Drug Susceptibility of *Haemophilus pleuropneumoniae* Strains Isolated from Pigs

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(Received 5 September 1983/Accepted 19 December 1983)

Haemophilus pleuropneumoniae strains isolated from pigs during the period from 1974 to 1982 were serotyped and examined for their susceptibility to 21 antimicrobial drugs. Of 109 field isolates in Japan and surrounding countries (Japan; 105, Korea; 2, Taiwan; 2), 105 strains were of serovar 2, two were of serovar 3, and the remaining two were of serovar 5. The great majority of the field isolates, 107 strains, were highly susceptible to penicillins, chloramphenicol, tetracyclines, rifampicin, trimethoprim and furazolidon. These strains were also susceptible to cephalexin and colistin. However, susceptibilities to aminoglycosides, macrolides, sulfamonomethoxine and nalidixic acid were not so high. The remaining two strains were found to be drug-resistant; strain Hpl was resistant to penicillin, tetracycline, streptomycin and sulfonamide, and strain 966 was resistant to streptomycin and sulfonamide. The former was identified as serovar 3 and the latter was serovar 5. All the 105 strains of serovar 2 were drug-sensitive, and no increase in the resistance of this organism had been observed during the period from 1974 to 1982.—Key words: Antibigram, *Haemophilus pleuropneumoniae*, Pig.

Jpn. J. Vet. Sci. 46(2): 175–180, 1984

Haemophilus pleuropneumoniae is the causative agent of porcine pleuropneumonia which is recognized as a serious economic problem in pig producing industries in many countries [15, 16]. Also in Japan, outbreaks of this disease have been reported from early 1970's [2]. Until now, many efforts have been made to control this disease by vaccination [7, 11, 13, 14], and some vaccines were experimentally proved to be protective to the disease. However, the effective commercial vaccine has not yet been supplied sufficiently for the pig producing field.

Good effects of chemotherapy of this disease have been reported by some workers [3, 16] and chemotherapy is considered to be one of the useful methods to control the disease. The present investigation was performed to know the *in vitro* drug susceptibility of *H. pleuropneumoniae* strains isolated in Japan and surrounding countries during the period

from 1974 to 1982 by an agar dilution method.

MATERIALS AND METHODS

Strains of H. pleuropneumoniae: Five strains listed in Table 1 were used as reference strains for serovar identification. A total of 109 field isolates were identified according to the method of Biberstein *et al.* [1], serotyped by tube agglutination test with rabbit immune sera prepared against the reference strains [2], and subjected to the assay of drug susceptibility. Of them, 19 strains had been identified and serotyped for merely [2]. Shope 4074, S1536, S1421 and six Danish isolates (1123, 2374, 2610, 2934, 3187 and 5570), which were kindly supplied by Dr. R. Nielsen of State Veterinary Serum Laboratory, Copenhagen, Denmark, were used in the drug susceptibility test as control strains.

Media: Sensitivity Test Broth (Nissui, Tokyo, Japan) and Sensivity Test Agar (Nissui) supplemented with β -nicotinamide adenine dinucleotide (Sigma) at the final concentration of 25 μ g/ml were used as liquid and solid media, respectively.

Drugs and susceptibility test: Drugs used in this study are listed in Table 3. Drug susceptibility test was carried out by an agar dilution method standardized and revised by the Japan Society of Chemotherapy [12]. A broth culture of each strain incubated at 37°C for 18 hours was diluted 1,000-fold with buffered saline with gelatin [12] and spotted onto solid media containing serial two-fold dilutions of each drug by the multiplanter. The minimum inhibitory concentration (MIC) was determined after aerobic incubation at 37°C for 18 hours.

RESULTS

Results of serovar identification of 109

field isolates are listed with their origins in Table 2. Of them, 105 strains were identified as serovar 2, two were serovar 3, and the remaining two were serovar 5. Though strain Hpl of group A cross-reacted completely with the reference strain S1421 and was typed as serovar 3 initially [2], it lost the agglutinability to antiserum against strain S1421 during storage. Most strains isolated in Japan belonged to serovar 2.

MICs of antimicrobial drugs employed in

Table 1. Reference strains used for serotyping

Serotype	Strain	Source
1	Shope 4074 (ATCC 27088)	J. Nicolet ^{a)}
2	S1536 (ATCC 27089)	
3	S1421 (ATCC 27090)	
4	M62	K. Kume ^{b)}
5	K17	

a) Institute of Veterinary Bacteriology, University of Bern, Bern, Switzerland.

b) The Kitasato Institute, Tokyo, Japan.

Table 2. Serotypes of field isolates of *Haemophilus pleuropneumoniae*

Group	Prefecture or country	Year	No. of strains	Sero-type	Source or reference
A	Japan	1974	18	2	[2]
			1	3	
B	Taiwan	1974	2	5	C. Chan ^{a)}
C	Ibaraki	1976	2	2	Our isolates
D	Saitama	1976	1	2	Our isolate
E	Chiba	1977	18	2	Our isolates
F	Ibaraki	1977	1	2	Our isolate
G	Hokkaido	1977	1	2	S. Serikawa ^{b)}
H	Fukuoka	1978	24	2	Y. Morita ^{c)}
I	Gifu	1979	11	2	Our isolates
J	Tokyo	1979	1	3	Our isolate
K	Chiba	1981	1	2	Our isolate
L	Korea	1981	2	2	Our isolates
M	Miyagi	1982	2	2	A. Kifune ^{d)}
N	Iwate	1982	24	2	Our isolates
Total			109		

a) Animal Industry Research Institute, Taiwan Sugar Corporation, Chunan, Miaoli, The Republic of China.

b) Takikawa Animal Husbandary Experimental Station of Hokkaido, Takikawa, Hokkaido.

c) Fukuoka Meat Inspectors Station, Fukuoka, Fukuoka.

d) Sendai Livestock Hygiene Service Center, Sendai, Miyagi.

this study against *H. pleuropneumoniae* strains are shown in Table 3. The great majority of 118 strains tested, i.e. 116 strains including nine control strains, were susceptible to all of the 21 drugs though the degree of susceptibility varied. Among β -lactam antibiotics, penicillin G (PCG), ampicillin (APC), and mecillinam (MPC) were highly active (MIC: 0.02 to 0.39 $\mu\text{g/ml}$) whereas cephalixin (CEX) showed lower activity (MIC: 0.78 to 3.13 $\mu\text{g/ml}$). Potency of aminoglycosides, streptomycin (SM), kanamycin (KM), furadiomycin (FRM), gentamicin (GM), and apramycin (AP); and macrolides, erythromycin (EM) and tylosin (TS), showed relatively low activity (MIC: 0.39 to 50 $\mu\text{g/ml}$). Chloramphenicol (CP); tetracyclines, oxytetracycline (OTC) and chlortetracycline (CTC); and rifampicin (RFP) had also low

MICs (MIC: 0.02 to 0.78 $\mu\text{g/ml}$). Colistin (CL) was moderately active (MIC: 0.39 to 1.56 $\mu\text{g/ml}$) and tiamuline (TM) showed a wide range of MICs (0.78 to 25 $\mu\text{g/ml}$). Of four synthetic antimicrobial drugs, trimethoprim (TMP) and furazolidon (FZ) showed lower MICs (0.02 to 0.20 $\mu\text{g/ml}$) than sulfamonomethoxine (SMM) and nalidixic acid (NA) (0.78 to 12.5 $\mu\text{g/ml}$).

Of 109 field isolates, two strains were resistant to two or four drugs. Table 4 shows the resistance levels of these isolates to seven drugs. Strain Hpl was resistant to PCG, APC, SM, OTC, CTC and SMM. As described above, this strain had been isolated in 1974 from a porcine lung lesion (group A in table 2) and identified as serovar 3, but lost its agglutinability to antiserum against strain S1421, the reference strain of serovar 3. The

Table 3. Susceptibility of 118 strains of *Haemophilus pleuropneumoniae* to various antimicrobial drugs

Drug tested ^{a)}	Minimum inhibitory concentration (MIC; $\mu\text{g/ml}$)													
	0.02	0.05	0.10	0.20	0.39	0.78	1.56	3.13	6.25	12.5	25	50	100	>100
PCG		1 ^{b)}	16	99	1					1				
APC	2	18	88	9					1					
MPC	6	111			1									
CEX						10	107	1						
SM									3	12	99	2		2
KM								5	7	104	2			
FRM						2	2	4	97	13				
GM					1	1	5	111						
AP									3	101	13	1		
CP				15	100	3								
OTC			24	86	7			1						
CTC			22	95				1						
EM								30	85	3				
TS									3	47	66	2		
TM						2	2	15	55	39	5			
RFP	2	1	18	97										
CL					10	53	55							
TMP	2	9	99	8										
SMM						4	8	27	76	1				2
FZ		1	116	1										
NA						37	76	5						

a) PCG; penicillin G, APC; ampicillin, MPC; mecillinam, CEX; cephalixin, SM; streptomycin, KM; kanamycin, FRM; furadiomycin, GM; gentamicin, AP; apramycin, CP; chloramphenicol, OTC; oxytetracycline, CTC; chlortetracycline, EM; erythromycin, TS; tylosin, TM; tiamuline, RFP; rifampicin, CL; colistin, TMP; trimethoprim, SMM; sulfamonomethoxine, FZ; furazolidon, NA; nalidixic acid.

b) Number of strains showing the respective MIC.

Table 4. Resistance level of 2 resistant strains of *Haemophilus pleuropneumoniae*

Drug tested ^{a)}	Minimum inhibitory concentration (MIC; $\mu\text{g/ml}$)				
	Reference strain			Resistant strain	
	Shope 4074 (ATCC 27088)	S1536 (ATCC 27089)	S1421 (ATCC 27090)	Hpl	966
PCG	0.2	0.2	0.2	12.5	0.1
APC	0.1	0.1	0.1	6.25	0.1
MPC	0.1	0.05	0.05	0.39	0.05
SM	25	25	25	>100	>100
OTC	0.2	0.2	0.39	3.13	0.2
CTC	0.2	0.2	0.2	3.13	0.2
SMM	6.25	3.13	1.56	>3,200	>3,200

a) Abbreviations are listed in Table 3.

other resistant strain, 966, which was isolated in Taiwan in 1974 (group B) and identified as serovar 5, was resistant to two drugs, SM and SMM. The remaining 107 strains including all the 105 strains of serovar 2 were susceptible to any drugs employed in this study. There was no tendency of increase in the resistance level against antimicrobial drugs throughout the period.

DISCUSSION

The *in vitro* susceptibility of *Haemophilus pleuropneumoniae* to 17 antibiotics and 4 synthetic drugs were determined by the agar dilution method standardized and modified by the Japan Society of Chemotherapy [12]. The use of a small inoculum size (10^5 colony forming units/ml) enabled the stable MIC determination of SMM. Most of the strains used in the present study were highly susceptible to PCG, APC, MPC, CP, OTC, CTC, RFP, TMP and FZ, and were moderately susceptible to CEX, CL and NA. On the other hand, these strains were relatively insensitive to aminoglycosides, macrolides, TM and SMM. Similar results were obtained by Nicolet and Schifferli [10]. The *in vitro* high susceptibility of this species to PCG and CP corresponds with the therapeutic efficacy of these drugs [16]. Thus the antibiogram of *H. pleuropneumoniae* was different from that of *Enterobacteriaceae* and similar to that of

other *Haemophilus* species (*H. influenzae* [9] and *H. somnus* [19]) in respect of high susceptibility to PCG and relatively low susceptibility to SM and other aminoglycoside antibiotics.

In the present investigations, only two strains of 109 field isolates were drug-resistant. Strain Hpl was resistant to penicillin (PCG and APC), SM, tetracycline (OTC and CTC), and SMM, and strain 966 was resistant to SM and SMM. It is noteworthy that these resistant strains belonged to serovar 3 or 5, which is the exceptional serovar in Japan. None of 105 isolates of serovar 2 showed any resistance. Nicolet and Schifferli [10] also reported that 98 strains belonging to serovar 2 were not resistant to any 12 drugs tested. On the other hand, Libal and Gates [6] found many strains resistant to several drugs among field isolates in U.S.A. by a disk diffusion technique. Though serotyping was not carried out by the authors, Schultz and Ross [17] reported the predominant serovar of *H. pleuropneumoniae* isolated in Iowa and surrounding states was serovar 5 and that they could not find the organisms of serovar 2. In these situations, it may be necessary to make clear the serovar distribution in drug resistant organisms of *H. pleuropneumoniae*.

Because of the widespread use of antibiotics as growth promoting feed additives as well as therapeutics for domestic animals,

many bacterial species isolated from livestock were recognized to show the antimicrobial resistance, and great majority of which were mediated by R plasmids. Among bacteria of porcine origins, extensive prevalence of R plasmids have been demonstrated in *Escherichia coli* [5, 8] and *Bordetella bronchiseptica* [18, 20] isolated in Japan. It is a curious phenomenon that no increase in the incidence of the drug-resistance occurred in *H. pleuropneumoniae* during the period from 1974 to 1982 under the same antibiotic pressure as in *E. coli* and *B. bronchiseptica*. We should continue the surveillance of the emergence of drug-resistance of *H. pleuropneumoniae* and that mediated by conjugative R plasmids.

Hirsh *et al.* [4] detected two strains of *H. pleuropneumoniae* which beared non-conjugative plasmids coding antimicrobial resistance. Genetic and biochemical studies on two drug-resistant strains, Hpl and 966, are now in progress and will be described elsewhere.

ACKNOWLEDGEMENT. We wish to express our appreciation to Mr. Katsuhiko Ono for his technical assistance. This work was supported in part by a grant from the Ministry of Education, Science, and Culture, Japan, No. 58560283.

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要 約

豚から分離された *Haemophilus pleuropneumoniae* の薬剤感受性について：井上 玲・山本孝史¹⁾・平野紀夫・村上敏明（岩手大学農学部家畜微生物学教室，¹⁾東京大学 医学部 動物実験施設）——1974～1982年に豚から分離された *Haemophilus pleuropneumoniae* 109株（日本；105株，韓国；2株，台湾；2株）の血清型別を行なうとともに，21種類の化学療法剤に対する感受性を寒天平板希釈法によって測定した。血清型別の結果，105株が2型，2株が3型，残りの2株が5型であった。このうち107株はペニシリン系薬剤（ペニシリンG，アンピシリンおよびメシリナム），クロラムフェニコール，テトラサイクリン系薬剤（オキシおよびクロルテトラサイクリン），リファムピシン，トリメトプリム，およびフラゾリドンに高度に感受性であり，セファレキシンとコリスチンにはそれに準ずる感受性を示した。一方，アミノ配糖体系薬剤（ストレプトマイシン，カナマイシン，フラジオマイシンおよびゲンタマイシン）とマクロライド系薬剤（エリスロマイシンとタイロシン），スルファモノメトキシ，およびナリジクス酸に対する感受性は低かった。残りの2株は薬剤耐性株であった。Hp 1株は1974年に日本で分離された3型の株で，ペニシリン系，テトラサイクリン系，ストレプトマイシン，およびサルファ剤に耐性，また966株は1974年に台湾で分離された5型の株で，ストレプトマイシン，およびサルファ剤に耐性であった。2型の株105株は全て感受性株であり，またこの期間中に *H. pleuropneumoniae* の各種薬剤に対する最小発育阻止濃度（MIC）の上昇は認められなかった。