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Antimicrobial Susceptibility Testing of Two *Lawsonia intracellularis* Isolates Associated with Proliferative Hemorrhagic Enteropathy and Porcine Intestinal Adenomatosis in South Korea▽

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This study represents the first published data on antimicrobial susceptibility of Asian isolates of *Lawsonia intracellularis*. We assessed MICs of 16 antimicrobials for two isolates of *L. intracellularis* recovered from diseased pigs in South Korea, one from a finisher pig with acute proliferative hemorrhagic enteropathy in 2002 and the other from a grower pig with porcine intestinal adenomatosis in 2010. Tylosin and tilmicosin were found to be the most active against *L. intracellularis* both intracellularly (MICs, 0.25 to 0.5 μg/ml and 0.125 μg/ml, respectively) and extracellularly (MICs, 0.25 to 0.5 μg/ml and 1 μg/ml, respectively).

Lawsonia intracellularis can cause acute intestinal hemorrhage (proliferative hemorrhagic enteropathy [PHE]) in naïve adult pigs and a wasting disease (porcine intestinal adenomatosis [PIA]) in growing pigs (7, 11). *In vitro* studies of the antimicrobial susceptibility of this obligate intracellular bacterium necessitate complicated cell culture systems (3, 5, 6, 12), and only a few laboratories in the world perform antimicrobial susceptibility testing, because few *L. intracellularis* strains have been successfully isolated and maintained in culture (17). Thus, while knowledge of the antimicrobial susceptibility of *L. intracellularis* is important for management and treatment decisions, published data on its *in vitro* antimicrobial sensitivity are very limited. There is only one previous field study of the antimicrobial resistance and susceptibility of *L. intracellularis* in the context of its treatment and control in Asia (8). The study demonstrated that the administration of tylosin in the animal feed reduced infection rates on farms (8). In this study, we compared the inhibitory activities of 16 antimicrobial agents against two *L. intracellularis* isolates from South Korea that were collected 8 years apart, with an emphasis on antimicrobials commonly used in pig production.

The following antimicrobial agents were purchased as pure chemicals from Sigma-Aldrich Korea (Yong-In Si, Republic of Korea): lincomycin hydrochloride, carbadox, ampicillin, penicillin G potassium salt, tiamulin hydrogen fumarate, chlortetracycline hydrochloride, bacitracin, polymyxin (colistin) sulfate, tylosin tartrate, gentamicin sulfate, kanamycin sulfate, neomycin sulfate, spectinomycin dihydrochloride, streptomycin sulfate, and enrofloxacin. Tilmicosin was supplied as a pure chemical by Elanco Animal Health Korea, Ltd. (Seoul, Republic of Korea). The *L. intracellularis* field isolates were obtained from infected pigs at farms located near each other in the same province (Hongseong-gun and Chungnam, Republic of Korea). A strain of *L. intracellularis*, termed the PHE/KK421 strain, was isolated from a finisher pig with acute PHE in 2002 (19), and another strain, PIA/MyCoyL1, was isolated from a grower pig with PIA in 2010. In this study, isolates were prepared in McCoy cells and harvested as previously described (4). *L. intracellularis* was quantified as follows. First, 10-fold serial dilutions of the samples were made in phosphate-buffered saline (PBS; pH 7.2). Then, 12-well glass slides were coated with 10 μl of each dilution and dried at 37°C for 30 min. Finally, the slides were fixed with cold acetone and stained by indirect immunoperoxidase activity using antiserum against *L. intracellularis*. Different sample dilutions were evaluated under the light microscope, and the dilution that could be accurately counted (50 to 500 bacteria) was recorded. Quantification of *L. intracellularis* was performed in duplicate. The concentrations of the *L. intracellularis* inocula were between 5.9×10^5 and 7.1×10^6 bacteria/ml. The MICs of each antimicrobial against *L. intracellularis* were determined using antimicrobial susceptibility testing and data analysis methodology as previously described (17).

The susceptibilities of *L. intracellularis* to each of the antimicrobials are displayed in Table 1. For both isolates, tilmicosin and tylosin displayed the greatest activity with MICs of 1.0 μg/ml, while lincomycin, the peptide-type antimicrobials, and most of the aminoglycosides had the weakest activity. For the 2002 *L. intracellularis* isolate, tilmicosin, tylosin, carbadox, and tiamulin displayed the greatest intracellular activities, with MICs of 0.5 μg/ml. In the extracellular activity assays, carbadox, ampicillin, penicillin G, tiamulin, chlortetracycline, and enrofloxacin all displayed moderate activities against this strain, with MICs ranging from 4 μg/ml to 32 μg/ml, whereas

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TABLE 1. Intracellular and extracellular MICs for various antimicrobials against two strains of *L. intracellularis* (PHE/KK421 at passages 21 and 22 and PIA/MyCoy L1 at passages 11 and 12)

Antimicrobial class and antimicrobial agent(s)	MIC (μg/ml)[a]			
	PHE/KK421 (2002)		PIA/MyCoyL1 (2010)	
	Intracellular activity	Extracellular activity	Intracellular activity	Extracellular activity
Lincosamide				
Lincomycin hydrochloride	16	64	>128	>128
Carbadox	0.25	4	0.25–0.5	4–8
Penicillin				
Ampicillin	0.5	8	2–4	16
Penicillin G potassium salt	1–2	2–4	4	16
Pleuromutilin				
Tiamulin hydrogen fumarate	0.25–0.5	4–8	2	32
Tetracycline				
Chlortetracycline hydrochloride	2–4	16	8	64
Peptide				
Bacitracin, polymyxin	>128	>128	>128	>128
Fluoroquinolone				
Enrofloxacin	2	8	2–4	16
Macrolide				
Tilmicosin	0.125	0.5	0.125	0.25–0.5
Tylosin tartrate	0.25–0.5	1	0.25	1
Aminoglycoside				
Gentamicin, kanamycin, neomycin, streptomycin	>128	>128	>128	>128
Spectinomycin dihydrochloride	8–16	64	32	128

[a] MIC is defined as the minimum antimicrobial concentration necessary to inhibit 99% of the growth of *L. intracellularis* relative to a drug-free control. MICs were measured twice (as independently prepared replicates at 5 days of incubation) using a tissue culture system.

tilmicosin and tylosin displayed MICs of 0.5 μg/ml and 1.0 μg/ml, respectively. The MICs of the 2010 isolate using lincomycin, ampicillin, penicillin G, tiamulin, chlortetracycline, and enrofloxacin were higher than those for the 2002 isolate. However, there was no difference in the MIC values of these strains when tilmicosin, tylosin, and carbadox were used (Table 1). Data on *in vitro* antimicrobial susceptibility for *L. intracellularis* are very limited, because the difficulty of maintaining and culturing *L. intracellularis* has resulted in a limited number of available strains. Reports regarding this organism involve low numbers of isolates from Europe and/or North America (12, 17). Thus, McOrist et al. included only three isolates of *L. intracellularis* in a European study (12), and Wattanaphansak et al. tested six isolates from North America and four from Europe in a U.S. study (17). The present study represents the first antimicrobial susceptibility testing of *L. intracellularis* isolates from Asia. Given that South Korea has a high *L. intracellularis* prevalence, ranging from 40% to 100% herd prevalence (8, 16), and that a high herd prevalence is present in other regions of Asia (e.g., Vietnam, 77%; China and the Philippines, 85 to 86%; Japan, 94%; Malaysia and Thailand, 100%) (10), our data are important preliminary first steps in assisting management and treatment decisions.

Reports from clinical trials supplying efficacy and pharmacokinetic data on antimicrobial compounds against *L. intracellularis* are still limited in Asia. Thus, the use of antimicrobial medication for the purpose of preventive medicine and treatment is supported by only a few clinical trial papers for lincomycin (1, 2, 14), tylosin (9, 13), tiamulin (15), and chlortetracycline (18). A wide range of antimicrobials has been recommended for the treatment of pigs with *L. intracellularis* infection in Asia, but the recommendations have not been based on susceptibility testing *in vitro*. Therefore, this preliminary study gives the first insight into the relationship of *in vitro* antibiotic activity and clinical effectiveness reports in South Korea and, taken together with previously published data (12, 17), should be used only as a guide to determine which antimicrobials could be effective in reducing or treating *L. intracellularis* infection and disease. Further *in vivo* studies are needed to confirm the antimicrobial efficacies against Asian isolates of *L. intracellularis*.

The intracellular and extracellular activities of lincomycin, tiamulin, chlortetracycline, spectinomycin, enrofloxacin, and the penicillin class of antimicrobials were two to eight times less effective against the 2010 isolate than against the isolate collected in 2002. With regard to susceptibility changes over time, these results contradict those of Wattanaphansak et al. (17), which reveal no pattern of increased resistance over time among the North American and European strains of *L. intracellularis*.

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