicrobial therapy remains the only treatment available. Tiamulin, tylosin, lincomycin, and chlortetracycline have been commonly recommended and used in the field (McOrist et al, 1995; Marsteller et al, 2001) and oxytetracycline, valnemulin, doxycycline, josamycin, and leucomycin were also known as effective (Tzika et al, 2009; Larsen et al, 2016) according to field experiences, not from the exact *in vitro* antimicrobial susceptibility testing (AST). However, AST could not be easily performed for *L. intracellularis* because it requires complicated cell culture system and particular at-

*Corresponding author: Ho-Seong Cho, Tel. +82-63-270-4872, Fax. +82-63-270-3780, E-mail. hscho@jbnu.ac.kr
*Corresponding author: Yeonsu Oh, Tel. +82-33-250-8792, Fax. +82-33-259-5625, E-mail. yeonoh@kangwon.ac.kr
These first two authors contributed equally to this work.

mosphere for its growth and proliferation (McOrist et al, 1995; Yeh et al, 2011).

In Korea, the antimicrobial susceptibility of *L. intracellularis* was tested 2006～2017 (Yeh et al, 2006; Yeh et al, 2011; Seo et al, 2019). Therefore, the aim of this study was to update *in vitro* antimicrobial sensitivities of newly isolated *L. intracellularis* in Korea.

MATERIALS AND METHODS

A recently isolate of *L. intracellularis* was obtained from hemorrhagic region of the small intestine from a finisher pigs with PHE (JBNU010) in 2019. The isolates were prepared in IEC-18 cells (CRL 1589, ATCC, VA, USA) and harvested as previously described elsewhere (Lawson et al, 1993).

The AST was conducted by determining minimum inhibitory concentrations (MICs) of each antimicrobial against *L. intracellularis*. The MICs and its results were studied according to previous studies (Seo et al, 2019). Briefly, antimicrobial agents used for the MICs were amoxicillin, penicllin G, chlortetracycline, oxytetracycline, colistin, enrofloxacin, marbofloxacin, florfenicol, lincomycin, tiamulin, tylosin (Sigma-Aldrich, MO, USA) and tylvalosin (Santa Cruz Biotechology, TX, USA), and all agents were serially diluted from 0.125 to 256 μg/mL. Briefly, to determine the intracellular MIC (InMIC), 100 μL of bacterial suspension was inoculated and incubated for 24 h in a 96-well plate which the IEC-18 cells were cultured. Antimicrobial stock solutions were added at 1, 2 and 3 day post inoculation (dpi) when the medium was freshly replaced. For the extracellular MIC (ExMIC), after exposure to each concentration of antimicrobials for 2 h, bacterial cells were infected to IEC-18 cells and cultured for 24 h. Then, the medium was replaced in new DMEM supplemented with L-glutamine and FBS (7%, v/v) and each antimicrobial agent to be tested at 1, 2, and 3 dpi. After that, the 96-well plates were fixed with cold acetone/methanol (1:1 v/v) and counted the number of heavily infected cells (HICs; > 30 bacteria per cell) using an immunoperoxidase monolayer assay.

RESULTS AND DISCUSSION

The results were determined by taking the median value from a set of triplicate 96-well plates and performed in duplicate. The InMIC and ExMIC values to each of the antimicrobials are displayed in Table 1. In the present study, the InMICs of the *L. intracellularis* isolate was lower than ExMICs, which was consistent with previous study (Seo et al, 2019). For isolate, Enrofloxacin (InMIC ; 1～2 μg/mL and ExMIC; 16 μg/mL) still presented the most notable antimicrobial susceptibility, and marbofloxacin (2 μg/mL and 8 μg/mL) was followed. Colistin (0.25 μg/mL and 2 μg/mL) presented a susceptibility followed by tylvalosin (1 μg/mL and 2 μg/mL). Florfenicol and lincomycin had the weakest susceptibility and amoxicillin, penicillin G, chlortetracycline, oxytetracycline, tiamulin, tilmicosin, and tylosin displayed weak susceptibility. Although some antibiotics showed decreased susceptibility patterns, they showed similar patterns to recent antibiotic susceptibility patterns in Korea. In addition, these results could be one of contributions in clinical fields.

The result postulated that macrolides could be still a good option of treatments for *L. intracellularis* in Korea. Previously, six field isolates of *L. intracellularis* in Korea presented higher intracellular and extracellular susceptibility to almost all antimicrobials including amoxicillin, penicillin G, chlortetracycline, lincomycin, tiamulin, tilmicosin, and tylosin (Yeh et al, 2011; Seo et al, 2019). However, those macrolides but tylvalosin were displayed a weak susceptibility to this isolate. Lincomycin presented very weak susceptibility to those five isolates from North America consistent with our results. In addition, tylvalosin oral treatment was studied to be effective to control PHE associated with *L. intracellularis* in pig farms (Canning et al, 2016; Seo et al, 2019), consistent with our *in vitro* MIC results. Fluoroquinolones showed the greatest susceptibility and were used to control PHE outbreaks in the very farm where the isolates came from. However, the case was not fully controlled with the antimicrobial. Colistin showed a strong susceptibility to the *L. intracellularis* isolates, being the first MIC report of colistin to the best of authors' knowledge. However, it is considered that the use

Table 1. Intracellular and extracellular MICs for 13 antimicrobial agents against recently isolated *L. intracellularis* strain isolated from Korea

Antimicrobial class	Antimicrobial agent (s)	MIC (μg/mL)[a]													
		CBNU001 (2013)[b]		CBNU002 (2014)[b]		CBNU004 (2016)[b]		CBNU006 (2017)[b]		JBNU010 (2019)		PHE/KK421 (2002)[c]		PIA/MyCoyL1 (2010)[c]	
		InMIC[d]	ExMIC[e]	InMC	ExMIC	InMC	ExMIC	InMC	ExMIC	InMC	ExMIC	InMIC	ExMIC	InMIC	ExMIC
Penicillins	Amoxicillin	8	32	8	32	8	32	16	32	8	16	0.5	8	2∼4	16
	Penicillin G	32	64	16	64	16	64	16	16	64	64	1∼2	2∼4	4	16
Tetracyclines	Chlortetracycline	16	64	32	64	16	64	32	64	8	32	2∼4	16	8	64
	Oxytetracycline	16	64	16	64	16	64	8	64	16	32				
Polypeptides	Colistin	2	2	0.125	4	0.125	2	0.5	2	0.25	2				
Fluoroquinolones	Enrofloxacin	0.25	16	0.125	2	0.125	4	0.25	4	1∼2	16	2	8	2∼4	16
	Marbofloxacin	0.5	32	0.5	4	0.25	4	0.5	8	2	8				
Phenicols	Florfenicol	>256	>256	>256	>256	64	>256	128	>256	128	>256				
Lincosamide	Lincomycin	>256	>256	128	>256	64	128	128	>256	128	>256	16	64	>128	>128
Pleuromutilins	Tiamulin	8	16	16	64	32	64	8	32	16	32	0.25∼0.5	4∼8	2	32
Macrolides	Tilmicosin	4	32	4	32	16	64	16	32	8∼16	16	0.125	0.5	0.125	0.25∼0.5
	Tylosin	16	64	8	64	32	64	16	32	4	32	0.25∼0.5	1	0.25	1
	Tylvalosin	1	2	0.5	2	1	4	1	4	1	2				

[a]The MIC data of each antimicrobial were determined using the median value from a set of triplicate 96-well plates and performed in duplicate on each *L. intracellularis* isolates independently.
[b]Seo et. al., 2019; [c]Yeh et. al., 2011; [d]The intracellular MIC; eThe extracellular MIC.

of colistin should be cautious to prevent the transmission of multidrug resistance genes to other bacteria in the same or different animals, of the food chain, and to the human community (Lim et al, 2016).

Although some antibiotics showed decreased susceptibility patterns, they showed similar patterns to recent antibiotic susceptibility patterns in Korea. In addition, these results could be one of contributions in clinical fields.

ACKNOWLEDGEMENTS

This research was supported by Technology Development Program (Project No. 315046-2) for Bio-industry, Ministry for Agriculture, Food and Rural Affairs, Republic of Korea.

REFERENCES

Canning P, Bates J, Hammen K, Coetzee J, Wolf L, Rajewski S, Wang C, Karriker L. 2016. Concentrations of tylvalosin and 3-O-acetyltylosin attained in the synovial fluid of swine after administration by oral gavage at 50 and 5 mg/kg. J Vet Pharmacol Therap 39: 621-624.

Hossain MM, Oh Y, Cho HS. 2016. Prevalence of antibody to and of *Lawsonia intracellularis* in samples from wild animals in Korea. J Wildl Dis 52: 803-808.

Larsen I, Nielsen SS, Olsen JE, Nielsen JP. 2016. The efficacy of oxytetracycline treatment at batch, pen and individual level on Lawsonia intracellularis infection in nursery pigs in a randomised clinical trial. Prev Vet Med 124: 25-33.

Lawson GH, Gebhart CJ. 2000. Proliferative enteropathy. J Comp Pathol 122: 77-100.

Lawson GH, McOrist S, Jasni S, Mackie RA. 1993. Intracellular bacteria of porcine proliferative enteropathy: cultivation and maintenance *in vitro*. J Clin Microbiol 31: 1136-1142.

Lim SK, Kang HY, Lee K, Moon DC, Lee HS, Jung SC. 2016. First detection of the *mcr-1* gene in *Escherichia coli* isolated from livestock between 2013 and 2015 in South Korea. Antimicrob Agents Chemother 60: 6991-6993.

Marsteller T, Winkelman N, Gebhart C, Armbruster G, Weldon W, Muller PR, Weatherford PJ. Symanowski J. 2001. Efficacy of intramuscular tylosin for the treatment and control of porcine proliferative enteropathy caused by *Lawsonia intracellularis*. Vet Ther 2: 51-60.

McOrist S, Mackie RA, Lawson GH. 1995. Antimicrobial susceptibility of ileal symbiont intracellularis isolated from pigs with proliferative enteropathy. J Clin Microbiol 33: 1314-1317.

Oh Y, Hossain MM, Cho HS. 2017. Prevalence of antibody to and DNA of *Lawsonia intracellularis* in samples from healthy horses in Korea. Thai J Vet Med 47: 543-549.

Park BY, Shim KS, Kim WI, Hossain MM, Kim BS, Kwon JK, Park CK, Cho SJ, Jo IH, Cho HS. 2015. Rapid and sensitive detection of *Lawsonia intracellularis* in pigs by real-time loop-mediated isothermal amplification. Acta Veterinaria-Belgrade 65: 20-29.

Seo BJ, Moon SH, Lee SM, Lee SY, Jung BY, Kim WI, Lee CS, Oh Y, Cho HS. 2019. Recent antimicrobial susceptibility of *Lawsonia intracellularis* field isolates from pigs with proliferative hemorrhagic enteropathy in Korea. Thai J Vet Med 49: 81-85.

Tzika E, Papatsiros V, Kyriakis S, Alexopoulos C, Lymberopoulos A, Kyriakis C. 2009. Efficacy of in-feed valnemulin hydrochloride for the treatment and control of ileitis in weaning and growing pigs. J Appl Anim Res 35: 181-184.

Yeh JY, Lee JH, Yeh HR, Kim A, Lee JY, Hwang JM, Kang BK, Kim JM, Choi IS, Lee JB. 2011. Antimicrobial susceptibility testing of two Lawsonia intracellularis isolates associated with proliferative hemorrhagic enteropathy and porcine intestinal adenomatosis in South Korea. Antimicrob Agents Chemother 55: 4451-4453.