屠場豚の肺病巣から分離されたActinobacillus(Haemophilus)pleuropneumoniaeの薬剤感受性

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Antimicrobial Susceptibility of *Actinobacillus (Haemophilus) pleuropneumoniae* Isolated from Pigs with Pleuropneumonia

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Actinobacillus (Haemophilus) pleuropneumoniae is the causative agent of porcine pleuropneumonia which causes a great economic loss in pig production. Antibiotic treatment is still commonly used to control this disease, although commercial vaccine is provided in Japan. Recently, antimicrobial resistance in *A. pleuropneumoniae* has been reported by many investigators [1–7,9]. The present investigation was performed to know the *in vitro* antimicrobial susceptibility of *A. pleuropneumoniae.*

A total of 190 strains of *A. pleuropneumoniae* was submitted for the assay of antimicrobial susceptibility. They were isolated from the lung lesions of slaughtered pigs during the period from 1986 to 1987 in Japan [10]. Of them, 6 (3.2%), 178 (93.7%), 2(1.1%) and 4 (2.2%) isolates belonged to serovar 1,2,5 and 7, respectively. Antimicrobial agents studied were: benzylpenicillin (PCG), ampicillin (ABPC), cloxacillin (CX), mecillinam (MPC), cephalexin (CEX), streptomycin (SM), kanamycin (KM), gentamicin (GM), chloramphenicol (CP), oxytetracycline (OTC), erythromycin (EM), tylosin (TS), rifampicin (RFP), colistin (CL), sulfadimethoxine (SDMX) and nalidixic acid (NA). The minimum inhibitory concentrations (MICs) were determined by an agar dilution method. A 10^{-2} dilution of 6 hr-broth culture was inoculated by microplanter onto Mueller-Hinton agar (Difco) for SDMX or trypticase soy agar (Difco) for the remaining drugs, containing 25 μg/m*l* of β-nicotineamide adenine dinucleotide (Oriental Yeast) and serial twofold dilution of the test drugs. The plates were incubated for 16 hr at 37°C under the aerobic condition. The MIC was defined as the lowest concentration of antimicrobial agent that prevented macroscopic growth. MICs are shown in Table 1. Almost all of the isolates were highly susceptible to penicillins. With 0.78 U/m*l* of PCG or 0.2 μg/m*l* of ABPC, more than 98% of strains tested were inhibited their growth. There were 3 peaks in the distribution of their MICs against MPC, indicating 3 groups of the strains (MIC, 0.05–0.1 μg/m*l*, 1.56 μg/m*l*, and 12.5–25 μg/m*l*). With 0.78 μg/m*l* of CP and RFP, more than 98% of the strains were inhibited their growth. One hundred and seventy four of 190 strains were moderately susceptible to CEX (MIC, 1.56 μg/m*l*). The susceptibility of the strains against EM, CL or NA was relatively moderate, and the MICs of almost all strains were less than 3.13 μg/m*l*. SM, KM and GM showed relatively low activity against the strains (MIC, 3.13–12.5 μg/m*l*). The distribution of their MICs against OTC and SDMX showed wide ranges (MIC, 0.39−>100 μg/m*l*). However, the growth of almost strains was inhibited with 0.78 μg/m*l* of OTC. CX and TS were low activity against the strains (MIC, 25 μg/m*l*).

The frequencies of isolation of resistant strains to each drugs were as follows: SM (3.7%), OTC (2.1%), CP (1.6%), PCG (1.1%), ABPC (1.1%), CX (1.1%), MPC (1.1%) and KM (1.1%). The relationship between antimicrobial resistance patterns and serotypes of isolates is shown in Table 2. Six different resistance patterns were observed. A total of 10 (5.3%) strains were resistant to SM, CP, OTC, PCG, ABPC, CX, MPC or KM. Of the 10 resistant strains, 2 and 3 strains belonged to serotype 5 were resistant to OTC. Of the three isolates of serotype 7, one strain was resistant to SM and CP and the other two were resistant to PCG, ABPC, CX, MPC, SM and KM.

Most of the isolates tested in the present study were highly susceptible to PCG, ABPC, CP and RFP, and were moderately susceptible to CEX, EM, CL and NA. On the other hand, SM, KM and GM showed relatively low activity against the strains. OTC and SDMX showed wide range of MICs. These results were nearly in agreement with those by Inoue *et al.* [4] and by Shimizu *et al.* [8]. However, 10 of 190 strains (5.3%) were resistant to drugs. Five of 178 strains of serotype

Table 1. Antimicrobial susceptibility of the 190 *A. pleuropneumoniae* isolates

Drug	Minimum Inhibitory Concentration ($\mu g/ml^{a)}$)														MIC break-point of resistance	No. of resistant strains (%)
	≤0.025	0.05	0.1	0.2	0.39	0.78	1.56	3.13	6.25	12.5	25	50	100	>100		
PCG				3[b)]	61	124						2			6.25	2 (1.1)
ABPC	1	1	23	157	6						2				3.13	2 (1.1)
CX									1	87	96	4	2		100	2 (1.1)
MPC	6	32	37	10	4	7	22	3	7	18	42			2	100	2 (1.1)
CEX					1	15	173	1								
SM									76	61	40	6	1	6	100	7 (3.7)
KM								1	69	117	1			2	100	2 (1.1)
GM							5	101	84							
CP				1	120	66		1	1	1					3.13	3 (1.6)
OTC					22	149	3	10	1	1	2	2			25	4 (2.1)
EM					1		103	86								
TS									1	6	158	25				
RFP			4	13	131	42										
CL						10	45	134		1						
SDMX					1	2	3	24	62	52	26	7	8	5		
NA						51	123	15	1							

a) Units per m*l* for PCG.
b) Number of isolates.

Table 2. Relationship between resistance patterns and serotypes of 190 *A. pleuropneumoniae* isolates

Drug		Serotype				Total (%)
		1	2	5	7	
SM			1[a)]			1 (0.5%)
CP			1			1 (0.5%)
OTC				2		2 (0.5%)
SM, CP			1		1	2 (1.1%)
SM, OTC			2			2 (1.1%)
PCG, ABPC, CX, MPC, SM, KM					2	2 (1.1%)
Total	Resistant	0	5	2	3	10 (5.3%)
	Susceptible	6	173	0	1	180 (94.7%)

a) Number of isolates.

2 were resistant to SM, CP or OTC. Two strains of serotype 5 and 7, respectively. The two isolates of 5 were resistant to OTC, and 2 strains of serotype 7 were multidrug resistant. Recently, antimicrobial resistance of *A. pleuropneumoniae* was reported by many investigators [1–7,9]. In Japan, pigs are usually fed food containing various antibiotics for the purpose of growth stimulation. It seems, therefore, that longterm administration of antibiotics gives a selective advantage to such antimicrobial-resistant strains of *A. pleuropneumoniae*. Inoue *et al.* [4] reported that 2 strains belonged to serovar 3 and 5 were resistant strains, although none of 105 isolates of serovar 2 showed any resistance. According to their data and ours, it seems that the increase of incidence of the antimicrobial-resistance dose not easily occur in the serotype 2 strains. However, it is not clear whether the serotype is concerned with the antimicrobial-resistance in *A. pleurop-*

neumoniae, because only small number of strains belonged to the other serotype except serotype 2 could be studied in our investigation. Therefore, further investigation should be carried out to make it clear.

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要　約

屠場豚の肺病巣から分離された *Actinobacillus* (*Haemophilus*) *pleuropneumoniae* の薬剤感受性（短報）：鈴木祥子・大前憲一[1]・大石弘司・村松昌武・高橋敏雄（農林水産省動物医薬品検査所，[1]農林水産省衛生課）——1986～1987年に，屠場豚の肺病巣から分離された *A. pleuropneumoniae* 190株（血清型 1，2，5，7）の薬剤感受性を調べたところ，ほとんどの株は，供試した薬剤の多くに感受性であった．一方，190株中10株（5.3%）に，SM，CP，OTC，PCG，ABPC，CX，MPC，KM に対する単剤あるいは多剤耐性が認められた．