BRIEF NOTE

**Antibiotic Susceptibility of *Haemophilus pleuropneumoniae*
and *Pasteurella multocida* Isolates from Swine**

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Recently outbreak of pneumonia caused by *Haemophilus* and *Pasteurella* infections in swine has been reported with increasing tendency in Japan [7, 16]. Kato and Morozumi [5] reported that 50 to 80% of serum specimens from swine showed specific agglutination with heat-treated cells of *H. pleuropneumoniae* or *H. parasuis* suggesting widely distributed *Haemophilus* infections in the country. Approximately 80% of swine tested revealed to have antibody against *H. pleuropneumoniae* in a recent survey by the cooperative research project of livestock hygiene service centers (Kito, S.: personal communication). Some data on antibiotic susceptibility of both species have been reported by Kato [6], Oda [9], and Suzuki [15] and incidence of multiple drug-resistant strains of *P. multocida* [3] and some R plasmids in *P. multocida* [1, 4, 13] and *H. influenzae* [2] have been reported. We have surveyed the recent epidemiological situation in regard to the drug susceptibility of bacteria from livestock. This paper describes the in vitro antibiotic susceptibility of *H. pleuropneumoniae* and *P. multocida* strains isolated from swine in 1979. Of thirteen lung specimens, *H. pleuropneumoniae* and *P. multocida* strains were

isolated in three specimens (23.1%) and seven specimens (53.8%), respectively as shown in Table 1. Only one specimen contained both species. All isolates of *H. pleuropneumoniae* were catalase-negative as pointed out by Kato [8] although described as catalase-positive in Bergey's Manual [17]. Minimal inhibitory concentrations (MICs) of drugs were determined by the agar dilution method standardized by the Japan Society of Chemotherapy [10]. Gas Pak system (BBL) was used for anaerobic cultivation. A standard method for MIC tests for *Haemophilus* species has not been established in the country, and MIC values varied with employed media [11]. Reproducible MIC values were obtained by heart infusion agar (HIA, Difco) or Mueller-Hinton agar (Eiken) plates containing 10 µg of β-NAD (Nakarai Chemicals) per ml. As shown in Table 2, the most active drugs (MIC: 0.2 to 0.8 µg/ml) were CBD (carbadox, under anaerobic condition), TMP (trimethoprim), TRP (trioprim), APC (ampicillin), CER (cephaloridin), CP (chloramphenicol), OTC (oxytetracycline), CL (colistin), RFP (rifampicin), and FZ (frazolidon). The moderate active drugs (MIC: 1.6 to 6.3 µg/ml) were PCG (penicillin G), MCI-PC (cloxacillin),

Table 1. Isolation of bacteria from swine lung specimens

Bacteria	Isolation of bacteria in pig No.												
	1	2	3	4	5	6	7	8	9	10	11	12	13
<i>P. multocida</i>	+	+	—	—	—	—	+	—	+	—	+	+	+
<i>H. pleuropneumoniae</i>	+	—	—	+	—	+	—	—	—	—	—	—	—

Table 2. Antimicrobial susceptibility of 24 strains of *H. pleuropneumoniae*

Drug	Minimal inhibitory concentration ($\mu\text{g/ml}$)												
	<0.2	0.2	0.4	0.8	1.6	3.1	6.3	12.5	25	50	100	>100	
CBD ^{a)}				24 ^{b)}									
SDM											13	11	
TMP	24												
TRP	24												
PCG					2	22	2						
APC			20	4									
MCI-PC					21	3							
CER		24											
CP				24									
OTC				20	4								
DSM								21	3				
KM								24					
SPC									24				
CL		10	3	11									
BC												24	
EM						3	21						
KT									5	19			
SP									2	3	19		
NA					16	8							
RFP			24										
FZ			13	9	2								
PZ								24					

^{a)} CBD was assayed under anaerobic condition.^{b)} Number of strains.

EM (erythromycin), and NA (nalidixic acid). Most of strains had a low susceptibility to SDM (sulfadimethoxin) and BC (bacitracin) showing MIC values of more than 100 $\mu\text{g/ml}$. Table 3 shows drug susceptibility of *P. multocida* strains under aerobic and anaerobic condition. The most active drugs (MIC: 0.2 to 0.8 $\mu\text{g/ml}$) were CBD (under anaerobic condition), PCG, MCI-PC, CER, CP, and OTC. The moderate active drugs (MIC: 1.6 to 6.3 $\mu\text{g/ml}$) were TMP, GM (gentamicin), EM, NA, RFP, FZ, PZ (panazon) and CL. Under anaerobic condition,

CBD increased the activity whereas aminoglycoside antibiotics decreased the activity on bacteria. The other drugs showed similar activity both under aerobic and anaerobic condition. Any strains of both species examined did not show a typical bimodal distribution of MIC values. Both species had a low susceptibility to aminoglycoside antibiotics such as DSM (dihydrostreptomycin), KM (kanamycin), SPC (spectinomycin) and FM (fradiomycin) which seemed characteristic of these species when compared with *Escherichia coli* and other enteric

Table 3. Antimicrobial susceptibility of 45 strains of *P. multocida*

Drug	Assay ^{a)}		Minimal inhibitory concentration ($\mu\text{g/ml}$)										
	A	AN	0.2	0.4	0.8	1.6	3.1	6.3	12.5	25	50	100	>100
CBD	+						2 ^{b)}	13	23	1	6		
		+		34	11								
SDM	+										8	10	27
TMP	+				5	21	19						
		+			8	22	15						
BCZ	+									16	7	15	7
		+								15	28	2	
PCG	+			39	6								
MCI-PC	+				22	23							
APC	+			20	25								
		+			24	21							
CER	+			4	41								
		+		7	38								
CP	+		5	32	2	4	2						
		+	3	36	4	2							
OTC	+		3	41	1								
		+	8	37									
DSM	+							15	19	11			
		+								2	23	20	
KM	+							2	43				
		+							5	34	5		
FM	+								25	20			
		+							2	12	31		
SPC	+								29	15	1		
		+									38	7	
GM	+					30	3	12					
		+					1	2	41	1			
EM	+				3	13	27	2					
		+			6	7	24	8					
KT	+							6	12	8	19		
		+						5	5	21	14		
SP	+								13	7	10	13	2
TS	+						2	6	8	24	5		
LCM	+							2	37	6			
NA	+					2	5	38					
		+				3	7	35					
RFP	+				3	4	7	31					
FZ	+		3	2	2	8	7	21	2				
PZ	+				14	24	7						
CL	+			2	3	22	8	6	4				

^{a)} A: aerobic, AN: anaerobic.^{b)} Number of strains.

bacteria. On the contrary, it is notable that PCG was pretty active on *P. multocida* strains although less active on most of Gram-negative bacteria. BCZ (bicozamycin), LCM (lincomycin) and macrolide antibiotics

such as KT (kitasamycin), SP (spiramycin) and TS (tylosin) showed less activity on both species. It seems curious that both species as well as *Bordetella bronchiseptica* strains [12] retained in high susceptibility

to OTC which has been extensively used in swine husbandary or in veterinary medicine in the country. This may mean that drug selection pressure in bacteria is weak in respiratory tracts of swine. Our results were in good agreement with those by Kato [6], and by Suzuki [15] in *H. pleuropneumoniae*, and those by Stevens et al. [14] in *P. multocida*. However, Chang et al. [3] reported that 84.0% of porcine isolates and 79.1% of bovine isolates of *P. multocida* were resistant to at least one antibiotic and resistance patterns of 75 porcine isolates were SM (56.0%), SM.TC (10.6%), SM.PCG (8.0%) and SM.PCG.TC (9.3%). Our data were different from their results which showed a broad range of MICs of SM (0.4 to 100 µg/ml). Recently nonconjugative R plasmids from *P. multocida* [1, 13] and conjugative R plasmids from *P. multocida* [4] and *H. influenzae* [2] were reported. Therefore, resistance determinants to SDM or DSM in 11 strains of *H. pleuropneumoniae* and 27 strains of *P. multocida* was tried to transfer to the recipients *H. pleuropneumoniae* 7 Rif (a rifampicin-resistant mutant of clinical isolate), *P. multocida* 2 Nal (a nalidixic acid-resistant mutant of clinical isolate), and *E. coli* ML1410 (nal, met) by incubating mating-mixtures of donor and recipient culture overnight at 30°C and selecting for transconjugants on agar plates containing RFP (50 µg/ml) or NA (50 µg/ml) and SDM (50 µg/ml) or DSM (12.5 µg/ml). None of strains tested were shown to transfer their resistance determinants. We most recently isolated some strains of *H. pleuropneumoniae* which showed high MIC values of more than 100 µg of SM per ml, and isolation of nonconjugative R plasmid is now under study. As to drug resistance levels in *P. multocida*, Silver et al. [13] described that TC resistance level of a plasmid from *P. multocida*

was expressed lower (MIC: 32 µg/ml) in *P. multocida* than in *E. coli* C600 (MIC: 256 µg/ml), whereas SM resistance level of a plasmid was expressed higher (MIC: 256 µg/ml) in *P. multocida* than in *E. coli* C600 (MIC: 64 µg/ml). Thus host specificity for expression of drug resistance levels by R plasmids will be an interesting problem in drug susceptibility tests.

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References

- [1] Berman, S. M., and Hirsh, D. C. (1978). *Antimicrob. Agents Chemother.* **14**, 347-352.
- [2] Brayan, L. E. (1978). *Antimicrob. Agents Chemother.* **14**, 154-156.
- [3] Chang, W. H., and Carter, G. R. (1976). *J. Am. Vet. Med. Assoc.* **169**, 710-712.
- [4] Curtis, P. E., and Ollerhead, G. E. (1979). *Vet. Rec.* **104**, 327.
- [5] Kato, K., and Morozumi, T. (1975). *In Proc. 80th Meet. Jpn. Soc. Vet. Sci.* 15 (in Japanese).
- [6] Kato, K. (1981). *In Proc. 7th Symp., 1980. Report of the Japanese Society on Drug Resistance in Bacteria from Animals*, No. 2, 1-4 (in Japanese).
- [7] Kato, K. (1979). *J. Jpn. Vet. Med. Assoc.* **32**, 63-68 (in Japanese).
- [8] Kato, K. (1978). *In Science of Swine*, Kumagai, T., Namioka, S., Niwa, T., and Sasahara, J., editors, Kindai Shuppan, Tokyo, 447-457 (in Japanese).
- [9] Oda, S. (1981). *In Proc. 7th Symp., 1980. Report of the Japanese Society on Drug Resistance in Bacteria from Animals*, No. 2, 5-11 (in Japanese).
- [10] Okoshi, M. (1974). *Chemotherapy* **22**, 1126-1129 (in Japanese).
- [11] Saruwatari, K., Mochida, S., Iori, F., Hayashi, A., Nasu, M., Shigeno, Y., Horiuchi, N., Nakatomi, M., Saito, A., and Hara, K. (1978). *Chemotherapy* **28**, 105 (in Japanese).
- [12] Shimizu, M., Kuninori, K., Inoue, M., and Mitsuhashi, S. (1981). *Microbiol. Immunol.* **25**, 773-786.
- [13] Silver, R. P., Leming, B., Garon, C. F., and Hjerpe, C. A. (1979). *Plasmid* **2**, 493-497.
- [14] Stevens, D. L., Higbee, J. W., Oberhofer, T.

- R., and Everette, E. D. (1979). *Antimicrob. Agents Chemother.* **16**, 322-324.
- [15] Suzuki, T. (1981). In Proc. 7th Symp., 1980. Report of the Japanese Society on Drug Resistance in Bacteria from Animals, No. 2, 12-19 (in Japanese).
- [16] Suzuki, T. (1975). *Chikusan no Kenkyu*, **29**, 47-52 (in Japanese).
- [17] Zinneman, K., and Biberstein, E. L. (1974). In Bergey's Manual of Determinative Bacteriology, 8th ed., Buchanan, R. E., and Gibbons, N. E., editors, The Williams and Wilkins Co., Baltimore, Md., 364-368.

要 約

豚由来 *Haemophilus pleuropneumoniae* および *Pasteurella multocida* の抗生物質感受性 (短報): 清水幹夫・国則健二・阪野哲也・寺島豊明 (全農飼料畜産中央研究所)——豚由来の *H. pleuropneumoniae* 24株および *P. multocida* 45株の薬剤感受性を調べた。最も高い活性を示した薬剤 (MIC: 0.2~0.8 $\mu\text{g/ml}$) は前者に対して CBD (嫌気条件), TMP, TRP, APC, CER, CP, OTC, CL, RFP および FZ であり, 後者に対しては CBD (嫌気条件), PCG, APC, MCI-PC, CER, CP および DTC であった。供試菌株のうち, 薬剤感受性分布に明確な 2 峰性を示すものは認められなかった。