Antibacterial Activity of Tilmicosin against *Pasteurella multocida* and *Actinobacillus pleuropneumoniae* Isolated from Pneumonic Lesions in Swine

Tamio INAMOTO, Katsuaki KIKUCHI, Hiroaki IJIMA, Yasunaga KAWASHIMA, Yutaka NAKAI, and Keiji OGIMOTO

Department of Animal Microbiology and Parasitology, Tohoku University, 1-1 Tsutsumidori Amamiyacho, Aoba-ku, Sendai 981, Japan

(Received 2 December 1993/Accepted 15 June 1994)

ABSTRACT. Sixty one strains of *Pasteurella multocida* and 35 strains of *Actinobacillus pleuropneumoniae* isolated from pneumonic lesions of porcine lungs during the period from 1985 to 1989 in Japan were tested for antibiotic susceptibility to chlortetracycline (CTC), thiamphenicol (TP), tylosin (TS), acetylisovaleryl-tylosin (AIV-TS), tilmicosin (TMS), mirosamycin (MRM). Most strains of both species were sensitive to CTC, TP and TMS. Growth of fifty-one strains (83.6%) and forty-six strains (75.4%) of *P. multocida* were inhibited with 3.13 µg/ml of CTC and 0.78 µg/ml of TP, respectively. TS showed low activity against almost all strains (MIC ≥ 6.25 µg/ml). Fifty-eight (95.1%), twenty-three (37.7%) and fifty (82%) of *P. multocida* showed MICs of ≥ 6.25 µg/ml against AIV-TS, TMS and MRM, respectively. The MICs of *A. pleuropneumoniae* against CTC were less than 1.56 µg/ml. Thirty-two strains (91.4%) and 33 strains (94.3%) of *A. pleuropneumoniae* were inhibited with 3.13 µg/ml of TP and TMS respectively. However, TS, AIV-TS and MRM showed low activity against all of *A. pleuropneumoniae* (MIC ≥ 6.25 µg/ml). Three different resistance patterns were observed in *P. multocida* and two in *A. pleuropneumoniae* isolates, respectively.—**KEY WORDS:** *Actinobacillus pleuropneumoniae*, antibiotic susceptibility, *Pasteurella multocida*, swine, tilmicosin.

J. Vet. Med. Sci. 56(5): 917–921, 1994

Pasteurella multocida and *Actinobacillus pleuropneumoniae* are the etiologic agents for respiratory diseases of swine [2, 16]. Economic losses caused by these infectious diseases are enormous in Japan and Southeast Asian countries. Although a commercial vaccine against *A. pleuropneumoniae* is available in Japan, it does not inhibit the infection, and a vaccine against *P. multocida* has not been developed yet. Therefore, antibiotic treatment is still a practical means of prophylaxis against diseases caused by these bacterial species.

Since macrolide antibiotics are effective against *Mycoplasma*, which is also one of the etiologic agents of respiratory diseases of swine, several macrolide derivatives have been developed recently to expand their antimicrobial spectrum. One of these newly developed derivatives, tilmicosin (20-deoxo-20-(3,5-dimethylpiperidin-1-yl) desmycosin) (TMS) reportedly inhibited the *in vitro* growth of both *A. pleuropneumoniae* and *P. multocida* [17]. It is also reported that TMS effectively prevented the transmission of atrophic rhinitis [1] and development of induced pneumonic pasteurellosis [18] in the U.S.A., but no information was provided regarding these species isolated in Japan.

To evaluate potential ability of TMS as a drug for prophylaxis and treatment against respiratory diseases of swine in Japan, the *in vitro* antibacterial activity of TMS, several other macrolides and antibiotics against *P. multocida* and *A. pleuropneumoniae* isolated from pneumonic lesions of porcine lungs in Japan were investigated.

MATERIALS AND METHODS

Bacterial isolates: Sixty-one strains of *P. multocida* were

isolated from the lungs of pigs from 28 farms in Miyagi and 5 other prefectures in Japan by several meat inspection offices, private veterinary service stations and our laboratory in 1985, 1988 and 1989 (Table 1). Three of these isolates belonged to Carter's capsular serotype D, while the remainder to Carter's serotype A. Thirty-five strains of *A. pleuropneumoniae* were isolated from the pleuropneumonic lungs of pigs from 21 farms in Miyagi and 9 other prefectures in Japan by various district livestock hygiene service centers and private veterinary service stations during the period from 1986 to 1989 (Table 2). There were 33 isolates of serotype 2, one isolate of serotype 1 and one isolate of serotype 5.

Bacterial isolation from porcine lungs showing lesions of pneumonia (consolidation and pleuritis) and identification procedures of the isolates at meat inspection offices in Miyagi prefecture were performed as follows. Pneumonic lesions were soaked with 100% ethanol, cut into 1 cm cubes and homogenized with 9 ml of 1% casamino acids (Difco Lab., Detroit, Michigan, U.S.A.) in the

Table 1. Source and serotypes of 61 *Pasteurella multocida* isolates

Prefecture	No. of farms	No. of strains		
		Serotype A	Serotype D	Total
Miyagi	13	35		35
Tokyo	3	3		3
Kanagawa	8	8		8
Kyoto	1	1		1
Tottori	1		3	3
Kagoshima	2	11		11
Total	28	58	3	61

Table 2. Source and serotypes of 35 *Actinobacillus pleuropneumoniae* isolates

Prefecture	No. of farms	No. of strains			
		Serotype 1	Serotype 2	Serotype 5	Total
Iwate	1	1	2		3
Miyagi	1		4		4
Gunma	3		4		4
Tochigi	2		2		2
Saitama	3		8	2	10
Tokyo	4	1	3		4
Kanagawa	3		3		3
Nagano	1		1		1
Tottori	1		1		1
Kagoshima	2		3		3
Total	21	2	31	2	35

homogenizer. After serial tenfold dilution of homogenized lesion in casamino acids, 0.1 ml of the dilution was inoculated onto a Tryptosoya agar (Nissui Co., Tokyo, Japan) plate containing 5% horse blood for *P. multocida* or onto a chocolate agar plate containing 10% horse blood for *A. pleuropneumoniae*. The former was incubated aerobically at 37°C for 24 hr and the latter in the presence of 10% CO₂ at 37°C for 48 hr. By morphological observation and gram stain, colonies were chosen and spread on new medium. After incubation pure cultures were examined for biochemical characteristics. *P. multocida* was identified by characterization of hemolysis, growth on Desoxycholate Hydrogen Sulfide Lactose Agar (Nissui), indole and urease production, gas production from carbohydrates, acid formation from lactose and mannitol. The capsular serotypes of *P. multocida* isolates were determined by acriflavine flocculation test [4] and/or hyaluronidase decapsulation test [3]. *A. pleuropneumoniae* was identified by characterization for V-factor requirement, CAMP reaction, and urease production. The serotypes of *A. pleuropneumoniae* isolates were determined by the agar gel diffusion test [24] and/or the rapid plate agglutination test [15].

Stock cultures of low passaged organisms were lyophilized in 10% skim milk (Difco) and stored at -80°C until tested for drug susceptibility.

Antimicrobial agents: Drug susceptibility tests were performed on both species with chlortetracycline (CTC), thiamphenicol (TP), tylosin (TS), acetylisovaleryl-tylosin (AIV-TS), TMS, mirosamycin (MRM). CTC, TP and TS were obtained from the National Veterinary Assay Laboratory, Kokubunji, Tokyo. AIV-TS, TMS and MRM were kindly provided by Sanraku Co., Tokyo, Eli Lilly and Co., Kobe, Toyo Jozo Co., Ltd., Tokyo, respectively. All antibiotics were supplied as standard powders and dissolved in dimethyl sulfoxide (DMSO) except CTC and TS, which were dissolved in distilled water. The final concentration of DMSO was less than 4% in each medium.

Susceptibility test: The agar dilution method standardized by the Japan Society of Chemotherapy [14] was used for the determination of minimal inhibitory concentration (MIC) values. Briefly, both species of organisms were inoculated into Mueller-Hinton broth (Difco) supplemented with 5% Fildes enrichment (Difco) and incubated for 18 hr at 37°C. The inocula were adjusted to 10⁶ colony forming units/ml using a spectrophotometer. A five microliter aliquot of these suspensions was deposited onto agar plates containing antibiotics using an inoculator system (MIT-P; Sakuma Co., Ltd., Tokyo, Japan). Blood agar plates were used for *P. multocida* and chocolate agar plates for *A. pleuropneumoniae*. These agar plates were prepared from Sensitivity Disc Agar-N (Nissui) containing 5% horse blood and serial twofold dilutions of the test drugs (concentration range from 100 µg/ml to 0.025 µg/ml with non-antibiotic growth control plates). After inoculation, the blood agar plates were incubated for 18 hr at 37°C under aerobic conditions and the chocolate agar plates were incubated for 18 hr at 37°C in the presence of 10% CO₂. *Escherichia coli* NIHJ-JC-2 and *Staphylococcus aureus* 209P were used as gram negative and positive reference strains for quality control in each experiment, respectively. The lowest concentration of the drug inhibiting macroscopic growth was regarded as the MIC of the drug.

RESULTS

MICs of various antibiotics of *P. multocida* are shown in

Table 3. Antimicrobial susceptibility of the 61 *Pasteurella multocida* isolates

Drugs ^{a)}	Minimal inhibitory concentrations (µg/ml)													MIC break-point of resistance	No. of resistant strains (%)
	≥100	50	25	12.5	6.25	3.13	1.56	0.78	0.39	0.2	0.1	0.05	≤0.025		
CTC		1 ^{b)}	3	5	1	1	12	18 ^{c)}	6	9	5			6.25	10(16.4)
TP	6	2		3	2	2		35	5				6	1.56	15(24.6)
TS	10	20	24	4	2							1		6.25	60(98.4)
AIV-TS	26		5	5	22	2				1					
TMS	1		1	9	12	29	6	1	1		1				
MRM		1	10	5	34	3		1	1				6		

a) CTC=chlortetracycline, TP=thiamphenicol, TS=tylosin, AIV-TS=acetylisovaleryl-tylosin. TMS=tilmicosin, MRM=mirosamycin.

b) Number of strains.

c) Underlines represent mode of MICs of the antibiotics.

Table 4. Antimicrobial susceptibility of the 35 *Actinobacillus pleuropneumoniae* isolates

Drugs ^{a)}	Minimal inhibitory concentrations (μg/ml)													MIC break-point of resistance	No. of resistant strains (%)
	≥100	50	25	12.5	6.25	3.13	1.56	0.78	0.39	0.2	0	0.05	≤0.025		
CTC							1 ^{b)}	5	7	22 ^{c)}					
TP	1		1	1		1	2	29						6.25	3(8.6)
TS	26	3	6												
AIV-TS	31	4													
TMS			2			10	22	1						6.25	2(5.7)
MRM	2	16	12	4	1										

a), b), c) See the footnotes in Table 3.

Table 3. There were 3 peaks in the distribution of MICs against TP, indicating 3 groups of strains ($\text{MIC} \leq 0.78 \mu\text{g/ml}$, $3.13\text{--}12.5 \mu\text{g/ml}$ and $\geq 50 \mu\text{g/ml}$). Growth of forty-six (75.4%) strains of *P. multocida* were inhibited by $0.78 \mu\text{g/ml}$ of TP. Six of 61 strains showed extremely low MIC ($\leq 0.025 \mu\text{g/ml}$) against TP and MRM, simultaneously. MICs of AIV-TS also showed a trimodal distribution ($0.2 \mu\text{g/ml}$, $3.13\text{--}25 \mu\text{g/ml}$ and $\geq 100 \mu\text{g/ml}$). There were bimodal distribution of MICs against CTC and TS, indicating 2 groups of the strains ($\text{MIC} \leq 3.13 \mu\text{g/ml}$ and $\geq 6.25 \mu\text{g/ml}$). Growth of fifty-one (83.6%) of strains were inhibited with $3.13 \mu\text{g/ml}$ of CTC. Tylosin showed low activity against almost all strains ($\text{MIC} \geq 6.25 \mu\text{g/ml}$). On the other hand, only one of 61 strain showed an extremely low MIC ($0.05 \mu\text{g/ml}$) against TS. This strain also had a low MIC ($0.1\text{--}0.39 \mu\text{g/ml}$) against all of the TS derivatives. Fifty-eight (95.1%) and 50 (82%) strains had MICs of $6.25 \mu\text{g/ml}$ against AIV-TS and MRM respectively. Growth of thirty-eight (62.3%) strains were inhibited with $3.13 \mu\text{g/ml}$ of TMS. The distribution of MICs against TMS and MRM showed a wide range ($0.1\text{--}\geq 100 \mu\text{g/ml}$ and $\leq 0.025\text{--}50 \mu\text{g/ml}$ respectively), and the modal MICs of these drugs were $3.13 \mu\text{g/ml}$ and $6.25 \mu\text{g/ml}$ respectively. Thirty, twenty-six, one and one strains had MICs of $\geq 50 \mu\text{g/ml}$ against TS, AIV-TS, TMS and MRM respectively.

MICs of various antibiotics to *A. pleuropneumoniae* are shown in Table 4. Strains of *A. pleuropneumoniae* were moderately sensitive to CTC and the MICs were less than $1.56 \mu\text{g/ml}$. There were 3 peaks in the distribution of MICs against TP, indicating 3 groups ($\text{MIC } 0.78\text{--}3.13 \mu\text{g/ml}$, $12.5\text{--}25 \mu\text{g/ml}$ and $\geq 100 \mu\text{g/ml}$) of *P. multocida* strains. Thirty-two strains (91.4%) of *A. pleuropneumoniae* were inhibited their growth with $3.13 \mu\text{g/ml}$ of TP. Tylosin, AIV-TS and MRM showed low activity against the strains ($\text{MIC} \geq 6.25 \mu\text{g/ml}$). However, MICs of TMS showed a bimodal distribution ($1.56 \mu\text{g/ml}$ and $25 \mu\text{g/ml}$), and more than 94% of the strains were inhibited their growth with $3.13 \mu\text{g/ml}$ of TMS.

Drug resistance patterns of *P. multocida* are shown in Table 5. Three different resistance patterns of *P. multocida* were observed. A total of 19 strains of *P. multocida* were resistant to CTC or TP. Of them, 7 were simultaneously resistant to CTC and TP, 3 were resistant to CTC, while the remaining 9 were resistant to TP. These multiple resistant strains showed relatively low susceptibi-

Table 5. Relationship between resistance patterns and serotypes of 61 *Pasteurella multocida* isolates

Drugs ^{a)}	Serotype		
	A	D	Total (%)
CTC, TP	7 ^{b)}		7(11.5)
CTC	2	1	3 (4.9)
TP	9		9(14.8)
Total	Resistant Susceptible	18 40	1 2
			19(31.1) 42(68.9)

a), b) See the footnotes in Table 3. TS resistant strains were omitted from this table to compare with other reports.

Table 6. Relationship between resistance patterns and serotypes of 35 *Actinobacillus pleuropneumoniae* isolates

Drugs ^{a)}	Serotype			
	1	2	5	Total (%)
TP		2 ^{b)}	1	3(8.6)
TMS		2		2(5.7)
Total	Resistant Susceptible	0 2	4 27	1 1
				5(14.3) 30(85.7)

a), b) See the footnotes in Table 3.

lities to macrolides. Eighteen (31.0%) of 58 strains of serotype A showed multiple or single drug resistance against CTC and TP, while one (33.3%) of three strains of serotype D was resistant to CTC. Drug resistance patterns of *A. pleuropneumoniae* are shown in Table 6. A total of 5 (14.3%) strains were resistant to TP or TMS. Of them, 2 strains each belonging to serotype 2 were resistant to TP or TMS, and 1 strain belonging to serotype 5 was resistant to TP.

DISCUSSION

P. multocida and *A. pleuropneumoniae* were highly sensitive to penicillins, tetracyclines (TC) and chloramphenicol (CP) [10–12, 21, 23, 27]. From our present

results, TC and CP derivatives (CTC and TP) were still sufficiently active against *P. multocida* and *A. pleuropneumoniae* as reported before [5, 9–12, 21, 23, 27]. Recently, however, antimicrobial resistance of both bacterial species has been reported [5, 7, 9–13, 21, 23, 25, 27]. Although our results in the distribution of MICs of *P. multocida* against CTC and TS were comparable to other reports [10, 25, 27], highly resistant strains against TP were detected. Unexpectedly, a TS sensitive strain of *P. multocida* was detected in the present study, but no highly sensitive strains were found in other reports [10, 12, 21, 25, 27]. Therefore, TS non-sensitive strains may not be considered as TS resistant strains to compare drug resistance patterns with other reports. Activities of TS against both species were well known, but the break points of AIV-TS, TMS and MRM against both species have not been reported yet. Also, a limited number of strains of both species were tested in this experiment. Therefore, MIC break points of AIV-TS, TMS and MRM could not be defined in this experiment, except TMS against *A. pleuropneumoniae*. Instead of defining each break point, a comparison of activities were based on break point of TS. The frequencies of resistant *P. multocida* strains were higher in the serotype D than in serotype A. These results were nearly in agreement with those by Iwamatsu *et al.* [12]. The present results and other reports [10, 12, 25, 27] indicate that CTC and TP are losing some effectiveness against *P. multocida* by the development of resistant strains.

On the other hand, strains of *A. pleuropneumoniae* in the present study were more sensitive to CTC than in the other reports [5, 13, 23], and consistent with previous report by Fukuyasu [5] in TP. However, TS was less effective against our strains than previous reports [10, 11, 23]. Furthermore, in the present investigations, 5 of 35 *A. pleuropneumoniae* isolates were drug-resistant. Four of these resistant strains belonged to serotype 2, while remainder belonged to serotype 5. Many investigators discussed the correlation between the antimicrobial resistance and the serotype of *A. pleuropneumoniae* [5, 9, 11, 13, 23]. Similar results were also obtained in this study. Therefore, strains of both species in the present study may be suitable for estimating antibacterial activities of TS derivatives against isolates of both species in Japan, although a small number of strains of both species were tested.

TMS was reported to be effective *in vitro* against *A. pleuropneumoniae*, *P. multocida*, *Mycoplasma* sp. and *Bordetella bronchiseptica* [17], and reduced clinical scores and extent of pneumonic consolidation in calves by *Pasteurella* sp. and *Mycoplasma bovis* [6, 19]. Furthermore, it was reported that TMS at the level of 200 ppm of feed effectively minimized the transmission of atrophic rhinitis from sick to healthy pigs [1], and that the 55 ppm TMS level added to the feed reduced the effects of induced pneumonic pasteurellosis in young pigs [18]. In our previous study [8], it was suggested that TMS had an equal activity as erythromycin, josamycin, kitasamycin

and spiramycin against *Mycoplasma hyopneumoniae* isolated from swine in Japan. Therefore, it is expected to be used for prophylaxis and treatment of mixed infection with both bacteria and *Mycoplasma* in swine and cattle.

In general, the antimicrobial spectrum of TS covered gram positive bacteria and *Mycoplasma* species [20], but not gram negative enteric bacteria. Therefore, in order to expand this antimicrobial spectrum, many derivatives of TS such as AIV-TS, TMS and MRM have been developed. The present study demonstrated that TMS was more effective than TS, AIV-TS and MRM against the isolates of *P. multocida* and *A. pleuropneumoniae*. Therefore, TMS may have a potential as a drug for prophylaxis and treatment of respiratory infections in pigs in Japan. MICs of TMS against *P. multocida* in this study were similar to the isolates in the U.S.A. [17]. Therefore, further investigations such as determination of blood concentration, half life, tissue concentration of the drug, laboratory trials and field trials against *P. multocida* should be done to clarify the effectiveness of the drug. Since two resistant strains of *A. pleuropneumoniae* against TMS were detected in the present study, the presence of less sensitive strains also should be monitored when using this antibiotic on swine farms.

Acetylisovaleryl-tylosin and MRM had less activity against both bacterial species, but AIV-TS had better *in vitro* [26] and *in vivo* [22] activities compared with TS against macrolide-resistant strains of *Mycoplasma*, and MRM had also better activity against *Mycoplasma hyopneumoniae* [8] *in vitro*. Therefore, they may also be useful against mycoplasmal pneumonia in animals.

ACKNOWLEDGEMENTS. The authors thanks Dr. Shoko Suzuki of the National Veterinary Assay Laboratory for her kind providing a part of *A. pleuropneumoniae* isolates, Mr. Koji Nakamoto of Shionogi and Co., Ltd. for his kind providing a part of *A. pleuropneumoniae* and *P. multocida* isolates and Dr. Masayuki Nakamura of the National Veterinary Assay Laboratory for his helpful suggestion.

REFERENCES

1. Backstrom, L., Chung, W. B., Collins, M. T., and Ose, E. 1988. Efficacy of tilmicosin for control of atrophic rhinitis. p. 161. In: Proc. 10th Cong. IPVS.
2. Baskerville, A. 1981. Pneumonia of pigs: a review. *N. Z. Vet. J.* 29: 216–218.
3. Cater, G. R., and Rundell, S. W. 1975. Identification of type A strains of *P. multocida* using staphylococcal hyaluronidase. *Vet. Rec.* 12: 343.
4. Cater, G. R. and Subronto, P. 1973. Identification of type D strains of *Pasteurella multocida* with acriflavine. *Am. J. Vet. Res.* 34: 293–294.
5. Fukuyasu, T. 1993. Serotype and antimicrobial susceptibility of *Actinobacillus pleuropneumoniae* isolated from pigs during 1989 to 1991. pp. 7–12. In: Proceedings of the Japanese Society of Antimicrobials for Animals (in Japanese with English abstract).
6. Gourlay, R. N., Thomas, L. H., Wyld, S. G., and Smith, C. J. 1989. Effect of a new macrolide antibiotic (tilmicosin) on

- pneumonia experimentally induced in calves by *Mycoplasma bovis* and *Pasteurella haemolytica*. *Res. Vet. Sci.* 47: 84–89.
7. Hirsh, D. C., Martin, L. D., and Libal, M. C. 1982. Plasmid-mediated antimicrobial resistance in *Haemophilus pleuropneumoniae*. *Am. J. Vet. Res.* 43: 269–272.
 8. Inamoto, T., Takahashi, H., Yamamoto, K., Nakai, Y., and Ogimoto, K. 1994. Antibiotic susceptibility of *Mycoplasma hyopneumoniae* isolated from swine. *J. Vet. Med. Sci.* 56: 393–394.
 9. Inoue, A., Yamamoto, K., Hirano, N., and Murakami, T. 1984. Drug susceptibility of *Haemophilus pleuropneumoniae* strains isolated from pigs. *Jpn. J. Vet. Sci.* 46: 175–180.
 10. Ishii, H., Mokudai, K., Seki, T., Matsumoto, T., Kameda, M., Kurihara, O., Araki, Y., Iyobe, S., and Hashimoto, H. 1990. Drug-susceptibility of *Pasteurella multocida* isolated from swine from 1987 to 1989. *Jpn. J. Vet. Sci.* 52: 399–402.
 11. Ishii, H., Nakasone, Y., Shigehara, S., Honma, K., Araki, Y., Iyobe, S., and Hashimoto, H. 1990. Drug-susceptibility and isolation of a plasmid in *Haemophilus (Actinobacillus) pleuropneumoniae*. *Jpn. J. Vet. Sci.* 52: 1–9.
 12. Iwamatsu, S., Mukouhara, and Sawada, T. 1991. Antibiotic susceptibility of *Pasteurella multocida* isolated from swine from 1983 to 1986. *J. Jpn. Vet. Med. Assoc.* 44: 478–481 (in Japanese with English abstract).
 13. Kawahara, K., Asano, M., Nakai, T., Kume, K., and Danbara, H. 1989. Antibiotic susceptibility of serotype 2 and 5 strains of *Actinobacillus (Haemophilus) pleuropneumoniae* isolated from swine from 1974 to 1986. *Jpn. J. Vet. Sci.* 51: 359–363.
 14. Mitsuhashi, S., Goto, S., Jo, K., Kawakita, T., Kozakai, N., Nishino, T., Osawa, N., and Tanami, H. 1981. Third edition of standard method for determining minimum inhibitory concentrations of antibiotics against bacteria. *Chemotherapy (Tokyo)* 29: 76–79 (in Japanese).
 15. Mittal, K. R., Higgins, R., and Lariviere, S. 1982. Evaluation of slide agglutination and ring precipitation tests for capsular serotyping of *Haemophilus pleuropneumoniae*. *J. Clin. Microbiol.* 15: 1019–1023.
 16. Muirhead, M. R. 1979. Respiratory disease in pigs. *Br. Vet. J.* 135: 497–508.
 17. Ose, E. E. 1987. *In vitro* antibacterial properties of EL-870, a new semi-synthetic macrolide antibiotic. *J. Antibiot.* 40: 190–194.
 18. Ose, E. E. and Tonkinson, L. V. 1988. Tilmicosin in feed for the control of induced pneumonic pasteurellosis in pigs. p. 62. *In: Proc. 10th Cong. IPVS.*
 19. Picavet, T., Muylle, E., Devriese, L. A., and Geryl, J. 1991. Efficacy of tilmicosin in treatment of pulmonary infections in calves. *Vet. Rec.* 129: 400–403.
 20. Sakakibara, H. and Omura, S. 1984. Chemical modification and structure activity relationship of macrolides. pp. 85–125. *In: Macrolide Antibiotics. Chemistry, Biology, and Practice (Omura, S. ed.), Academic Press, Inc.*
 21. Shimizu, M., Kuninori, K., Sakano, T., and Terashima, T. 1982. Antibiotic susceptibility of *Haemophilus pleuropneumoniae* and *Pasteurella multocida* isolates from swine. *Jpn. J. Vet. Sci.* 44: 359–363.
 22. Skelly, B. J., Andersen, D., Pruss, M., and Pellegrino, R. 1986. Prophylactic efficacy of 3-acetyl-4"-isovaleryl tylosin in a *Mycoplasma gallisepticum*-induced airsacculitis infection. *Avian Dis.* 30: 505–509.
 23. Suzuki, S., Ohmae, K., Ohishi, K., Muramatsu, M., and Takahashi, T. 1989. Antimicrobial susceptibility of *Actinobacillus (Haemophilus) pleuropneumoniae* isolated from pigs with pleuropneumonia. *Jpn. J. Vet. Sci.* 51: 450–452.
 24. Suzuki, S., Takahashi, T., Muramatsu, M., Ohishi, K., Nakajima, M., and Yamashita, M. 1988. Serotyping of *Actinobacillus (Haemophilus) pleuropneumoniae* isolates from pigs in slaughter-house. *Jpn. J. Vet. Sci.* 50: 1264–1267.
 25. Takahashi, I., Yoshida, T., Higashide, Y., and Sawada, T. 1989. Susceptibility of animal isolates of *Pasteurella multocida* to ofloxacin and commonly used antimicrobial agents. *Chemotherapy (Tokyo)* 37: 399–405 (in Japanese with English abstract).
 26. Tsuchiya, M., Hamada, M., Takeuchi, T., Umezawa, H., Yamamoto, K., Tanaka, H., Kiyoshima, K., Mori, S., and Okamoto, R. 1982. Studies of tylosin derivatives effective against macrolide-resistant strains: synthesis and structure-activity relationships. *J. Antibiot.* 35: 661–672.
 27. Ushijima, T. 1993. Drug susceptibility and R plasmids of *Pasteurella multocida* isolated from nasal cavities of swine. pp. 23–31. *In: Proceedings of the Japanese Society of Antimicrobials for Animals.* (in Japanese with English abstract).