MAJOR ARTICLE

# Clinical and Bacteriological Characteristics of IMP-Type Metallo-β-Lactamase–Producing *Pseudomonas aeruginosa*

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**IMP-type metallo-β-lactamase–producing bacteria have recently emerged worldwide. We conducted a case-control study in which 69 inpatients harboring $bla_{IMP}$-positive *Pseudomonas aeruginosa* and 247 control subjects with $bla_{IMP}$-negative pathogens were investigated. Prolonged hospitalization, antineoplastic chemotherapy, corticosteroid therapy ($P = .001$), and indwelling urinary catheters ($P = .04$) were risk factors for isolation of $bla_{IMP}$-positive pathogens. The predominant source was urine ($P = .001$). The duration of antibiotic treatment and the total dose (including of carbapenems) were significantly greater among case patients than among control subjects ($P < .01$). $bla_{IMP}$-positive *P. aeruginosa* isolates were more frequently resistant to multiple drugs ($P = .001$) and caused more infections ($P = .001$) than $bla_{IMP}$-negative pathogens. There were no significant differences in bacteriological outcome ($P = .94$); however, infection-related death was more frequent among case patients than among control subjects ($P = .023$). These results suggest that precautionary measures against the spread of $bla_{IMP}$-positive isolates are needed, because, for most of such pathogens, no antibiotic is potent enough to be used as a single agent in treatment of infection.**

*Pseudomonas aeruginosa* is a common nosocomial pathogen, particularly among immunocompromised patients. Carbapenems are the β-lactams that are most potent against *P. aeruginosa* [1, 2], as a result of their high affinity for penicillin-binding protein 2; their stability against most serine β-lactamases, including extended-spectrum β-lactamases; and their excellent permeability across bacterial outer membranes [3–5]. Recently, however, a novel metallo-β-lactamase, IMP-1, was identified in *P. aeruginosa* [6–9] and Enterobacteriaceae in Japan [8–12]. The $bla_{IMP}$ genes responsible for IMP-1 production are usually mediated by integrons carried by transferable large plasmids [11].

Metallo-β-lactamases such as IMP-1 belong in the molecular class B of Ambler [13] and in group 3 of the Bush-Jacoby-Medeiros functional classification [14]; they hydrolyze carbapenems, as well as a variety of penicillins and cephalosporins. The appearance of metallo-β-lactamase genes and their potential spread among bacterial pathogens is a matter of major concern with regard to the future of antimicrobial chemotherapy, because the metallo-β-lactamases are not inhibited by the currently approved β-lactamase inhibitors, such as clavulanic acid, sulbactam, and tazobactam [15]. IMP-1, which currently is the most predominantly identified carbapenemase in Japan, has been found in

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scattered hospitals in various regions of Japan, and it is estimated that ~1.3% of *P. aeruginosa* strains have already acquired the ability to produce IMP-1 [12].

However, this situation is currently changing. IMP-1 and its closely related enzymes have recently been identified in isolates from several European (the United Kingdom [16] and Italy [17, 18]) and Asian (Japan [5, 19], South Korea [20], Singapore [21], Taiwan [22, 23], and Hong Kong [24]) regions. It has been reported more recently that *P. aeruginosa* isolates from an outbreak in Canada produced a novel metallo-β-lactamase, IMP-7 [25]. Furthermore, a second family of acquired metallo-β-lactamases, the VIM types, has been detected in several countries (Italy [26], Greece [4], France [27], and Taiwan [22]). Importantly, *P. aeruginosa* and related pathogens producing IMP-1, IMP-7, VIM-1, and VIM-3 have been reported to be associated with clonal spread and hospital outbreaks [4, 7, 9, 22, 25]. Furthermore, there are no potent antibiotics for the treatment of infections caused by metallo-β-lactamase–producing *P. aeruginosa* isolates that exhibit resistance to aminoglycosides and fluoroquinolones.

We have reported elsewhere that gram-negative rods carrying $bla_{IMP}$ were found in 80 patients at Nagasaki University Hospital (Nagasaki, Japan) between 1991 and 1996 [9]. That study is, to our knowledge, the only report of surveillance of IMP-type metallo-β-lactamase–producing bacteria in a single hospital, and the number of patients harboring $bla_{IMP}$-positive *P. aeruginosa* that was detected was the largest thus reported worldwide. In our hospital, *P. aeruginosa* carrying $bla_{IMP}$ was frequently isolated from patients with cancer, from patients receiving antineoplastic chemotherapy, or from patients who had undergone surgery [9]; however, even nosocomial pathogens without specific resistance determinants commonly cause infections in these immunocompromised hosts, and this finding may not be due to particular characteristics of $bla_{IMP}$-positive bacterial strains.

To determine the risk factors involved in the isolation of *P. aeruginosa* carrying $bla_{IMP}$ and to clarify the clinical and bacteriological characteristics of such isolates, we conducted a case-control study in which 69 case patients and 247 control subjects were examined in whom $bla_{IMP}$-positive *P. aeruginosa* and $bla_{IMP}$-negative *P. aeruginosa,* respectively, were isolated.

## SUBJECTS, MATERIALS, AND METHODS

***Detection of* bla$_{IMP}$ *in clinical isolates of* P. aeruginosa.** The present study was conducted at Nagasaki University Hospital, an 829-bed hospital in Nagasaki, Japan. All clinical isolates obtained through culture since 1991 were saved in our laboratory until the beginning of this study, in 1996. All clinical isolates of *P. aeruginosa* obtained from January 1991 through December 2000 were examined for $bla_{IMP}$, which was detected retrospectively until 1996 and prospectively from that point onward. *P. aeruginosa* isolates that were highly resistant to ceftazidime, with a ceftazidime MIC of ⩾64 μg/mL, were screened for $bla_{IMP}$ by PCR, using methods reported previously [8, 9]. *P. aeruginosa* ATCC 27853 was used as a negative control for PCR and for quality control of the antimicrobial susceptibility test. It should be noted that the PCR-positive isolates could have some product variants that are very close to IMP-1, such as IMP-3 [19] and IMP-6 [5], because the differences between these β-lactamases are only 1 to a few amino acid substitutions. Total DNA was prepared from $bla_{IMP}$-positive *P. aeruginosa* isolates obtained between 1997 and 2000 and digested with the restriction enzyme *Spe*I for PFGE, as described elsewhere [9]. Isolates obtained between 1991 and 1996 were genotyped in another study, and the results have been reported elsewhere [9]. In patients in whose samples multiple $bla_{IMP}$-positive isolates were found, the first isolate was examined for antimicrobial susceptibility and included in the analysis; isolates from such patients were confirmed to be the same clone by PFGE in all cases. These patients were counted once in the case-control study.

***Study subjects.*** During the study period, $bla_{IMP}$-positive *P. aeruginosa* was isolated from 70 patients. Data from 1 of the patients were insufficient for inclusion in the analysis. The remaining 69 patients were enrolled in the present study as case patients. Because $bla_{IMP}$-positive *P. aeruginosa* was isolated from inpatients only, control subjects were also selected, at random, from among inpatients; these subjects were patients from whose samples $bla_{IMP}$-negative *P. aeruginosa* was isolated during the study period. The number of control subjects recruited each year was matched to the case group identified in the same year, because the type of antibiotics used and the total amount of injectable antibiotics used each year in the hospital dynamically changed during the study period. A total of 247 control subjects were included in the present study. The ward distribution and the time of bacterial isolation varied among control subjects. To confirm the variability of strains in the control group, a proportion of isolates from these subjects was preliminarily confirmed by PFGE to be genetically different.

***Antibacterial susceptibility testing.*** MICs were determined by the microdilution method, using cation-adjusted Mueller-Hinton broth, according to the recommendations of the NCCLS [28]. MICs of potent antibiotics against *P. aeruginosa,* including 4 β-lactams (piperacillin, ceftazidime, aztreonam, and imipenem), an aminoglycoside (gentamicin), and a fluoroquinolone (ciprofloxacin), were determined. The prevalence of nonsusceptible isolates (those considered to be resistant or intermediate by NCCLS breakpoints [28]) among isolated $bla_{IMP}$-positive *P. aeruginosa* was compared with that of $bla_{IMP}$-negative *P. aeruginosa* for each antibiotic agent. The rel-

**Table 1. Numbers of *Pseudomonas aeruginosa* isolates and subjects in a study of IMP-type metallo-β-lactamase–producing bacteria at Nagasaki University Hospital, 1991–2000.**

| Year | Total no. of isolates | No. (%) of isolates highly resistant to ceftazidime[a] | No. of case patients | Percentage of all case patients enrolled[b] | No. of control subjects[c] |
|---|---|---|---|---|---|
| 1991 | 928 | 85 (9.2) | 9 | 12.9 | 32 |
| 1992 | 779 | 127 (16.3) | 18 | 25.7 | 63 |
| 1993 | 780 | 115 (14.7) | 6[d] | 8.6 | 21 |
| 1994 | 615 | 60 (9.8) | 10 | 14.3 | 35 |
| 1995 | 779 | 75 (9.6) | 3 | 4.3 | 11 |
| 1996 | 798 | 70 (8.8) | 8 | 11.4 | 28 |
| 1997 | 765 | 49 (6.4) | 5 | 7.1 | 18 |
| 1998 | 850 | 8 (0.9) | 2 | 2.9 | 7 |
| 1999 | 691 | 7 (1.0) | 1 | 1.4 | 4 |
| 2000 | 801 | 35 (4.4) | 8 | 11.4 | 28 |

[a] MIC ⩾64 μg/mL.
[b] The no. of case patients enrolled each year as a percentage of the total no. of case patients enrolled over the course of the study.
[c] The no. of control subjects recruited each year was matched to the case group.
[d] One patient was excluded from further analysis because of lack of sufficient data.

ative proportion of multiple drug–resistant (MDR) isolates was also determined for the 2 groups.

***Data collection.*** Detailed information on the patients and isolates was collected from clinical records and computer databases. This included age, sex, days of hospitalization until isolation of *P. aeruginosa,* source of isolate, presence of malignant diseases and related treatment factors, previous antibiotic use, systemic and local signs and symptoms of inflammation, the results of serial bacterial examination, and clinical outcomes. Patients were divided into 2 categories of clinical significance as described elsewhere [9]: (1) infected, if the patient showed both an acute systemic inflammatory response (e.g., fever and elevation of C-reactive protein levels) and local infectious signs or symptoms (e.g., purulent sputum and pyuria), and (2) colonized or "unknown," if the patient displayed no local infectious signs or symptoms, even if the patient had a systemic inflammatory response (e.g., a urinary isolate from a patient without pyuria), or if sufficient information was not available from the patient.

***Statistical analysis.*** Data are presented as means or as counts or proportions. All analyses were performed by SAS software version 6.12 (SAS Institute). The difference in the age of the 2 study populations was evaluated by *F* test. For continuous variables, we used the Mann-Whitney *U* test. A 2-tailed $\chi^2$ test was used to assess the statistical significance of the associations among variables, as well as to compare categorical variables between the 2 groups. ORs and 95% CIs were also calculated. $P < .05$ was considered to be statistically significant.

## RESULTS

***Identification of patients infected with* bla$_{IMP}$*-positive* P. aeruginosa.** Table 1 shows the number of *P. aeruginosa* isolates, of pathogens highly resistant to ceftazidime, of patients harboring $bla_{IMP}$-positive *P. aeruginosa,* and of control subjects in each year of the study. The highest numbers of isolates that were highly resistant to ceftazidime (127 [16.3%] of the 779 *P. aeruginosa* isolates in that year) and patients with $bla_{IMP}$-positive *P. aeruginosa* isolates (18 [25.7%] of 70 patients) were seen in 1992. There was no significant increase in the number of isolates highly resistant to ceftazidime or of patients with $bla_{IMP}$-positive *P. aeruginosa* isolates. There were no significant differences in the ward distribution among case patients who were enrolled in different years. During the study period, 9 episodes of clonal spread of $bla_{IMP}$-positive *P. aeruginosa* in different patients were confirmed (8 were in 2 patients, and 1 was in 3 patients) by PFGE [9]. Because 5 of these clonal strains showed the same antibiograms, duplicated data were excluded from the antimicrobial susceptibility analysis.

***Case-control study.*** Table 2 summarizes patient characteristics and key variables. The age distribution of the case patients was not significantly different from that of the control subjects ($P = .58$), and there was no difference in the average ages ($P = .21$). In addition, there was no sex-related difference between the 2 groups ($P = .96$). Approximately one-half of the case patients had malignant diseases (52.2%); however, this was the same among the control subjects (48.6%) ($P = .60$). In approximately one-third of both groups of patients, *P. aeru-*

**Table 2. Characteristics of subjects and key variables in a study of IMP-type metallo-β-lactamase–producing bacteria at Nagasaki University Hospital, 1991–2000.**

| Variable | Case patients | Control subjects | OR (95% CI) | *P* |
|---|---|---|---|---|
| No. of subjects | 69 | 247 | | |
| Age, mean years ± SD (range) | 53.0 ± 24.1 (0–87) | 57.3 ± 22.9 (0–91) | | NS |
| Male sex | 39 (56.5) | 157 (63.6) | | NS |
| Malignant disease | 36 (52.2) | 120 (48.6) | 1.15 (0.67–1.97) | NS |
| Surgery | 24 (34.8) | 82 (33.2) | 1.01 (0.57–1.78) | NS |
| Antineoplastic therapy | 14 (20.3) | 4 (1.6) | 15.46 (4.90–48.79) | .001 |
| Corticosteroid therapy | 12 (17.4) | 12 (4.9) | 4.12 (1.76–9.65) | .001 |
| Urinary catheter | 17 (24.6) | 35 (14.2) | 1.98 (1.03–3.80) | .04 |
| Intravenous hyperalimentation catheter | 16 (23.2) | 78 (31.6) | 0.65 (0.35–1.21) | NS |
| Interval between hospitalization and isolation of the pathogen, mean days ± SD (range) | 94.8 ± 122.4 (2–808) | 52.3 ± 115.4 (0–579) | | .001 |
| Source of isolates | | | | |
| Respiratory sample | 18 (26.1) | 153 (61.9) | 0.22 (0.12–0.39) | .001 |
| Urine sample | 27 (39.1) | 23 (9.3) | 6.26 (3.28–11.95) | .001 |
| Abscess, pus, or wound sample | 13 (18.8) | 44 (17.8) | 1.07 (0.54–2.13) | NS |
| Other | 11 (15.9) | 27 (10.9) | 1.54 (0.72–3.29) | NS |

**NOTE.** Data are no. (%) of subjects, unless otherwise indicated. NS, not significant.

*ginosa* was isolated after a surgical procedure ($P = .80$). Systemic antineoplastic chemotherapy ($P = .001$), corticosteroid therapy ($P = .001$), and indwelling urinary catheters ($P = .04$) were more often noted in the case patients than the control subjects, whereas there was no difference in the frequency of use of intravenous hyperalimentation catheters ($P = .18$). The 14 case patients who were receiving antineoplastic agents systemically were in 10 different wards. The interval between admission to the hospital and isolation of *P. aeruginosa* was significantly longer among the case patients than among control subjects ($P = .001$), although the ranges varied widely in both groups. The predominant sources of *P. aeruginosa* isolates were the urinary tract for case patients (39.1% among case patients vs. 9.3% among control subjects; $P = .001$) and the respiratory tract for control subjects (26.1% among case patients vs. 61.9% among control subjects; $P = .001$) (table 2).

There was no significant difference in the use of any class of antibiotics in the 2 groups. The proportion of patients who did not receive any antibiotics before the isolation of *P. aeruginosa* was higher in the case group than in the control group (21.7% vs. 12.6%; $P = .06$). However, the duration of antibiotic use and the total dose used to treat case patients were significantly greater than for control subjects for several classes of antibiotics, including carbapenems but not third-generation cephems (e.g., ceftazidime) (table 3). The duration of treatment regimens that included penicillins ($P = .001$), carbapenems ($P = .016$), or tetracyclines ($P = .011$) was significantly longer among case patients than among control subjects. Total doses of antibiotics among the case patients were also significantly greater that those among the control subjects for penicillins ($P = .001$), second-generation cephems (e.g., cefotiam; $P = .002$), carbapenems ($P = .006$), and tetracyclines ($P = .006$). In contrast, there were no differences between the 2 groups in duration of treatment and total dose for other classes of antibiotics, including first-generation cephems (e.g., cefazolin), aminoglycosides, fluoroquinolones, and third-generation cephems.

Although many of the *P. aeruginosa* isolates in both groups did not cause infection, $bla_{IMP}$-positive *P. aeruginosa* caused infection more frequently than did $bla_{IMP}$-negative *P. aeruginosa* (46.4% vs. 9.3%, respectively; OR, 8.42; 95% CI, 4.44–15.95; $P = .001$). Furthermore, the majority of infections occurred in the urinary tract (43.8% of case patients vs. 13.0% of control subjects; OR, 5.18; 95% CI, 1.27–21.03; $P = .02$), skin or soft tissue (21.9% vs. 17.4%; $P = .68$), and lower respiratory tract (pneumonia in 5 patients and bronchitis in 1 patient) among the case patients. In contrast, most infections in the control group occurred in the lower respiratory tract (18.8% of case patients vs. 52.2% of control subjects; OR, 0.21; 95% CI, 0.06–0.70; $P = .009$), including pneumonia in 3 patients.

Serial bacterial examination was not performed in 37 (53.6%) of 69 case patients and 140 (56.7%) of 247 control subjects. There were no significant differences in bacteriological outcome between the groups ($P = .94$). Of the 32 patients with $bla_{IMP}$-positive *P. aeruginosa* in whom follow-up bacterial examination was performed, the bacteria was found to have been eliminated in 14 patients (43.8%) after antibiotic treatment, after removal

**Table 3. Antibiotic therapy used before the isolation of *Pseudomonas aeruginosa* in a study of IMP-type metallo-β-lactamase–producing bacteria at Nagasaki University Hospital, 1991–2000.**

| Antibiotic group | Duration of use, mean days ± SD | | | Total dose, mean μg ± SD | | |
|---|---|---|---|---|---|---|
| | Case patients | Control subjects | *P* | Case patients | Control subjects | *P* |
| β-lactams | | | | | | |
| Penicillins | 15.1 ± 12.7 | 6.1 ± 5.9 | .001 | 51.1 ± 45.3 | 14.6 ± 22.3 | .001 |
| First-generation cephems (e.g., cefazolin) | 5.0 | 6.6 ± 4.2 | NS | 2.0 | 12.5 ± 8.5 | NS |
| Second-generation cephems (e.g., cefotiam) | 7.5 ± 5.9 | 5.9 ± 5.8 | .07 | 25.6 ± 26.9 | 12.1 ± 12.0 | .002 |
| Third-generation cephems (e.g., ceftazidime) | 9.9 ± 11.2 | 8.8 ± 6.6 | NS | 24.4 ± 38.5 | 18.4 ± 15.2 | NS |
| Monobactams | 5.5 ± 2.1 | 1.5 ± 0.7 | .12 | 13.0 ± 1.4 | 2.5 ± 2.1 | .12 |
| Carbapenems | 12.8 ± 5.9 | 8.9 ± 7.3 | .016 | 14.1 ± 7.9 | 9.3 ± 8.5 | .006 |
| Extended-spectrum β-lactams[a] | 17.2 ± 16.3 | 10.9 ± 11.4 | .001 | 34.3 ± 51.8 | 18.8 ± 20.8 | .011 |
| Any β-lactam | 18.2 ± 16.6 | 12.1 ± 12.2 | .003 | 44.5 ± 46.8 | 21.5 ± 25.8 | .001 |
| Tetracyclines | 12.1 ± 8.7 | 4.7 ± 8.9 | .011 | 2.5 ± 1.6 | 0.9 ± 0.7 | .006 |

**NOTE.** NS, not significant.

[a] Piperacillin, second- and third-generation cephems, and carbapenems.

of the urinary catheter without antibiotics, or without any treatment. In contrast, $bla_{IMP}$-positive *P. aeruginosa* was persistently isolated in 18 (56.3%) of 32 patients who received any antibiotics. The rate of persistent isolation, however, was not different for the 2 groups (56.3% of case patients vs. 59.4% of control subjects for whom serial bacterial examination was done; $P = .79$).

Of the 21 patients (30.4%) who died after isolation of $bla_{IMP}$-positive *P. aeruginosa,* infection was thought to have been the possible cause of death in 4 patients (5.8% of all case patients): pneumonia, in 1 patient with adult T cell leukemia who was receiving therapy with antineoplastic agents; clinical sepsis, in 1 patient with chronic renal failure; and terminal pneumonia, in 2 patients with esophageal cancer who died postoperatively while receiving antineoplastic chemotherapy. Of 63 (25.5%) of 247 control subjects who died after isolation of $bla_{IMP}$-negative *P. aeruginosa,* 3 were thought to have died of *P. aeruginosa* infections (1.2% of the all control subjects). All of these were terminally ill patients with pneumonia and malignant lymphoma, hepatic cancer, or gastric cancer. The incidence of death did not differ between the 2 groups ($P = .41$); however, infection-related death was more frequent among the case patients than the control subjects (5.8% vs. 1.2%, respectively; OR, 5.00; 95% CI, 1.09–22.9; $P = .02$).

***Antimicrobial susceptibilities and MDR isolates of* bla$_{IMP}$*-positive and -negative* P. aeruginosa.** Table 4 shows $MIC_{50}$ and $MIC_{90}$ values and the number and proportion of *P. aeruginosa* isolates resistant to each antibiotic used in the study, as well as the proportion of MDR isolates. $bla_{IMP}$-positive *P. aeruginosa* isolates were more likely to be resistant to any single antibiotic than were $bla_{IMP}$-negative isolates ($P = .001$). The proportion of resistance to 3 classes of antibiotics (β-lactams, gentamicin, and ciprofloxacin) was greater among $bla_{IMP}$-positive than -negative *P. aeruginosa* isolates ($P = .001$). Furthermore, isolates with resistance to 2 classes of antibiotics, such as β-lactams and gentamicin ($P = .001$) or β-lactams and ciprofloxacin ($P = .001$), were more frequently $bla_{IMP}$ positive than $bla_{IMP}$ negative.

## DISCUSSION

The present study examined what was the largest, to our knowledge, group of patients harboring $bla_{IMP}$-positive *P. aeruginosa* in the world. Our results indicate that malignant diseases and surgery per se were not risk factors for isolation of $bla_{IMP}$-positive *P. aeruginosa.* Instead, cancer chemotherapy was identified as a risk factor; 3 of 4 patients who died of infection due to $bla_{IMP}$-positive *P. aeruginosa* had terminal malignant diseases and were receiving chemotherapy. Systemic administration of corticosteroids and use of an indwelling urinary catheter were also found to be risk factors associated with isolation of the resistant pathogens. That long-term hospitalization was another risk factor may be explained by the fact that most patients with advanced malignancies received chemotherapy postoperatively, thus necessitating longer hospitalization. There were quite large differences between the case and control groups in the sources of isolates. In particular, percentages for urinary tract and respiratory tract isolates were strikingly different: $bla_{IMP}$-positive *P. aeruginosa* was mostly isolated from urine samples, which

**Table 4. Susceptibility of 64 *bla*$_{IMP}$-positive (*bla*$_{IMP}^{+}$) and 247 *bla*$_{IMP}$-negative (*bla*$_{IMP}^{-}$) *Pseudomonas aeruginosa* isolates to different antibiotics.**

| Antibiotic(s) | $MIC_{50}$, μg/mL | | $MIC_{90}$, μg/mL | | Nonsusceptible isolates, no. (%)[a] | | | |
|---|---|---|---|---|---|---|---|---|
| | *bla*$_{IMP}^{+}$ isolates | *bla*$_{IMP}^{-}$ isolates | *bla*$_{IMP}^{+}$ isolates | *bla*$_{IMP}^{-}$ isolates | *bla*$_{IMP}^{+}$ isolates | *bla*$_{IMP}^{-}$ isolates | OR (95% CI) | *P* |
| Piperacillin | ≥64 | 4 | ≥64 | ≥64 | 48 (75.0) | 34 (13.8) | 18.79 (9.6–36.79) | .001 |
| Ceftazidime | ≥64 | 4 | ≥64 | 16 | 64 (100) | 28 (11.3) | ND | .001 |
| Aztreonam | 16 | 8 | ≥64 | ≥64 | 35 (54.7) | 77 (31.2) | 2.66 (1.52–4.66) | .001 |
| Imipenem | 8 | 1 | ≥64 | 8 | 39 (60.9) | 43 (17.4) | 7.40 (4.06–13.48) | .001 |
| Gentamicin | ≥64 | 1 | ≥64 | 8 | 59 (92.2) | 24 (9.7) | 109.64 (40.12–299.63) | .001 |
| Ciprofloxacin | 16 | ≤0.5 | ≥64 | ≤0.5 | 49 (76.6) | 31 (12.6) | 17.96 (9.15–35.24) | .001 |
| All 6 antibiotics examined | | | | | 16 (25.0) | 2 (0.8) | 40.83 (9.09–183.4) | .001 |
| Any β-lactam, gentamicin, and ciprofloxacin | | | | | 49 (76.6) | 10 (4.0) | 72.42 (32.80–182.45) | .001 |
| Any β-lactam and gentamicin | | | | | 50 (78.1) | 31 (12.6) | 24.88 (12.33–50.21) | .001 |
| Any β-lactam and ciprofloxacin | | | | | 59 (92.2) | 19 (7.7) | 141.60 (50.70–395.00) | .001 |
| Gentamicin and ciprofloxacin but not β-lactams | | | | | 0 (0.0) | 0 (0.0) | ND | NS |

**NOTE.** ND, not determined; NS, not significant.

[a] Isolates judged to be resistant or intermediate by NCCLS breakpoints [28].

may correlate with the use of indwelling urinary catheters (table 2).

A reasonable hypothesis for the appearance and spread of metallo-β-lactamase–producing bacteria is the compromising use of carbapenems; the carbapenem class of antibiotics is the market leader among parenteral β-lactams in Japan, where several metallo-β-lactamases have been found [29]. Analysis of the antibiotics used before the isolation of *P. aeruginosa* showed that selective pressure from antibiotics is not always necessary for the isolation of *bla*$_{IMP}$-positive *P. aeruginosa* and that resistant pathogens were isolated from a considerable proportion (21.7%) of antibiotic-free patients. No antibiotic was administered more frequently than others among the case patients; however, several classes of antibiotics, including carbapenems, were administered for a longer duration and in larger doses (table 3).

In our study, *bla*$_{IMP}$-positive *P. aeruginosa* was found to have caused infection more frequently than did *bla*$_{IMP}$-negative pathogens, and the proportion of patients who subsequently died of infection was also higher among the case patients than among the control subjects. In contrast, there were no differences in bacteriological outcomes between the 2 groups. These paradoxical results suggest that the clinical condition of the case patients infected with *bla*$_{IMP}$-positive *P. aeruginosa* was more severe than that of the control subjects. To determine the virulence of *bla*$_{IMP}$-positive *P. aeruginosa* in detail, we are conducting in vitro and in vivo animal studies that use *P. aeruginosa*–carrying plasmids with and without the *bla*$_{IMP}$ gene.

Our previous study [9] and those of others [5, 24, 30] showed that *P. aeruginosa* isolates producing IMP-type metallo-β-lactamases were also resistant to meropenem, which is another carbapenem and has activity that is generally greater than that of imipenem against *P. aeruginosa* [1, 2]. The present antimicrobial susceptibility studies clearly demonstrated that *bla*$_{IMP}$-positive *P. aeruginosa* not only was resistant to β-lactams but also was frequently resistant to other classes of antibiotics. In fact, >75% of *bla*$_{IMP}$-positive *P. aeruginosa* isolates were MDR, which suggests the involvement of other antibiotic-resistant mechanisms, such as active efflux. In addition, the responsible genes were often mediated by integrons, where aminoglycoside acetyltransferase genes also exist [11].

In conclusion, our results show that possible risk factors for infection or colonization with such pathogens include long-term hospitalization, administration of antineoplastic agents or corticosteroids, use of indwelling urinary catheters, and long-term antibiotic use, in particular of β-lactams. We also have found that MDR isolates were more frequently of *bla*$_{IMP}$-positive than *bla*$_{IMP}$-negative strains. Although synergistic combinations of antibiotics still may be effective in treating some of these isolates, we have no such antibiotics available at present that are potent as single agents against MDR *bla*$_{IMP}$-positive *P. aeruginosa,* which emphasizes the need for precautionary measures against the spread of these resistant strains worldwide.

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