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Short Communication

Antimicrobial Resistance of *Pseudomonas aeruginosa* Isolated from Dogs and Cats in Primary Veterinary Hospitals in Japan

Shoichiro Yukawa[1]*, Yuzo Tsuyuki[2], Tomomi Sato[3], Akira Fukuda[3], Masaru Usui[3], and Yutaka Tamura[3]

[1]*Department of Comparative Animal Science, College of Life Science, Kurashiki University of Science and The Arts, Okayama 712-8505;*
[2]*Sanritsu Zelkova Veterinary Laboratory, Kanagawa 213-0032; and*
[3]*Laboratory of Food Microbiology and Food Safety, School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido 069-8501, Japan*

SUMMARY: We collected 200 *Pseudomonas aeruginosa* isolates from dogs and cats in primary veterinary hospitals in Japan to investigate their antimicrobial resistance. Resistance rates against ciprofloxacin, cefotaxime, gentamicin, amikacin, and fosfomycin were 9%, 12.5%, 4.5%, 2.5%, and 35.5%, respectively. One strain displayed resistance (0.5%) to ceftazidime. We did not detect any imipenem-resistant or multidrug-resistant *P. aeruginosa* strains as defined by the Japanese Ministry of Health, Labour, and Welfare Law Concerning the Prevention of Infections and Medical Care for Patients with Infections. In addition, we did not find any *P. aeruginosa* isolates that produced metallo-β-lactamase, the aminoglycoside 6′-N-acetyltransferase AAC(6′)-Iae, or the aminoglycoside acetyltransferase AAC(6′)-Ib.

Pseudomonas aeruginosa is a gram-negative, non-glucose-fermenting, aerobic bacterium. It is an opportunistic pathogen frequently involved in canine otitis, pyoderma, and urinary tract infections (1,2). The bacterium can horizontally acquire resistance by incorporating mobile genetic elements such as integrons (3,4). Thus, susceptibility testing should be a crucial step in the selection of appropriate antimicrobial therapy for both human and veterinary use. Multidrug-resistant *P. aeruginosa* (MDRP) that produces the aminoglycoside 6′-N-acetyltransferase AAC(6′)-Iae or the aminoglycoside acetyltransferase AAC(6′)-Ib is widespread in Japan (5). However, at present, there are scant epidemiological data on the antimicrobial resistance profiles of MDRP of canine and feline origin in veterinary hospitals in Japan (6). Furthermore, there are no epidemiological data on such profiles in only primary veterinary hospitals in Japan. The objectives of this study were 2-fold: i) to determine the prevalence and antimicrobial resistance profiles of *P. aeruginosa* isolates in samples from infected dogs and cats in primary veterinary hospitals in Japan, and ii) to assess their production of metallo-β-lactamase (MBL), AAC(6′)-Iae, and AAC(6′)-Ib.

We investigated 200 *P. aeruginosa* isolates from dogs ($n = 168$) and cats ($n = 32$) with bacterial infections between September 2014 and February 2015. Clinical specimens were sent to the Sanritsu Zelkova Veterinary Laboratory by primary veterinary hospitals located in 21 prefectures of Japan (Table 1). There was no selection of isolates, and only a single isolate was extracted from each animal. Each animal was numbered so that its name was unknown. No information was available regarding the previous antimicrobial treatments of the animals. The specimens were isolated from various anatomical locations assessed as being sites of bacterial infection by the clinical veterinarians. These sites included the ear canal ($n = 78$), skin ($n = 56$), urine ($n = 26$), genitals ($n = 2$), respiratory organs ($n = 2$), nasal cavity ($n = 23$), oral cavity ($n = 5$), bile ($n = 2$), body fluids ($n = 2$), and eye ($n = 1$). All confirmed *P. aeruginosa* strains were stored at −80°C in 10% skim milk.

Antimicrobial susceptibility testing was performed using the agar dilution method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. CLSI resistance breakpoints (7) were used to determine the minimum inhibitory concentration (MIC) of ciprofloxacin (CIP; Sigma-Aldrich, St. Louis, MO, USA), cefotaxime (CTX; Sigma-Aldrich), gentamicin (GEN; Sigma-Aldrich), amikacin (AMK; Wako Pure Chemical Industries, Tokyo, Japan), fosfomycin (FOM; Wako), ceftazidime (CAZ; Sigma-Aldrich), and imipenem (IPM; Wako). All susceptibility testing was carried out using Mueller Hinton II agar (Becton, Dickinson and Company, Le Pont de Claix-Cedex, France). *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as quality control strains.

The MIC criteria for the first screening of MBL producers (8 μg/mL IPM or 16 μg/mL CAZ) were used for all isolates as reported previously (3). Simultaneously, we performed a sodium mercaptoacetic acid (SMA) inhibition test to detect MBL producers from all isolates using 2 commercially prepared Kirby-Bauer disks: 1 containing 30 mg CAZ and 1 containing 3 mg SMA (Eiken Chemical, Tokyo, Japan), as described by the manufacturer. This procedure was almost identical to that for the 2-mercaptopropionic acid inhibition test (8). We employed the definition of MDRP provided by the Law Concerning the Prevention of Infections and Med-

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*Corresponding author: Mailing address: Department of Comparative Animal Science, College of Life Science, Kurashiki University of Science and The Arts, 2640 Tsurajimacho Nishinoura Kurashiki, Okayama 712-8505, Japan. Tel: +81-86-440-1137, Fax: +81-86-440-1132, E-mail: yukawa@sci.kusa.ac.jp

Table 1. *P. aeruginosa* isolates used in this study classified by region of origin

Region	Hokkaido/ Tohoku	Kanto/ Koshinetsu	Tokai	Hokuriku/ Kinki	Chugoku/ Shikoku	Kyushu	Okinawa	Total
No. (%)	1 (0.5)	126 (63)	45 (22.5)	17 (8.5)	6 (3)	3 (1.5)	2 (1)	200 (100)

Table 2. Minimum inhibitory concentration (MIC) distribution and resistance rates among *P. aeruginosa* strains isolated from dogs and cats (n = 200)

			MIC (μg/mL)													No. resistant (%)
Antimicrobial	MIC_{50}	MIC_{90}	≤0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	>256	
Ceftazidime	2	4	1	1	2	30	91	58	13	4	1					1 (0.5)
Cefotaxime	16	64	2			1		5	27	102	39	18	2	5		25 (12.5)
Imipenem	1	1	1	2	19	165	7	4	1							0 (0)
Gentamicin	2	4	1		7	54	105	24	3	1	1		2	2		9 (4.5)
Amikacin	4	8	1		2	8	49	93	29	13	3	2				5 (2.5)
Ciprofloxacin	0.125	2	109	25	20	12	11	5	3	4	3	1		2		18 (9)
Fosfomycin	32	64					5	8	16	18	82	58	7	6		71 (35.5)

Vertical lines show the break point for each antimicrobial agent.

ical Care for Patients with Infections of the Japanese Ministry of Health, Labour, and Welfare; resistance to IPM was defined as MIC ≥ 16 μg/mL, to AMK as MIC ≥ 32 μg/mL, and to CIP as MIC ≥ 4 μg/mL (9). To detect AAC(6′)-Iae and AAC(6′)-Ib, all strains that showed resistance to aminoglycosides (GEN and AMK) were subjected to immunochromatographic assays (Mizuho Medy, Saga, Japan) (10,11).

Resistance to one or more of the tested antimicrobials was observed in 30 (41.1%) of the *P. aeruginosa* isolates. We did not detect any strains resistant to IPM or any MDRP strains. The MICs of all the tested antimicrobials were broadly distributed (Table 2), suggesting various degrees of susceptibility among the *P. aeruginosa* isolates examined. Overall, the rates of antibiotic resistance were low. Similar results were reported in a previous Japanese study (6) and confirmed in other countries (12,13).

Although 6 of the 200 *P. aeruginosa* isolates fulfilled the MIC criteria for MBL producers, each tested strain was negative for the SMA inhibition test. Of the 10 strains that showed resistance to aminoglycosides, none were positive for either AAC(6′)-Iae or AAC(6′)-Ib, as determined by the immunochromatographic assay. Unfortunately, the histories of previous antimicrobial treatments of the animals were not available in this study. The Ministry of Agriculture, Forestry, and Fisheries publishes the annual sales volume of veterinary antimicrobials for dogs and cats (14). However, in Japan, if veterinarians diagnose an animal disease that can be treated with a prescription medication used in humans, they are able to prescribe this medication to treat companion animals such as dogs and cats. Therefore, it is not possible to determine the exact quantity of antimicrobials consumed by dogs and cats in Japan without additional tracking mechanisms. An improved system is needed to clarify the consumption of antimicrobials by dogs and cats.

In contrast to the results of this study, MBL producers and MDRP strains have been found at considerably higher frequencies in previous studies of human isolates in Japan (3–5,9). Furthermore, IMP-45-producing *P. aeruginosa* of canine origin was isolated in China, suggestive of a human-to-dog transfer (15). As a result, when selecting antimicrobial drugs, veterinarians should consider the site-specific prevalence of antibiotic-resistant *P. aeruginosa* in companion animals. In addition, improved monitoring of antibiotic-resistant *P. aeruginosa* in companion animals is needed.

Conflict of interest None to declare.

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