Short Communication

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

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**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, ceftaxime, 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-acetyltranferase 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-acetyltranferase 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 (117), skin (56), urine (26), genitals (2), respiratory organs (2), nasal cavity (23), oral cavity (5), bile (2), body fluids (2), and eye (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), ceftaxime (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 Clai-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 mercaptoacetate 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-mercaptopyrimidinic acid inhibition test (8). We employed the definition of MDRP provided by the Law Concerning the Prevention of Infections and Med-
Table 1. *P. aeruginosa* isolates used in this study classified by region of origin

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

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

| Antimicrobial | MIC ≤ 0.125 | MIC ≥ 0.25 | MIC ≥ 0.5 | MIC ≥ 1 | MIC ≥ 2 | MIC ≥ 4 | MIC ≥ 8 | MIC ≥ 16 | MIC ≥ 32 | MIC ≥ 64 | MIC ≥ 128 | MIC ≥ 256 | MIC ≥ 512 | No. resistant (%) |
|---------------|-------------|------------|-----------|--------|--------|--------|--------|---------|---------|---------|---------|---------|---------|-----------|-------------------|
| Ceftazidime   | 2           | 4          | 1         | 1      | 2      | 30     | 91     | 58      | 13      | 4       | 1       | 1       | 1        | 0 (0)               |
| 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       | 0       | 0        | 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.

**Conflict of interest** None to declare.

**REFERENCES**


