Serotypes and Antimicrobial Susceptibility of *Pseudomonas aeruginosa* Strains Isolated from Diseased Dogs

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ABSTRACT. Strains of *Pseudomonas aeruginosa* isolated from diseased dogs in Tokyo area during 1983 through 1986 were serotyped and assayed for antimicrobial susceptibility to obtain an epizootiological aspect of canine *P. aeruginosa* infection. Major sources of specimens were ear and nasal discharges and urine. The results of O-antigen typing using a monoclonal antibody kit showed that the most predominant serotype was type M. Types G and B were also major serotypes. In a yearly distribution of serotypes, type I was almost limited in 1986, and was isolated mainly from surgical wounds, which showed an episode of nosocomial infection, whereas that of type M or B was dispersed during 4 years from 1983 to 1986. As a result of antimicrobial susceptibility test, most canine strains were considered to be susceptible to 4 drugs, which were commonly used in both human and veterinary clinics, in contrast to human isolates.—**KEY WORDS:** antimicrobial susceptibility, dog, *Pseudomonas aeruginosa*, serotype.

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Among Gram-negative glucose-non-fermenting bacteria (NFB) isolated from small animals, *Pseudomonas aeruginosa* is the organism isolated most frequently [15]. In human clinical bacteriology, *P. aeruginosa* is the most important opportunistic pathogen causing septicemia, pneumonia, and urinary tract infection, and so on [19]. In animals, *P. aeruginosa* has been assigned as the distinct causes of infections such as canine *otitis externa*, equine metritis, equine ulcerative keratoconjunctivitis, mink haemorrhagic pneumonia, and respiratory diseases of experimental animals [2, 4, 6]. *P. aeruginosa* infections in dogs and cats were generally recognized as opportunistic infections [15]. However, little is known about the epizootiology of *P. aeruginosa* infection in small animals.

P. aeruginosa can be readily isolated not only from environments such as soil and water [3], but also from the intestinal tract and mucous membrane as a member of the normal flora of animals in addition to

clinical specimens [4]. To understand the ecological diversity of opportunistic bacterial species in general, infrasubspecific ranking, i.e. typing, is necessary. Therefore, epizootiological study on the infection due to *P. aeruginosa* requires the typing of isolates based on various typing markers. In the studies of human *Pseudomonas* infection, serotyping of O-antigen, pyocine-typing, and phage-typing are used for such purposes [18]. Especially, serotyping is the most widely used and reliable method among them [8]. In this study, we carried out an epizootiological study of *P. aeruginosa* infection in dogs using an O-typing kit composed of monoclonal antibodies and examined the susceptibility of isolates against selected antibiotics. We also discussed their relation to diseases.

MATERIALS AND METHODS

Bacterial strains: In total, 91 isolates of *P. aeruginosa* were used in this study. All of

them were isolated from specimens collected from diseased dogs visiting The Veterinary Hospital, Faculty of Agriculture, The University of Tokyo during 1983 through 1986. Yearly distribution of isolates was expressed according to the fiscal year of Japan, which begins in April and ends in March. Identification of the bacteria was carried out according to the method of Gilardi [3].

The majority of isolates cultured on blood agar produced large, low convex colonies with an irregular surface and an edge that was translucent in comparison with pigmented center. A few isolates grew as smaller domed colonies. Most of the isolates showed a greenish blue pigment which diffused into the agar. All had a sweet odor similar to trimethylamine. The organisms were Gram-negative, motile, catalase and oxidase positive, did not grow at 4°C, but did grow in nutrient broth at 42°C. They utilized citrate, but did not split urea and were relatively inactive in carbohydrate media. Unless otherwise stated, the cultures were grown at 37°C. Clinical specimens from which *P. aeruginosa* strains were isolated were listed in Table 1 with the results

of serotyping.

For antibiotic susceptibility test, *Staphylococcus aureus* strain ATCC6538P and *Escherichia coli* strain NIHJ were used as reference strains.

Serotyping method: Strains of *P. aeruginosa* were typed with a set of monoclonal antibodies (MAbs) (MEI-ASSAY; Meiji Seika Kaisha Ltd., Tokyo, Japan) prepared against the serotype strains of the Homma scheme [9, 10]. This set is marketed as 17 vials each containing 2 ml of type-specific MAbs and three polyvalent reagents. For typing, strains were grown on a 5% horse blood agar at 37°C overnight, a portion of a colony was emulsified with a loop directly in a drop of polyvalent reagent on a microscope slide, and the slide was rotated manually for about 15 sec and examined for agglutination. Strains were then tested with the constituent antibodies of the pooled reagent, and the serotype was recorded.

Antibiotic susceptibility test: Minimal inhibitory concentration (MIC) was determined by an agar dilution method standardized and revised by Japan Society of Chemotherapy [5]. For broth and agar media, Sensitivity Test Broth Medium (Nis-

Table 1. Results of O-typing of *P. aeruginosa* isolates in correlation with isolation source

Isolation source	Serotypes												Total(%)
	M	I	G	B	E	A	D	F	C	Double ^{a)}	Non. ^{b)}	Spont. ^{c)}	
Ear	9 ^{d)}	1	3	6	2		2		1	1	1	2	28(30.8)
Urine	13	3	2	1	1	2							22(24.1)
External genitalia				1		1					1		3(3.3)
Uterus			1			1							2(2.2)
Nasal cavity	2	7	2	1	1			1		1	1		16(17.6)
Surgical wound	2	5			1						1		9(9.9)
Skin			2								1		3(3.3)
Others	2	2	2	1				1					8(8.8)
Total	28 (30.7)	18 (19.8)	12 (13.2)	10 (11.0)	5 (5.5)	4 (4.4)	2 (2.2)	2 (2.2)	1 (1.1)	2 (2.2)	5 (5.5)	2 (2.2)	91(100)

a) Double agglutinable isolate; b) Nonagglutinable isolate; c) Isolate agglutinating spontaneously; d) Number of isolates.

sui, Tokyo, Japan) and Sensitivity Test Agar Medium (Nissui), respectively, were used. Streptomycin (SM), gentamicin (GM), carbenicillin (CBPC), piperacillin (PIPC), and chloramphenicol (CP) were used for assays.

RESULTS

Distribution of P. aeruginosa in various specimens: The isolation frequency is shown in Table 1. Among the clinical specimens, isolates from ear discharge, urinary samples, and nasal discharge were predominant (30.8%, 24.1%, and 17.6%, respectively). In total, ca. 73% of strains were isolated from these specimens. Also, *P. aeruginosa* were isolated from surgical wound (9.9%), skin lesions (3.3%), external genitalia (3.3%), and so on.

Results of serotyping: Some 90% of isolates (82 strains) were typable into 9 serotypes by using the monoclonal serotyping kit. Slide agglutination reactions with MAbs were strong and rapid. Nine untypable strains included 2 strains showing spontaneous agglutination, 5 unagglutinable against any antibodies, and 2 agglutinable against two antibodies.

O-type distribution: Type M was the most predominant serotype (28 strains, 30.7%).

Type I and type G were also predominant (18 strains, 19.8%, and 12 strains, 13.2%, respectively). Strains belonging to types B (10 strains, 11.0%) and E (5 strains, 5.5%) were less frequently isolated. The correlation between serotypes and isolation sources is shown in Table 1 in detail. Although types M (9 strains) and B (6 strains) were predominant from the ear lesions, other serotypes were also isolated. On the other hand, from urinary specimens type M was most predominant (13 strains, 59.1%). Both from the nasal specimens and surgical wounds, type I was predominant.

Yearly distribution of serotypes is shown in Table 2. While strains of types M and G were relatively uniformly isolated, those of type I were isolated solely in the year of 1986 except one strain. The isolation frequency of type B showed the tendency of gradual increase.

Antimicrobial susceptibility of isolates: The distribution of MICs of drugs for *P. aeruginosa* isolates is shown in Table 3. Among the two aminoglycosides, MICs for streptomycin (SM) were widely distributed from 1.56 µg/ml to >200 µg/ml. However, a large number of strains showed MIC at >200 µg/ml, which indicated the ubiquitous prevalence of SM-resistant strains in *P. aeruginosa*. MIC₅₀ and MIC₉₀ for SM were

Table 2. Results of O-typing of canine isolates identified as *P. aeruginosa*

Fiscal year	Number of isolates	Serotypes						
		M	I	G	B	E	A	Others ^{a)}
1983/84	21 (100)	8 ^{b)} (38.1)		4 (19.0)	1 (4.8)	1 (4.8)	3 (14.3)	4 (19.0)
1985	31 (100)	13 (41.9)	1 (3.2)	5 (16.1)	2 (6.5)	3 (9.7)		7 (22.6)
1986	39 (100)	7 (17.9)	17 (43.6)	3 (7.7)	7 (17.9)	1 (2.6)	1 (2.6)	3 (7.7)

a) Serotypes other than I, M, G, A, E, and B.

b) Number of isolates showing agglutination with the relevant antibody. Percentage in parentheses.

Table 3. Antimicrobial susceptibility of *P. aeruginosa* isolates

Drug	Minimal inhibitory concentration (MIC, $\mu\text{g/ml}$)											
	<0.2	0.39	0.78	1.56	3.13	6.25	12.5	25	50	100	200	200<
SM				1	4	1	8	16	22	13	2	24
GM	5		2	8	21	31	19	5				
CBPC						5	14	58	13	1		
PIPC			7	14	56	8	5	1				
CP									7	75	9	

SM, streptomycin; GM, gentamicin; CBPC, carbenicillin; PIPC, piperacillin; CP, chloramphenicol.

Table 4. MIC distribution correlated with lesions

Drug	Lesion	MIC ($\mu\text{g/ml}$)											
		<0.2	0.39	0.78	1.56	3.13	6.25	12.5	25	50	100	200	200<
SM	Urinary tract					1		3	4	6		1	7
	Ear canal			1	1	1	3	8	3	11			
	Nasal cavity							1	1	7		1	6
GM	Urinary tract		1			2	5	6	7	1			
	Ear canal		2		1	3	6	7	5	4			
	Nasal cavity				1	1	5	6	3				
CBPC	Urinary tract							1	3	12	5	1	
	Ear canal						2	4	17	5			
	Nasal cavity								2	13	1		
PIPC	Urinary tract					5	12	3	2				
	Ear canal			3	2	21	1	1					
	Nasal cavity				1	2	12			1			
CP	Urinary tract											18	4
	Ear canal									2	25	1	
	Nasal cavity										2	13	1

SM, streptomycin; GM, gentamicin; CBPC, carbenicillin; PIPC, piperacillin; CP, chloramphenicol.

50 $\mu\text{g/ml}$ and >200 $\mu\text{g/ml}$, respectively. Also for gentamicin (GM), MICs were widely distributed from <0.2 $\mu\text{g/ml}$ to 25 $\mu\text{g/ml}$. MIC₅₀ and MIC₉₀ for GM were 6.25 $\mu\text{g/ml}$ and 12.5 $\mu\text{g/ml}$, respectively, indicating the absence of GM-resistant strains.

Among beta-lactams, for carbenicillin (CBPC) MICs against some 60% of strains were 25 $\mu\text{g/ml}$. MIC₅₀ and MIC₉₀ for CBPC were 25 $\mu\text{g/ml}$ and 50 $\mu\text{g/ml}$, respectively. For piperacillin (PIPC), 61.5% of strains showed MIC at 3.13 $\mu\text{g/ml}$.

Chloramphenicol (CP) was a relatively

ineffective drug for *P. aeruginosa*. MICs for CP ranged from 50 to 200 $\mu\text{g/ml}$. Especially, 82.4% of strains were distributed at the MIC of 100 $\mu\text{g/ml}$. MIC₅₀ and MIC₉₀ for CP were 100 $\mu\text{g/ml}$ and 200 $\mu\text{g/ml}$, respectively.

Correlation between isolation sites and antimicrobial susceptibility was not so obvious (Table 4).

On the other hand, between serotypes and antimicrobial susceptibility apparent correlation was recognized in some cases (data not shown). Both the 2 strains belonging to type D showed high sensitivity to

aminoglycosides: SM and GM. Strains of type I showed resistance to SM at high proportion. However, no correlation was seen between serotypes and antimicrobial susceptibility in other specimens.

DISCUSSION

The frequency of *P. aeruginosa* among Gram-negative glucose-non-fermenting bacteria (NFB) isolated from diseased dogs and cats was reported to be over 70% [15]. In our study, the frequency was 49.1% among NFB in 1983–1985 (unpublished data). However, details of canine or feline *Pseudomonas* infection have been known insufficiently. In particular, there was no report on seroepidemiological study. To elucidate the epidemiology in human infections due to *P. aeruginosa*, several typing schemes are used, namely, O-type [18, 25], phage type [18], pyocine type [18], plasmid profiling [18, 25], phage typing and pyocine typing are unstable due to dissociation of strains [20]. The usefulness of plasmid profiling is limited due to the presence of plasmidless strains [18]. Furthermore, protein patterns were available only when soluble proteins were prepared [18], though the procedures are time-consuming and somewhat complicated. Therefore, we chose O-typing of *P. aeruginosa* strains isolated from dogs as the available method for typing the strains at the first step of epizootiological studies.

Using a monoclonal antibody kit, we could type about 90% of strains into 9 serotypes. The monoclonal antibody set was reported to be an excellent tool in its good correspondence with the International Antigenic Typing Scheme (IATS) [23]. However, 9 strains were untypable. Among them, two were agglutinable against two antibodies, indicating the possibility of the strain bearing both epitopes reacting with each antibody. However, the reason is not known at present. Other 5 untypable strains

failed to agglutinate to any antisera used, which may indicate the existence of strains bearing so far unknown O-antigen type. To elucidate such problems, further serological studies will be required.

The results of serotyping showed the predominance of O-types M, I, G, and B. This tendency is inconsistent with that observed in human clinical bacteriology. In isolates from human specimens, serotypes G, E, and L were predominant [13, 18]. Gross *et al.* reported a post-operative septicemia due to *P. aeruginosa* in experimental dogs [7]. Yearly distribution of serotypes showed that strains of type I were almost exclusively isolated in the year of 1986. Because large proportion of them (5 isolates) were isolated from surgical wounds, the emergence of nosocomial infection seemed to occur. However, detailed comparisons among isolates are necessary to conclude the occurrence of nosocomial infection. Further, it is unknown whether strains bearing O-antigen M, G or B have pathogenicity to dogs at present. So far, in animal pseudomoniasis, the relationships between the O-type and the disease were established in a few cases. In mink pneumonia, O-types I and C were predominant [14]. Although in equine genital infections type A is predominant, it is also predominant among the normal flora of genital tract [1].

P. aeruginosa is known to cause various infections of dogs, i.e. *otitis externa*, ocular infection, urinary tract infection, tracheobronchitis or pneumonia, and so on [2]. So far, there has been no report on the relationship between serotypes and isolation sites in canine *Pseudomonas* infection. The specimens used for isolation of *P. aeruginosa* in this study were ear discharge, urinary samples, and nasal discharge and others. The sites from which the organisms were mainly isolated were reported to be major lesions caused by *P. aeruginosa* in dogs [4]. However, no association of a certain seroty-

pe with a distinct disease of dog was observed as seen in mink haemorrhagic pneumonia caused by *P. aeruginosa* [14], indicating the lack of such a form of virulent infection in canine pseudomoniasis.

In this study, the antimicrobiogram of canine isolates of *P. aeruginosa* of Japan was shown. The drugs used in this study except chloramphenicol (CP) were essentially effective to *P. aeruginosa* [21, 24]. Among human clinical isolates, a number of strains were reported to show antimicrobial resistance at a high MIC level, especially streptomycin (SM), CP, kanamycin (KM), carbenicillin (CBPC), and so on [11, 17, 22]. Furthermore, a number of strains bearing multiple drug resistance due to R plasmids were detected [11]. However in the present study using canine isolates, only SM-resistant strains (25 strains) and a CBPC-resistant strain were found. No strains showed high resistance (MIC: $>200\mu\text{g/ml}$) to GM, PIPC, and CP or no multiple drug resistant strains were found. At present, such situation may be advantageous for chemotherapy in small animal clinics. The tendency of relatively infrequent occurrence of drug resistant isolates, especially to more newly developed drugs, may be the reflection of the difference in the usage of chemotherapeutic drugs between man and small animals. In addition, the difference in ecological state of *P. aeruginosa* between man and dog should be considered. So far, most of the surveillances of drug resistant strains of human origin were carried out in hospitals, where resistant strains were accumulated due to the heavy application of drugs for patients. On the other hand, the environment of house dogs are thought to be less contaminated with drug resistant strains, especially in urban area [16]. Perhaps, both of circumstances described above might cause the lower frequency of drug resistant strains in dogs than in man.

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REFERENCES

1. Atherton, J. G. and Pitt, T. L. 1982. Types of *Pseudomonas aeruginosa* isolated from horses. *Equine Vet. J.* 14: 329–332.
2. Bush, B. M. 1983. *Pseudomonas* infections in small animals. *Vet. Annu.* 23: 162–167.
3. Gilardi, G. L. 1985. *Pseudomonas*. pp. 350–372. In: Manual of Clinical Microbiology, 4th ed. (Lennete, E. H., Balows, A., Hausler, W. J., Jr., and Shadomy, H. J. eds.), American Society for Microbiology, Washington, D. C.
4. Gillespie, J. H. and Timoney, J. F. 1981. The genus *Pseudomonas*. pp. 51–60. In: Hagan and Brunner's Diseases of Domestic Animals, 7th. ed. Ithaca, Comstock Publishing Associates, Cornell Univ. Press.
5. Goto, S. 1981. The revised standard method for determining minimal inhibitory concentrations of antibiotics against bacteria. *Chemotherapy* (Tokyo) 29: 76–79 (in Japanese).
6. Grono, L. R. and Frost, A. J. 1969. Otitis externa in the dog—The microbiology of the normal and affected external ear canal. *Aust. Vet. J.* 45: 420–422.
7. Gross, M. R., Cooper, J. E., and Needham, J. R. 1975. Observation on a post-operative septicaemia in experimental dogs with a particular reference to *Pseudomonas aeruginosa*. *J. Comp. Pathol.* 85: 445–451.
8. Hayer-Harting, A. 1948. The serology of *Pseudomonas pyocyanea*. *J. Gen. Microbiol.* 2: 31–39.
9. Homma, J. Y. 1979. Proposal of an international standard for the intraspecific serologic classification of *Pseudomonas aeruginosa*. *Jpn. J. Exp. Med.* 49: 89–94.
10. Homma, J. Y. 1982. Designation of the thirteen O-group antigens of *Pseudomonas aeruginosa*; An amendment for the tentative proposal in 1976.
11. Iyobe, S. 1985. *Pseudomonas aeruginosa*. In: Yakuzai-taisei-kinni yoru kankyou-osen (Mitsuhashi, S., Goto, S., and Shimizu, K. eds.), Gakkai-shuppan-center, Tokyo.
12. Janda, J. M. and Bottone, E. J. 1981. *Pseudomonas aeruginosa* enzyme profiling: Predictor of potential invasiveness and use as epidemiological tool. *J. Clin. Microbiol.* 14: 55–60.
13. Legakis, N. J., Aliferopoulou, M., Papavassiliou, J., and Papapetropoulou, M. 1982. Serotypes of *Pseudomonas aeruginosa* in clinical specimens in

- relation to antibiotic susceptibility. *J. Clin. Microbiol.* 16: 458-463.
14. Long, G. G., Gallina, A. M., and Gorham, J. R. 1980. *Pseudomonas aeruginosa* of mink: Pathogenesis, vaccination, and serologic studies. *Am. J. Vet. Res.* 41: 1720-1725.
 15. Mathewson, J. J. and Simpson, R. B. 1982. Glucose-non-fermenting Gram-negative bacilli associated with clinical veterinary specimens. *J. Clin. Microbiol.* 15: 1016-1018.
 16. Monaghan, C., Tierney, U., and Colleran, E. 1981. Antibiotic resistance and R-factors in the fecal coliform flora of urban and rural dogs. *Antimicrob. Agents Chemother.* 19: 266-270.
 17. Pederson, M., Marso, E., and Pickett, M. J. 1970. Non-fermentive bacilli associated with man: III. Pathogenicity and antibiotic susceptibility. *Am. J. Clin. Pathol.* 54: 178-192.
 18. Pitt, T. L. 1988. Epidemiological typing of *Pseudomonas aeruginosa*. *Eur. J. Clin. Microbiol.* 7: 238-247.
 19. Sabbath, L. D., ed. 1980. *Pseudomonas aeruginosa*: The Organism, Diseases It Causes, and Their Treatment. Hans Huber, Bern.
 20. Shinoyama, H. and Homma, J. Y. 1968. Dissociation in *Pseudomonas aeruginosa*. *Jpn. J. Exp. Med.* 38: 81-94.
 21. Smith, C. B., Wilfert, J. N., Dans, P. E., Kurrus, T. A., and Finland, M. 1970. *In vitro* activity of carbenicillin and results of treatment of infections due to *Pseudomonas* with carbenicillin singly and in combination with gentamicin. *J. Infect. Dis.* 124: Suppl. 14-25.
 22. Snelling, C. F. T., Ronald, A. R., Cates, C. Y., and Forsythe, W. C. 1971. Resistance of Gram-negative bacilli to gentamicin. *J. Infect. Dis.* 124: Suppl. 264-270.
 23. Strickland, M. A., Gaston, M. A., and Pitt, T. L. 1988. Comparison of polyclonal rabbit antisera with monoclonal antibodies for serological typing of *Pseudomonas aeruginosa*. *J. Clin. Microbiol.* 26: 768-769.
 24. Turck, M., Silverblatt, F., Clark, H., and Holmes, K. 1970. The role of carbenicillin in treatment of infections of the urinary tract. *J. Infect. Dis.* 122: Suppl. 29-33.
 25. Walia, S., Madhavan, T., Williamson, T., Kaiser, A., and Tewari, R. 1988. Protein patterns, serotyping and plasmid DNA profiles in the epidemiologic fingerprinting of *Pseudomonas aeruginosa*. *Eur. J. Clin. Microbiol.* 7: 248-255.

要 約

イヌ由来 *Pseudomonas aeruginosa* の血清型と抗生物質感受性：尾崎圭介・井上 玲¹⁾・跡部ヒサエ・高橋英司・小西信一郎（東京大学農学部獣医微生物学教室，¹⁾相模中央化学研究所）——東京地域で飼育されている犬の病例より1983年から1986年にかけて分離された *Pseudomonas aeruginosa* の血清型別と抗生物質感受性試験を行った。これら菌株の主要な分離部位は，耳疾患，尿路，および鼻汁であった。モノクローナル抗体を用いたO抗原型別の結果，M型が最も高頻度であった。GおよびB型も頻度の高い血清型であった。年次別分離頻度をみると，I型は1例を除いて1986年のみに分離されていた。さらにI型は主に手術創より分離されていたことから，この型による院内感染が強く疑われた。抗生物質感受性試験の結果，ヒトおよび獣医臨床で常用されている4薬剤には概して感受性であり，ヒト臨床における耐性菌の蔓延とは異なる傾向が明らかとなった。