J Infect Chemother (2005) 11:64–70
DOI 10.1007/s10156-005-0377-z

ORIGINAL ARTICLE

Akiyoshi Tsuji · Intetsu Kobayashi · Toyoko Oguri
Matsuhisa Inoue · Eiko Yabuuchi · Sachiko Goto
The *Pseudomonas aeruginosa* Epidemiological Research Group (PER2001; 37 medical institutes)

An epidemiological study of the susceptibility and frequency of multiple-drug-resistant strains of *Pseudomonas aeruginosa* isolated at medical institutes nationwide in Japan

Received: October 20, 2004 / Accepted: February 15, 2005

Abstract The susceptibility of 3233 strains of *Pseudomonas aeruginosa*, isolated primarily in 2001, as agents of infection at 37 medical institutes with various specialties in seven regions of Japan (ranging from Hokkaido to Kyushu/Okinawa), to 18 antipseudomonal agents known to be active against *P. aeruginosa* was evaluated, in accordance with the National Committee for Clinical Laboratory Standards (NCCLS) guidelines. Of the 18 antipseudomonal agents, including some combinations of β-lactamase inhibitors and antibacterial agents, ciprofloxacin had the lowest minimum inhibitory concentration $(MIC)_{50}$ (0.25 μg/ml) against *P. aeruginosa*, followed by meropenem, with an MIC_{50} of 0.5 μg/ml. The MIC_{50} of 7 of the examined antibacterial agents (ceftazidime, cefozopran, imipenem, biapenem, gentamicin, tobramycin, and levofloxacin) was between 1 and 2 μg/ml. Among the antipseudomonal agents tested, tobramycin showed the lowest MIC_{90} (2 μg/ml), which was not significantly different from its MIC_{50} (1 μg/ml). The MIC_{90} of the other antibacterial agents examined ranged from 8 to 64 μg/ml and more. The susceptibility of the 3233 strains to the 12 antibacterial agents covered by the NCCLS guidelines was determined according to the standard method of the NCCLS guidelines. The frequency of strains resistant to meropenem, gentamicin, or tobramycin was relatively low (7.5%–8.3%). The frequency of strains showing intermediate to severe resistance to tobramycin was particularly low (8.0%). The frequency of strains resistant to aztreonam, imipenem, or levofloxacin was 16.7%–19.0%, about twice as high as the frequency of strains resistant to tobramycin. The susceptibility pattern of the 3233 strains (isolated from seven regions of Japan) to five antibacterial agents (ceftazidime, piperacillin, imipenem, gentamicin, and ciprofloxacin) was evaluated in relation to the regions from which they were isolated. The MIC_{50} values of these antibacterial agents did not differ significantly among the regions. However, the MIC_{90} values of ceftazidime and gentamicin were higher for strains isolated from the Kansai region than for strains isolated from other regions. The MIC_{90} of ciprofloxacin was higher for strains isolated from the Tohoku, Kansai, and Kyushu/Okinawa regions than for strains isolated from other regions. Of the 3233 strains, 89 were classified as multiple-drug-resistant (imipenem, gentamicin, and ciprofloxacin) strains. Of these 89 strains, 42 were isolated from urine, 17 from sputum or pharyngeal mucus, 13 from pus, 8 from blood, 1 from cerebrospinal fluid, and 8 from other specimens. The frequency of multiple-drug-resistant strains was higher among strains isolated from the Tohoku and Kansai regions than in strains isolated from other regions.

Key words *Pseudomonas aeruginosa* · Susceptibility · Multi-drug-resistant · Epidemiology

A. Tsuji (✉)
Department of Infection Control and Prevention, School of Nursing, Faculty of Medicine, Toho University, 4-16-20 Omori-nishi, Ota-ku, Tokyo 143-0015, Japan
Tel. +81-3-3762-9881; Fax +81-3-3766-3914
e-mail: aki@med.toho-u.ac.jp

A. Tsuji · I. Kobayashi · T. Oguri · M. Inoue · E. Yabuuchi · S. Goto
The *Pseudomonas aeruginosa* Epidemiological Research Group (PER2001; 37 medical institutes)

Introduction

Pseudomonas aeruginosa is an important opportunistic pathogen in patients managed at various medical and surgical specialist institutions. Numerous studies from Europe and the United States have reported *P. aeruginosa* as a respiratory pathogen in patients with cystic fibrosis.[1,2] In immunocompromised hosts with intractable *P. aeruginosa* infection, the resistance of *P. aeruginosa* to treatment is not mediated by ordinary mechanisms[3,4] (development of direct resistance to antibacterial agents), but by other mechanisms, such as the formation of a biofilm at the site of infection,[5] transformation of the pathogen into a mucoid type of *P. aeruginosa*,[6] diverse changes in the surface structures of *P. aeruginosa*,[4] and infection with multiple organisms.[7] Efforts to develop effective antibacterial agents against *P. aeruginosa* have continued over the years. While many antibacterial agents of various families effective

against *P. aeruginosa* have been introduced for clinical use, *P. aeruginosa* has rapidly developed resistance to these antibacterial agents, via various mechanisms.[8–10] Furthermore, the antibacterial activity of the available agents against *P. aeruginosa* is therapeutically inferior as compared to that against other organisms. Thus, further advances in chemotherapy against *P. aeruginosa* infection would be highly desirable.

Under such circumstances, the *Pseudomonas aeruginosa* Epidemiological Research Group (PER2001) was organized for epidemiological surveys of the current status of *P. aeruginosa* infection in Japan. The group collected fresh clinical isolates of *P. aeruginosa* from the clinical microbiology laboratories at 37 institutes nationwide and tested their susceptibility to the available antipseudomonal agents. The results varied greatly, both among the different institutes and depending on the time of the survey. This epidemiological analysis of susceptibility was expected to reveal the current status of *P. aeruginosa* infection and to provide useful data for the selection of the optimal antibacterial agents for treating this infection in the clinical setting, as well as providing data that could lead to the development of new antibacterial agents effective against *P. aeruginosa*.

Materials and methods

Test strains

A total of 3233 *Pseudomonas aeruginosa* isolates from patients with pseudomonal infectious disease were tested. The isolates were collected from 37 hospitals in Japan, mainly in 2001. *P. aeruginosa* isolates were stored at −70°C in skim milk until they were used. The details of institutions that participated in this study are shown in the Appendix.

Antimicrobial susceptibility

The determination of the minimum inhibitory concentrations (MICs) of ceftazidime (CAZ), cefozopran (CZOP), cefoselis (CFSL), cefpirome (CPR), cefepime (CFPM), sulbactam/cefoperazone (SBT/CPZ), tazobactam/piperacillin (TAZ/PIPC), piperacillin (PIPC), aztreonam (AZT), imipenem (IPM), meropenem (MEPM), panipenem (PAPM), biapenem (BIPM), gentamicin (GM), tobramycin (TOB), isepamicin (ISP), ciprofloxacin (CPFX), and levofloxacin (LVFX) was done by the broth dilution method according to the National Committee for Clinical Laboratory Standards (NCCLS) guideline M7-A5.[11] A microbroth dilution method was performed with a FrozenPlate (Eiken Chemical, Tokyo, Japan).

The isolates were inoculated onto CLED medium (Oxoid, Hampshire, UK), and incubated for 18–48 h at 35°C. Mueller Hinton broth (MHB) suspension of a test strain, equivalent to 0.5 McFarland standard (containing 10^8 CFU/ml), was prepared from a subculture of Trypticase soy agar (Nippon Becton Dickinson, Tokyo, Japan), and was diluted ten times with MHB. A 5 μg/ml volume of diluted suspension was added to each well (5×10^4 CFU/well) of the plate, and the cultures were incubated at 35°C, for 16–20 h. The MIC was defined as the lowest concentration of the antibiotic which completely inhibited bacterial growth.

Interpretive standards were according to the NCCLS M100-S11.[12] The interpretive standard used for SBT/CPZ was that used for CPZ.

Multi-drug-resistant (MDR) strains were defined according to the "Law Concerning the Prevention of Infections and Medical Care for Patients with Infections" administered by the Japanese Ministry of Health, Labour, and Welfare; that is, MDR strains were resistant to IPM, amikacin (AMK; as a substitute for GM), and CPFX.

Pulsed-field gel electrophoresis (PFGE)

PFGE for the 89 strains found to be MDR was performed according to the method described by Takigawa et al.[13] The isolates, embedded in agarose gel, were lysed and the bacterial proteins were digested. After the DNA was digested with the restriction enzyme *Spe* I (Takara Shuzo, Shiga, Japan) at 37°C for 8 h, the resulting fragments were separated with a pulsed-field gel electrophoresis system (CHEF-DRII; Bio-Rad Laboratories, Hercules, CA, USA). Lambda Ladder (Bio-Rad Laboratories) and *Saccharomyces cerevisiae* chromosomes (Bio-Rad Laboratories) was used as the DNA size standard. The following conditions were used: 1% agarose gel, pulse time, 5–30 s; voltage, 200 V; electrophoresis time, 20 h. After the gels ware stained with ethidium bromide, the chromosomal DNA patterns of different strains of *P. aeruginosa* were classified according to Tenover's criteria.

Metallo β-lactamase

The metallo β-lactamase production of *P. aeruginosa* isolates was tested using Metallo β-lactamase SMA (Eiken Chemical).[14]

Results

Susceptibility of *P. aeruginosa* to antipseudomonal agents

Table 1 summarizes the susceptibility of the 3233 strains of *P. aeruginosa* to 18 antipseudomonal agents, including two combinations of a β-lactamase inhibitor and antibacterial agent. Of all the antibacterial agents examined, CPFX showed the highest antibacterial activity, with an MIC_{50} of 0.25 μg/ml, followed by MEPM (MIC_{50}, 0.5 μg/ml). The MIC_{50} values of 7 of the antibacterial agents examined (CAZ, CZOP, IPM, BIPM, GM, TOB, and LVFX) ranged from 1 to 2 μg/ml. The other nine antibacterial agents (CFSL, CPR, CFPM, SBT/CPZ, TAZ/PIPC, PIPC, AZT, PAPM and ISP) had lower activity than the above-mentioned antibacterial agents, with the MIC_{50} ranging from 4 to 8 μg/ml.

Table 1. Susceptibilities of *Pseudomonas aeruginosa* (3233 isolates) to individual antipseudomonal drugs

Drug	MIC range	MIC_{50}	MIC_{90}
Ceftazidime	0.12–>128	2	32
Cefozopran	≦0.06–>128	2	16
Cefoselis	≦0.5–>64	8	64
Cefpirome	≦0.5–>64	8	64
Cefepime	0.12–>128	4	32
Sulbactam/Cefoperazone	≦0.5–>64	8	64
Tazobactam/Piperacillin	≦0.5–>64	8	64
Piperacillin	≦0.5–>64	8	>64
Aztreonam	≦0.25–>32	8	32
Imipenem	≦0.06–>128	2	16
Meropenem	≦0.06–>128	0.5	8
Panipenem	≦0.06–>128	8	32
Biapenem	≦0.06–>128	1	16
Gentamicin	≦0.12–>128	2	8
Tobramycin	≦0.12–>16	1	2
Isepamicin	≦0.5–>64	4	16
Ciprofloxacin	≦0.06–>128	0.25	16
Levofloxacin	≦0.25–>32	1	32

MIC, μg/ml

Table 2. Drug resistance among *Pseudomonas aeruginosa* isolates (3233)

Drug	Susceptible	Intermediate	Resistant
Ceftazidime	84.7 (2739)[a]	3.2 (103)	12.1 (391)
Cefepime	81.4 (2633)	7.9 (255)	10.7 (345)
Sulbactam/ Cefoperazone	77.7 (2511)	11.4 (368)	10.9 (354)
Tazobactam/ Piperacillin	90.5 (2925)	–	9.5 (308)
Piperacillin	87.7 (2834)	–	12.3 (399)
Aztreonam	68.0 (2198)	15.3 (495)	16.7 (540)
Imipenem	76.2 (2463)	4.8 (156)	19.0 (614)
Meropenem	84.5 (2731)	7.2 (233)	8.3 (269)
Gentamicin	86.1 (2784)	6.3 (205)	7.5 (244)
Tobramycin	91.9 (2972)	0.3 (11)	7.7 (250)
Ciprofloxacin	82.2 (2658)	3.3 (108)	14.4 (467)
Levofloxacin	73.3 (2370)	9.3 (300)	17.4 (563)

Interpretive criteria were defined according to NCCLS M100-S11
[a] Percentages, with numbers of isolates in parentheses

Of all the antipseudomonal agents tested in this study, TOB showed the lowest MIC_{90} (2μg/ml). In addition, the MIC_{50} of TOB (1μg/ml) differed only slightly from its MIC_{90}. The second lowest MIC_{90} was recorded for MEPM and GM (8μg/ml). The MIC_{90} of the other antibacterial agents ranged from 16 to 64μg/ml and more.

Frequency of antipseudomonal agent-resistant strains

The susceptibility of the 3233 isolates of *P. aeruginosa* to the 12 antipseudomonal agents covered by the NCCLS guidelines was evaluated. Table 2 shows the frequency of strains resistant to each of these agents, and also shows the distribution of strains with intermediate resistance and susceptible strains. The frequency of strains resistant to GM, TOB, or MEPM was relatively low (7.5%, 7.7%, and 8.3%, respectively), and the frequency of strains resistant to TAZ/PIPC was 9.5%. Of the 3233 strains, fewer than 10% were resistant to all 4 of these antibacterial agents. On the other hand, the frequency of strains resistant to AZT, LVFX, or IPM was about twice as high as that of strains resistant to the above-mentioned 4 antibacterial agents (16.7% to AZT, 17.4% to LVFX, and 19.0% to IPM). The frequency of strains resistant to the remaining 5 antibacterial agents (CFPM, SBT/CPZ, CAZ, PIPC, and CPFX) ranged from 10.7% to 14.4%.

The frequency of strains with intermediate, was resistance lowest for TOB (0.3%), followed by that for CAZ (3.2%), CPFX (3.3%), and IPM (4.8%). The frequency of strains resistant to TAZ/PIPC and PIPC, for which no standards of evaluation for intermediate resistance were available, was 9.5% and 12.3%, respectively. Thus, the frequency of intermediate or more resistant strains was lowest with TOB (8.0%), and second lowest with GM (13.8%); these frequencies for CAZ, MEPM, CPFX, and CFPM were under 20% (15.3%–18.6%). The frequencies of strains susceptible to AZT, LVFX, IPM, and SBT/CPZ (68.0%–77.7%) were slightly lower than those for the other antibacterial agents.

Antibacterial susceptibility of strains isolated from respiratory tract and blood or cerebrospinal fluid

Table 3 shows comparisons of the susceptibilities of the 1059 strains isolated from the respiratory tract and 336 strains isolated from the blood or cerebrospinal fluid to the 18 antipseudomonal agents. The MIC_{50} values of each antipseudomonal agent for the two groups of isolated strains were similar. That is, susceptibility to the antipseudomonal agents did not differ between the two groups of isolates. As shown in Table 3, the lowest MIC_{50} of MEPM was 0.5μg/ml, for both groups of isolates, and the lowest MIC_{50} of CPFX was 0.5μg/ml for the strains isolated from the respiratory tract and 0.25μg/ml for the strains isolated from blood or cerebrospinal fluid. The MIC_{90} of the antipseudomonal agents differed little between the two groups. The MIC_{90} of TOB was lowest among the antipseudomonal agents tested (2μg/ml for the strains isolated from the respiratory tract and 4μg/ml for the strains isolated from blood or cerebrospinal fluid).

Comparison of the susceptibility of isolates of *P. aeruginosa* from seven regions of Japan to five antibacterial agents

Table 4 shows the MIC_{50} and MIC_{90} values of five antibacterial agents (CAZ, PIPC, IPM, GM, and CPFX) for the 3233 strains of *P. aeruginosa* isolated at 37 medical institutes with different specialties, from seven regions of Japan (from Hokkaido to Kyushu/Okinawa). The MIC_{50} of CAZ (2–4μg/ml) for the isolates differed little among the seven regions. However, the MIC_{90} of CAZ was higher for strains isolated from the Kansai region (128μg/ml) than for strains

Table 3. Susceptibilities of *Pseudomonas aeruginosa* isolated from respiratory tract and blood, or cerebrospinal fluid specimens

Drug	MIC_{50}		MIC_{90}	
	Respiratory[a] (1059)[c]	Blood or CSF[b] (336)	Respiratory (1059)	Blood or CSF (336)
Ceftazidime	2	2	32	32
Cefozopran	2	2	16	32
Cefoselis	8	8	64	64
Cefpirome	8	8	64	64
Cefepime	4	4	16	32
Sulbactam/ Cefoperazone	8	8	32	64
Tazobactam/ Piperacillin	8	8	64	64
Piperacillin	8	8	>64	>64
Aztreonam	8	8	32	32
Imipenem	2	2	16	32
Meropenem	0.5	0.5	8	16
Panipenem	8	8	32	32
Biapenem	1	1	16	16
Gentamicin	2	2	8	8
Tobramycin	1	1	2	4
Isepamicin	4	4	16	16
Ciprofloxacin	0.5	0.25	4	8
Levofloxacin	1	1	8	16

MIC, μg/ml
[a] Respiratory tract specimen
[b] Blood or cerebrospinal fluid specimen
[c] No. of isolates

Table 4. Susceptibility of *Pseudomonas aeruginosa* isolates from seven regions of Japan to five antipseudomonal drugs

Region (no. of isolates)	Ceftazidime		Piperacillin		Imipenem		Gentamicin		Ciprofloxacin	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
Hokkaido (224)	2	32	8	>64	2	16	2	8	0.25	4
Tohoku (439)	2	32	8	>64	2	16	2	16	0.5	32
Kanto (1341)	2	16	8	>64	2	16	2	8	0.25	8
Chubu (340)	2	32	8	>64	2	16	2	8	0.5	4
Kansai (126)	4	128	8	>64	2	16	2	64	0.5	64
Chugoku/Shikoku (372)	2	64	8	>64	2	32	2	8	0.5	8
Kyushu/Okinawa (391)	4	32	8	>64	2	32	2	8	0.5	32

MIC, μg/ml

isolated from the other regions (16–64 μg/ml). The MIC_{50} and MIC_{90} of PIPC ranged from 8 to 64 μg/ml and more, with no marked differences being observed for strains isolated from different regions. There were also no significant differences in the MIC_{50} values of IPM (MIC_{50}, 2 μg/ml; MIC_{90}, 16–32 μg/ml) among the different regions. The MIC_{50} of GM was 2 μg/ml for the strains isolated from each region, but the MIC_{90} of this antibacterial agent was markedly higher for the strains isolated from the Kansai region (64 μg/ml) than for the strains from other regions (8–16 μg/ml). The MIC_{50} of CPFX was similar (0.25–0.5 μg/ml) for strains isolated from each region, but the MIC_{90} of this antibacterial agent was highest for strains isolated from the Kansai region (64 μg/ml), and the MIC_{90} was higher for strains isolated from the Tohoku and Kyushu/Okinawa regions (32 μg/ml) than the values for strains isolated from the other four regions (4–8 μg/ml).

Frequency of MDR strains and PFGE patterns

Table 5 shows the frequency of MDR strains among the 3233 clinical isolates, according to the clinical sources from which they were isolated. Of the 3233 strains, 89 (2.8%) were MDR. The most frequent source of MDR strains was urine (42 strains; 47.2%), followed by the respiratory tract (sputum and pharyngeal mucus, 17 strains; 19.1%), pus (13 strains; 14.6%), blood (8 strains; 9.0%), cerebrospinal fluid (1 strain; 1.1%), and others (8 strains; 9.0%).

Table 6 shows the frequency of isolation of MDR strains in each of the seven regions of Japan, and the PFGE patterns of such strains. The largest number of MDR strains was isolated from the Kanto region (39 of 89 strains; 43.8%). The frequency of detection of such strains was highest in strains from the Kansai region (4.8%), followed by the Tohoku region (3.9%), while the percentage was

lowest in the Chubu region (0.9%; 3 strains). The PFGE pattern differed among all the 3 strains isolated in the Chubu region. The two resistant strains isolated from the Hokkaido region had the same PFGE pattern, which differed from that of the strains isolated from the Chubu region. Some of the MDR strains isolated from other regions showed somewhat similar patterns, but not all of the patterns were identical.

Metallo-β-lactamase production

Of the 614 IPM-resistant strains, 48 (7.8%) were demonstrated to be positive for metallo-β-lactamase production, using an SMA disk.

Discussion

Although *P. aeruginosa* is ubiquitously distributed in nature, it is a nosocomial pathogen responsible for infection in immunocompromised hosts. Aminoglycosides were first known as the most effective antibacterial agents against *P. aeruginosa* infection. Subsequently, β-lactam antibiotics of various families were developed to treat infections caused by *P. aeruginosa*. More recently, fluoroquinolones, all of which exert varying degrees of activity against *P. aeruginosa*, have been introduced clinically for the control of *P. aeruginosa* infection. In many cases, however, the activity of the antibacterial agents against *P. aeruginosa* in vitro was lower than that against other Gram-negative bacilli. Furthermore, *P. aeruginosa* rapidly developed resistance to these antibacterial agents via diverse and complex mechanisms,[3,4,8–10,15] as mentioned above, e.g., by the formation of a biofilm[11] or by the development of changes in the surface structures,[4,16] which decreased the antibiotics' ability to permeate the intracellular membranes.

For the successful treatment of *P. aeruginosa* infection, it is urgent to have adequate evaluation of the efficacy of currently available agents that are effective against *P. aeruginosa*, and to have knowledge of the current status of the drug resistance. To date, a number of reports have been published from Japan, as well as from overseas, concerning the susceptibility of clinical isolates of *P. aeruginosa* to the available antibacterial agents. The reported susceptibility patterns varied greatly depending upon the medical facility or the region from which the strains were isolated,[17] and depending on the era[18] of the surveys. It is suggested that several factors (e.g., differences in the selection of antibacterial agents tested among different institutes, and differences in the background variables of patients) may affect the efficacy of antibacterial agents against *P. aeruginosa*.

In the present study, we evaluated the susceptibility of 3233 strains of *P. aeruginosa*, isolated from patients at 37 medical institutes, with various specialties, in seven regions of Japan, to various clinically used antipseudomonal agents.

The MIC_{50} values of the antipseudomonal agents (analyzed as an indicator of the antibacterial agents' efficacy) against the 3233 strains of *P. aeruginosa* ranged from 0.25 to 8 μg/ml. This range was relatively similar to that (1–8 μg/ml) reported by Hikida et al.[19] for four carbapenems (IPM, PAPM, MEPM, and BIPM), CAZ, and AZT, against *P. aeruginosa* strains isolated in the year 2000 (not too long before the period of the present study). Hikida et al.[19] reported that the MIC_{90} of MEPM was 8 μg/ml but that the MIC_{90} of the other three carbapenems, CAZ and AZT, ranged from 16–32 μg/ml, consistent with the results of the present study. In a previous survey, conducted in 1995–1996 at multiple institutes in the vicinity of Fukushima prefecture,[20] the MIC_{50} of three carbapenems (IPM, PAPM, and MEPM) and three β-lactams (CPR, CAZ, and PIPC) against 216 strains of *P. aeruginosa* ranged from 0.78 to 12.5 μg/ml; the MIC_{90} values of these antibacterial agents against the same 216 strains did not differ markedly from

Table 5. Frequency of multi-drug-resistant *Pseudomonas aeruginosa* strains according to source of clinical specimen

Specimen	No. of isolates	Percentage
Urine	42	47.2
Sputum/Pharyngeal mucosa	17	19.1
Pus	13	14.6
Blood	8	9.0
Cerebrospinal fluid	1	1.1
Other	8	9.0
Total	89	100

Multi-drug-resistant (MDR); resistant to imipenem, gentamicin, and ciprofloxacin. There were 89 MDR isolates among the 3233 isolates

Table 6. Frequency of isolation and PFGE patterns of multi-drug-resistant *Pseudomonas aeruginosa* isolates from seven regions of Japan

Region (no. of isolates)	No. of MDR isolates (%)	PFGE pattern (no. of isolates, omit one isolate)
Hokkaido (224)	2 (0.9)	1 (2)
Tohoku (439)	17 (3.9)	2, 3 (2), 4, 5, 6, 7, 8, 9 (4), 10, 11 (2), 12, 13
Kanto (1341)	39 (2.9)	14 (5), 15, 16, 17, 18 (3), 19, 20, 21, 22, 23 (2), 24, 25, 26 (3), 27 (7), 28, 29, 30, 31, 32, 33, 34 (2), 35, 36
Chubu (340)	3 (0.9)	37, 38, 39
Kansai (126)	6 (4.8)	40 (2), 41 (4)
Chugoku/Shikoku (372)	10 (2.7)	42 (4), 43 (3), 44 (2), 45
Kyushu/Okinawa (391)	12 (3.1)	46 (3), 47, 48 (5), 49, 50, 51
Total (3233)	89 (2.8)	

Multi-drug-resistant (MDR), resistant to imipenem, gentamicin, and ciprofloxacin

the result obtained in the present study. However, Sasaki et al.,[21] who analyzed the annual changes in the frequency of strains resistant to IPM, CAZ, and GM among strains of *P. aeruginosa* isolated from inpatients at a surgical facility, reported that the frequency of resistant strains varied greatly from year to year, and they concluded that surrounding factors (antibacterial-therapeutic strategies, etc.) could strongly affect the development of *P. aeruginosa* resistance to antibacterial agents.

A remarkable finding in the present study was that the frequency of strains resistant to GM, TOB, and MEPM among the 3233 strains was relatively low (7.5%–8.3%). The exact cause for this finding is unclear, but it may due to the recent trend of a decrease in the use of aminoglycosides for the treatment of *P. aeruginosa* infection, with the development of a wide variety of other antibacterial agents effective against *P. aeruginosa* infection. The low frequency of MEPM-resistant strains may reflect the increasingly widespread use of IPM in recent years against IPM-resistant *P. aeruginosa*. Of the 614 strains resistant to IPM, only 48 strains (7.8%) tested positive for metallo-β-lactamase production. But, special attention should be paid to the frequency of *P. aeruginosa* that produced this enzymes.

There was little difference in the MIC_{50} or MIC_{90} values of the 18 antipseudomonal agents for strains isolated from the respiratory tract (sputum pharyngeal mucosa) and those isolated from blood or cerebrospinal fluid. Of the 89 MDR strains identified, 47.2% were isolated from urine, 19.1% from sputum and pharyngeal mucus, 14.6% from pus, and 9.0% from blood. MDR strains were most often isolated from urine, probably reflecting changes in the susceptibility of viable *P. aeruginosa*, which are often exposed to high concentrations of these antibacterial agents in patients with urinary tract infections. Burns et al.[6] investigated the susceptibility of 498 *P. aeruginosa* strains isolated from patients with cystic fibrosis in the United States, and reported high percentages of strains showing resistance to antibacterial agents (about 50% showing resistance to GM, PIPC, CAZ, or AZT, and 26%–39% showing resistance to CPFX, IPM, or MEPM), as compared to the data obtained from the institutes with various specialties in the present study. It seems likely that the long-term treatment of these patients with cystic fibrosis with antibacterial agents may be responsible for the changes in susceptibility and the development of resistance by *P. aeruginosa* to these multiple antibacterial agents.

We found that the MIC_{50} values differed little among the different geographical regions in this study, although the values for some of the antibacterial agents (CAZ, GM, and CPFX) were higher for strains isolated from the Kansai region, whereas those for others were higher for strains isolated from the Tohoku and Kyushu/Okinawa regions. The frequency of MDR strains was higher in the Kansai region (4.8%) than in the other regions of Japan. The exact cause of this difference is unknown, but it may be related to differences in various surrounding factors (antibacterial-therapeutic strategies, etc.) among the different institutes. Of the 3233 strains of *P. aeruginosa*, 89 strains (2.8%) were resistant to multiple antibacterial agents. The frequency of the MDR strains differed among the different regions; it was the highest (that is, 4.8%) in the Kansai region, a value which was about five times higher than the lowest percentage (0.9%) recorded in the Hokkaido and Chubu regions. When the 89 MDR strains were subjected to PFGE, 51 different PFGE patterns were observed. Some strains showed identical patterns; however, strains isolated from each region, excluding the two isolated from the Hokkaido region, showed variable PFGE patterns. The two strains isolated from the Hokkaido region showed an identical PFGE pattern (both isolated from the same institute). This regional specificity of the PFGE pattern suggests that these MDR strains may have acquired resistance at individual institutes or in the different regions. However, the frequency of susceptible strains among all the strains tested (including the MDR strains) ranged widely, from 68.0% (to AZT) to 91.9% (to TOB). This indicates that, while it may be difficult, the selection of suitable and efficacious antipseudomonal agents in the clinical setting is, however, not impossible. It cannot be overemphasized that the antipseudomonal activity of each agent in vitro cannot be directly extrapolated to its use in vivo. When selecting antipseudomonal agents, it is essential to choose the most suitable one from among the effective antipseudomonal agents, taking into account the pharmacodynamic profile of the antipseudomonal agent, its potential to enter the site of infection, and various other factors (tissue-invading, etc.).

The results of the present study are expected to facilitate the development of new combination antibacterial agent regimens against *P. aeruginosa* infections, to suppress the development of resistant strains, and/or to reinforce the activity of antibacterial agents against resistant strains, via the efflux mechanism.[9,15]

Appendix

The medical institutes belonging to PER2001 are as follows.

Hokkaido University Hospital, Sapporo City General Hospital, Muroran City General Hospital, Aomori Prefectural Central Hospital, Iwate Medical University Hospital, Tohoku University Hospital, Yamagata University School of Medicine Hospital, Kosirakawa-Shiseido Hospital, Yamanashi Prefectural Central Hospital, Gunma University Hospital, Shinshu University Hospital, Niigata University Medical and Dental Hospital, Jichi Medical School Hospital, Juntendo University Hospital, Toho University Oomori Hospital, Nihon University Itabashi Hospital, Surugadai Nihon University Hospital, The University of Tokyo Hospital, Social Insurance Central General Hospital, Toshiba Hospital, Tokyo Metropolitan Police Hospital, Teikyo University Hospital, Tokyo Women's Medical University Hospital, Mitsubishi Kagaku Bio-Clinical Laboratories, The University of Tokyo-The Institute of Medical Science, Kanto Medical Center NTT EC, Chiba University Hospital, Tokai University Hospital, Kitasato University Hospital, Yokohama City University Hospital,

Nagoya University Hospital, Fujita Health University Hospital, KKR Meijo Hospital, Tenri Hospital, Kyoto University Hospital, Anjo Kosei Hospital, KKR Otemae Hospital, Osaka General Medical Center, Kobe University Hospital, Toyama Medical and Pharmaceutical University Hospital, Kawasaki Medical School Hospital, Okayama University Hospital, Yamaguchi University Hospital, Ehime University Hospital, Kagawa Medical University Hospital, Kochi Medical School Hospital, Saga Medical School Hospital, Oita Medical University Hospital, Nagasaki University Medical Hospital, The Chemo-Sero-Therapeutic Research Institute, University of The Ryukyus University Hospital, Department of Microbiology, Kitasato University School of Medicine, and the Department of Infection Control and Prevention, School of Nursing Faculty of Medicine Toho University.

References

1. Boukadida J, De Montalembert M, Lenoir G, Scheinmann P, Veron M, Berche P. Molecular epidemiology of chronic pulmonary colonisation by *Pseudomonas aeruginosa* in cystic fibrosis. J Med Microbiol 1993;38:29–33.
2. Ramsey BW. Management of pulmonary disease in patients with cystic fibrosis. N Engl J Med 1996;335:179–88.
3. Nordmann P, Guibert M. Extended-spectrum β-lactamases in *Pseudomonas aeruginosa*. J Antimicrob Chemother 1998;42:128–31.
4. Kobayashi I, Hasegawa M, Nishida M. Alteration of serotype and drug susceptibility of some *Pseudomonas aeruginosa* isolates by exposure to human serum and polymorphonuclear leukocytes. Kansenshogaku Zasshi 1994;68:500–7.
5. Kobayashi H. Airway biofilm disease. Int J Antimicrob Agents 2001;17:351–6.
6. Burns JL, Saiman L, Whittier S, Larone D, Krzewinski J, Liu Z, et al. Comparison of agar diffusion methodologies for antimicrobial susceptibility testing of *Pseudomonas aeruginosa* isolates from cystic fibrosis patients. J Clin Microbiol 2000;38:1818–22.
7. Maeda K, Sawaki M, Mikasa K, Konishi M, Teramoto S, Mori K, et al. A clinical study of respiratory infections due to mucoid *Pseudomonas aeruginosa* diagnosed by transtracheal aspiration. Kansenshogaku Zasshi 1994;68:1472–8.
8. Shawar RM, MacLeod DL, Garber RL, Burns JL, Stapp JR, Clausen CR, et al. Activities of tobramycin and six other antibiotics against *Pseudomonas aeruginosa* isolates from patients with cystic fibrosis. Antimicrob Agents Chemother 1999;43:2877–80.
9. Lomovskaya O, Warren MS, Lee A, Galazzo J, Fronko R, Lee M, et al. Identification and characterization of inhibitors of multidrug resistance efflux pumps in *Pseudomonas aeruginosa*: novel agents for combination therapy. Antimicrob Agents Chemother 2001;45:105–16.
10. Senda K, Arakawa Y, Nakashima K, Ito H, Ichiyama S, Shimokata K, et al. Multifocal outbreaks of metallo-beta-lactamase-producing *Pseudomonas aeruginosa* resistant to broad-spectrum beta-lactams, including carbapenems. Antimicrob Agents Chemother 1996;40:349–53.
11. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, fifth ed. M7-A5: 2000. Wayre, PA: NCCL 5; 2000.
12. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing; eleventh informational supplement. M100-S11, 2001. Wayre, PA: NCCLS; 2001.
13. Takigawa K, Fujita J, Negayama K, Yamagishi Y, Yamaji Y, Ouchi K, et al. Nosocomial outbreak of *Pseudomonas cepacia* respiratory infection in immunocompromised patients associated with contaminated nebulizer devices. Kansenshogaku Zasshi 1993;67:1115–25.
14. Arakawa Y, Shibata N, Shibayama K, Kurokawa H, Yagi T, Fujiwara H, et al. Convenient test for screening metallo-β-lactamase-producing gram-negative bacteria by using thiol compounds. J Clin Microbiol 2000;38:40–3.
15. Li XZ, Livermore DM, Nikaido H. Role of efflux pump(s) in intrinsic resistance of *Pseudomonas aeruginosa*: resistance to tetracycline, chloramphenicol, and norfloxacin. Antimicrob Agents Chemother 1994;38:1732–41.
16. Kobayashi I, Hasegawa M, Miyazaki S, Nishida M, Goto S. In vitro and in vivo changes of serotype in *Pseudomonas aeruginosa* isolates by anti-pseudomonal drugs. J Antibiot (Tokyo) 1994;47:72–9.
17. Kouda M, Fukuhara J, Takeuchi M, Ohgawara M, Matsuzaki H, Tohi H, et al. Estimation of antibacterial activity of various antibiotics against *Pseudomonas aeruginosa* by score method. Jpn J Antibiot 1999;52:458–68.
18. Nakae M, Sugahara Y, Sasaki H, Yasui H, Imai C, Hasegawa Y, et al. Serotypes and drug susceptibility of *Pseudomonas aeruginosa* isolated from clinical specimens. Jpn J Antibiot 1997;50:187–94.
19. Hikida M, Terashima S, Sato Y, Okamoato R, Inoue M. Comparative antibacterial activity of carbapenems against *P. aeruginosa* (1). Jpn J Antibiot 2001;54:571–9.
20. Niitsuma K, Saitoh M, Kojimabara M, Kashiwabara N, Aoki T, Tomizawa M, et al. Antimicrobial susceptibility of *Pseudomonas aeruginosa* isolated in Fukushima Prefecture. Jpn J Antibiot 2001;54:79–87.
21. Sasaki M, Hiyama E, Takesue Y, Kodaira M, Sueda T, Yokoyama T. Clinical surveillance of surgical imipenem-resistant *Pseudomonas aeruginosa* infection in a Japanese hospital. J Hosp Infect 2004;56:111–18.