

1974年-1986年にかけてブタの鼻腔から分離された  
Actinobacillus(Haemophilus)pleuropneumoniaeの抗生  
物質感受性

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## Antibiotic Susceptibility of Serotype 2 and 5 Strains of *Actinobacillus (Haemophilus) pleuropneumoniae* Isolated from Swine from 1974 to 1986

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**ABSTRACT.** Antibiotic susceptibility of 129 isolates of *Actinobacillus (Haemophilus) pleuropneumoniae* was examined. All the strains were isolated in 1974 to 1986 from the nasal cavities of swine housed on 35 farms in 11 prefectures of Japan. All 28 strains of serotype 2 isolated before 1984 were susceptible to 10 antibiotics used. In contrast, more than one-half of 79 strains of serotype 2 isolated in 1985 and 1986 were resistant to aminoglycosides, tetracycline, tylosin and carbadox, and most of them showed multiple drug resistance. All the 22 strains of serotype 5 were isolated only in 1985 and 1986, and almost all the isolates were susceptible to the antibiotics used in this study. Distribution of minimal inhibitory concentration of serotype 2 strains were bimodal to aminoglycosides, tetracycline, or tylosin. The present results showed that the number of antibiotic resistant strains of *A. pleuropneumoniae* has increased recently in Japan.—**KEY WORDS:** *A. pleuropneumoniae*, antibiotic susceptibility.

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*Actinobacillus (Haemophilus) pleuropneumoniae* is the causative agent of porcine *Haemophilus pleuropneumonia* which is characterized by acute fibrinohemorrhagic or chronic localized necrotizing pneumonia with pleuritis, and is one of the major problems in swine industry. *A. pleuropneumoniae* strains are divided into 12 serotype strains [12], and distribution of serotype strains in the world varies from one country to another. Previous reports showed that serotype 2 strains were predominant in Japan [6, 7, 10]. However, we found that serotype 5 strains have also been isolated in recent Japanese field [11].

Shimizu *et al.* [14] and Inoue *et al.* [5] previously reported that antibiotic resistant strains of *A. pleuropneumoniae* were rare in

Japan, while they were commonly isolated outside of Japan [1, 2, 4, 9, 13].

The present study, *in vitro* antibiotic susceptibility test, demonstrated that the number of antibiotic resistant *A. pleuropneumoniae* strains has increased and they have spread to the wide area of recent Japanese field.

### MATERIALS AND METHODS

**Bacterial strains:** A total of 129 strains of serotype 2 (107 strains) and serotype 5 (22 strains) of *A. pleuropneumoniae* was used in this study (Table 1). They were isolated in 1974 to 1986 from the nasal cavities of swine housed on 35 farms in 11 prefectures of Japan. These strains were serotyped according to the method described previously [6].

**Test of antibiotic susceptibility:** Minimal inhibitory concentration (MIC) value was determined by agar-plate dilution method as

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follows: Organisms of each strain were streaked on S agar plates [8] using toothpick and incubated at 37°C for overnight. They were then replica-plated using velveteen cloth onto Mueller-Hinton agar plates (BBL, Maryland, U.S.A.) containing antibiotic, which are supplemented with  $\beta$ -nicotinamide adenine dinucleotide at the concentration of 20  $\mu\text{g/ml}$ . MIC values were determined after 48 hr incubation at 37°C in the presence of 5% CO<sub>2</sub>. The highest concentration of each antibiotic in Mueller-Hinton agar plates was 200  $\mu\text{g/ml}$ , and serial two-fold dilutions to 0.39  $\mu\text{g/ml}$  were prepared. Antibiotics used were as follows with abbreviations in parenthesis; ampicillin

(ABPC), carbadox (CBD), chloramphenicol (CP), fradiomycin (FRM), kanamycin (KM), lincomycin (LCM), streptomycin (SM), tetracycline (TC), tiamulin (TIM), and tylosin (TS). Strains showing the MIC values of 200  $\mu\text{g/ml}$  for LCM and TS, 50 to 200  $\mu\text{g/ml}$  for ABPC, KM, SM, FRM, TIM and CBD, and 25 to 200  $\mu\text{g/ml}$  for CP and TC were judged as antibiotic resistant in this study.

## RESULTS

*Antibiotic susceptibility of 129 strains of A. pleuropneumoniae:* Table 1 shows the results of *in vitro* susceptibility test of 129

Table 1. Susceptibility of 129 strains of *A. pleuropneumoniae* isolated from the nasal cavities of swine during 1974–1986

Sero-type	Year of isolation	Place of farm	Number of strains isolated	Number of strains resistant to									
				ABPC	KM	SM	LCM	FRM	CP	TC	TS	TIM	CBD
2	1974	Tokyo	1	0	0	0	0	0	0	0	0	0	0
2	1976	Ibaraki	1	0	0	0	0	0	0	0	0	0	0
2	1977	Chiba	1	0	0	0	0	0	0	0	0	0	0
2	1978	Fukuoka	2	0	0	0	0	0	0	0	0	0	0
2	1978	Shizuoka	1	0	0	0	0	0	0	0	0	0	0
2	1980	Chiba	2	0	0	0	0	0	0	0	0	0	0
2	1980	Tokyo	1	0	0	0	0	0	0	0	0	0	0
2	1980	Gifu	1	0	0	0	0	0	0	0	0	0	0
2	1982	Gunma	2	0	0	0	0	0	0	0	0	0	0
2	1982	Chiba	3	0	0	0	0	0	0	0	0	0	0
2	1982	Fukuoka	5	0	0	0	0	0	0	0	0	0	0
2	1983	Gunma	3	0	0	0	0	0	0	0	0	0	0
2	1983	Yamaguchi	5	0	0	0	0	0	0	0	0	0	0
2	1985	Miyagi	1	0	1	0	0	1	0	1	0	0	0
2	1985	Gunma	17	0	6	6	14	4	2	15	13	0	4
5	1985	Ibaraki	12	0	1	0	0	0	0	0	0	0	1
2	1985	Miyazaki	14	0	10	8	7	3	0	10	7	0	4
5	1985	Miyazaki	1	0	0	0	0	0	0	0	0	0	0
2	1986	Fukushima	7	0	0	0	7	0	0	7	4	0	2
5	1986	Fukushima	1	0	0	0	0	0	0	0	0	0	0
5	1986	Gunma	5	0	0	0	0	0	0	0	0	0	0
2	1986	Gunma	12	0	8	8	10	1	0	3	3	0	5
2	1986	Shizuoka	2	0	0	1	1	0	0	2	2	0	1
2	1986	Yamaguchi	20	0	5	6	20	2	1	20	16	0	13
2	1986	Miyazaki	6	1	4	6	4	1	0	4	4	0	2
5	1986	Miyazaki	3	0	0	0	0	0	0	0	0	0	0
Total			129	1	35	35	63	12	3	62	49	0	32

isolates of *A. pleuropneumoniae* to 10 antibiotics. As summarized in Table 2, all strains of serotype 2 isolated in 1974 to 1983 were susceptible to the antibiotics tested. In contrast, serotype 2 strains isolated in 1985 and 1986 were frequently resistant: *i.e.* more than half of 79 strains were resistant to LCM, TC and TS, and approximately one-half of the strains were resistant to KM, SM and CBD. The farms, from which the resistant strains were isolated, were geographically widely distributed. Serotype 5 strains were isolated only in 1985 and 1986. Only one out of 22 serotype 5 strains were resistant to KM and CBD, and others were all sensitive strains. Most of the 129 strains were susceptible to ABPC, CP and TIM.

Multi-resistance profiles of the serotype 2 strains isolated in 1985 and 1986 to KM, SM, LCM, TC, TS and CBD were examined. Eight (10%) out of 79 strains were resistant to all the six antibiotics, 13 (16%) were quintuple resistant, 19 (24%) were quadruple, 21 (27%) were triple, 9 (11%) were double, 3 (4%) were single, and 6 (8%) were sensitive to all the antibiotics.

*Distribution of MIC value of A. pleuropneumoniae strains:* Distribution of MIC

values of 129 *A. pleuropneumoniae* strains for KM, SM, LCM, TC and TS were bimodal. The typical patterns of KM and TC resistances were shown in Fig. 1. In the case of CP, two strains showed MIC value of 50  $\mu\text{g/ml}$ , one strain 25  $\mu\text{g/ml}$ , and all others 6.3  $\mu\text{g/ml}$  or lower than that. Only one strain showed MIC value of 200  $\mu\text{g/ml}$  to ABPC, and other strains were very susceptible to ABPC (MIC value lower than 0.39  $\mu\text{g/ml}$ ).

Table 2. Summary of antibiotic susceptibility test of *A. pleuropneumoniae* strains to 10 antibiotics

Antibiotics	Serotype 2		Serotype 2		Serotype 5	
	(1974-1983)		(1985-1986)		(1985-1986)	
	S	R	S	R	S	R
ABPC	28	0	78	1	22	0
KM	28	0	45	34	21	1
SM	28	0	44	35	22	0
LCM	28	0	17	62	22	0
FRM	28	0	67	12	22	0
CP	28	0	76	3	22	0
TC	28	0	17	62	22	0
TS	28	0	32	47	22	0
TIM	28	0	79	0	22	0
CBD	28	0	48	31	21	1

S: susceptible, R: resistant.

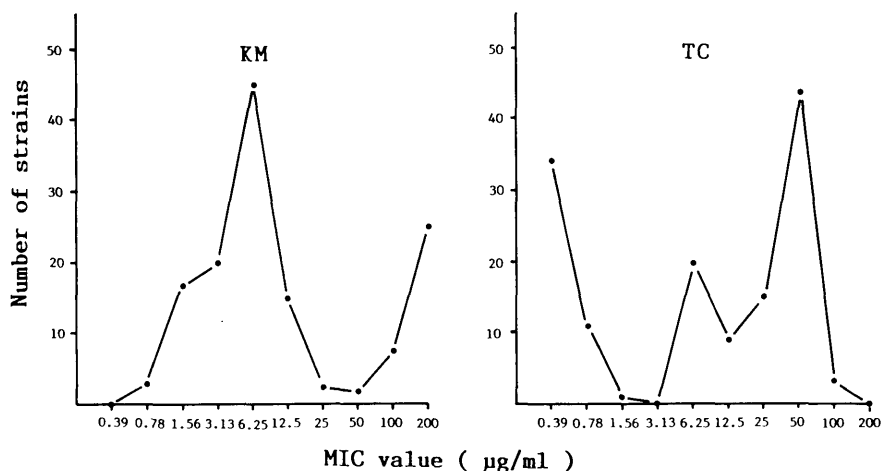


Fig. 1. Distribution of MIC values of *A. pleuropneumoniae* strains. A total of 129 *A. pleuropneumoniae* strains isolated in 1974-1986 were examined for their MIC values for kanamycin (KM) and tetracycline (TC) according to the method described in the text.

Those results suggest that the antibiotic resistance of the strains with higher MIC values is plasmid-mediated.

#### DISCUSSION

The present study, consistently with previous reports by Shimizu *et al.* [14] and Inoue *et al.* [5], showed that most of *A. pleuropneumoniae* strains isolated before 1984 in Japan were antibiotic susceptible. However, this study also showed that strains isolated recently, in 1985 and 1986, were frequently resistant to antibiotics, such as aminoglycosides, TC, TS and CBD. Yamamoto (Faculty of Medicine, University of Tokyo, Japan) *et al.* found that majority of serotype 5 strains and all of the untypable strains of *A. pleuropneumoniae*, which were isolated during 1984–1987 from porcine pneumonic lung tissue samples, were resistant to oxytetracycline (OTC) (Personal communication). Also in our study considerable number of serotype 5 strains isolated recently showed MIC value of 25 µg/ml to OTC (data not shown). These results suggest that antibiotic resistant strains of *A. pleuropneumoniae* are increasing gradually in recent Japanese field.

In contrast to our present study, Yamamoto *et al.* showed that most of *A. pleuropneumoniae* strains of serotype 2 isolated recently in Japan were antibiotic susceptible (Personal communication). At present, explanations for the isolation-frequency difference of antibiotic resistant serotype 2 strains between the results of Yamamoto *et al.* and of ours are speculative. One possibility is the different origins of the isolates: *i. e.* they isolated *A. pleuropneumoniae* strains from lung tissues of the diseased swine, whereas we isolated them from the nasal cavities of healthy swine. An alternative explanation might be the difference of geographical areas of farms, from which *A. pleuropneumoniae* strains were isolated.

*A. pleuropneumoniae* strains isolated outside of Japan are frequently resistant to sulfasoxazole, spectinomycin, SM, TC, and ABPC [2, 4, 9]. We found in this study that the strains isolated in Japan were frequently resistant to SM, KM, LCM, TC, TS, and CBD. These evidences demonstrate that strains resistant to the antibiotics as above had spread in worldwide. Accordingly, careful selection of the drugs is required for the effective chemotherapy of the disease caused by *A. pleuropneumoniae*.

Hirsh *et al.* isolated two species of non-conjugative drug resistance plasmid from *A. pleuropneumoniae* strains [3]. One is 3.6 megadaltons (Mdal) in size and mediates ABPC and sulfonamides (SA) resistance, and the other 2.3 Mdal and mediates SM and SA resistance. Huether *et al.* analyzed plasmid profiles of drug resistant *A. pleuropneumoniae* strains [4]. Plasmids varying the sizes from 1.7 to 7.0 Mdal were detected among them. They could not correlate plasmids to antibiotic resistance, because of the high incidence of resistance to some antibiotics and because of the large number of different plasmid sizes. *A. pleuropneumoniae* strains used in this study showed a typical bimodal distribution of MIC values of KM, SM, TC, or TS. The results suggest that some of the strains carry antibiotic resistance plasmids. Curing and reintroduction of the plasmids of antibiotic resistant *A. pleuropneumoniae* strains are now in progress in our laboratory in order to identify the antibiotic resistance plasmids.

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## 要 約

1974年～1986年にかけてブタの鼻腔から分離された *Actinobacillus* (*Haemophilus*) *pleuropneumoniae* の抗生物質感受性：川原一芳・浅野昌彦<sup>1)</sup>・中井豊次<sup>2)</sup>・久米勝巳<sup>2)</sup>・檀原宏文（北里研究所，<sup>1)</sup>北里大学薬学部微生物，<sup>2)</sup>北里研究所附属家畜衛生研究所）——1974年～1986年にかけて我が国11県に所在する計35の農場において、ブタの鼻腔から *A. pleuropneumoniae* の分離を行い、得られた計129株の、10種類の抗生物質に対する感受性を調べた。1983年以前に分離された計28株の2型菌はすべて、用いた抗生物質に感受性であった。これに対して、1985年～1986年に分離された計79株の2型菌は、その過半数がアミノグリコシド系抗生物質、テトラサイクリン、タイロシン、カルバドックスに耐性であり、そのうちの多くが多剤耐性を示した。計22株の5型菌は1985年以降に分離され、その殆どが感受性であった。2型菌のアミノグリコシド系抗生物質、テトラサイクリンおよびタイロシンに対する最小発育阻止濃度の分布は2峰性を示した。以上の結果は、最近になって抗生物質に耐性の *A. pleuropneumoniae* 菌株が我が国で増加していることを示すものである。