2.2. Detection of IMP-type metallo-β-lactamases and AAC(6′)-Iae

IMP-type MBLs and AAC(6′)-Iae were detected using an immunochromatographic assay kit (Mizuho Medy Co., Saga, Japan) designed for the detection of these enzymes [3,4].

2.3. Antimicrobial susceptibility

MICs of IPM (Banyu Pharmaceutical Co., Tokyo, Japan), AMK (Banyu Pharmaceutical Co.), CIP (Daiichi Pharmaceutical Co., Tokyo, Japan) and colistin (Sigma-Aldrich, St Louis, MO) were determined using the microdilution method as per the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [7]. Values of MICs at which 50% and 90% of the isolates were inhibited (MIC50 and MIC90, respectively) were determined. Isolates were tested for the presence of MBL by a double-disk synergy test with disks containing sodium mercaptoacetic acid (SMA) as described previously [8].

2.4. Detection of antibiotic resistance genes

The bla_{IMP} and *aac(6′)-Iae* genes were amplified using polymerase chain reaction (PCR) primers as described previously [9]. All of the PCR products were sequenced using an ABI PRISM 3130 sequencer (Applied Biosystems, Foster City, CA). The class 1 integron was amplified using the PCR primer set of 5′CS and 3′CS. All of the PCR products were sequenced to identify the contents of the genes [10].

2.5. Pulsed-field gel electrophoresis (PFGE)

DNA plugs were prepared and digested overnight at 37 °C with *Spe*I (Takara Bio, Otsu, Japan). PFGE analysis was performed as described previously [8]. Fingerprinting patterns were analysed by the unweighted pair-group method using Molecular Analyst Fingerprinting Plus software (Bio-Rad Laboratories, Hercules, CA) to create an average linkage-based dendrogram.

2.6. Multilocus sequence typing (MLST)

MLST was performed according to the protocols described on the *P. aeruginosa* MLST Database website (http://pubmlst.org/paeruginosa/). PCR and sequencing were performed for seven chromosomal genes (*acsA*, *aroE*, *guaA*, *mutL*, *nuoD*, *ppsA* and *trpE*). The nucleotide sequences of these genes were compared with the sequences submitted to the MLST database to determine the allelic numbers and sequence types (STs).

2.7. Serotyping

Serotypes of the isolates were determined using a slide agglutination test kit (Denka Seiken Co., Tokyo, Japan). Serotype O18 was determined using a sequence-based method [11].

3. Results

3.1. Screening of multidrug-resistant Pseudomonas aeruginosa producing IMP-type metallo-β-lactamases and AAC(6′)-Iae

In total, 217 MDR *P. aeruginosa* isolates were screened for production of IMP-type MBLs and AAC(6′)-Iae using an immunochromatographic assay. Of these, 145 isolates (66.8%) produced both IMP-type MBLs and AAC(6′)-Iae, 29 (13.4%) produced IMP-type MBLs but did not produce AAC(6′)-Iae and 6 (2.8%) produced AAC(6′)-Iae but did not produce IMP-type MBLs. The six isolates producing AAC(6′)-Iae but not IMP-type MBLs were negative for MBL by the SMA double-disk synergy test. Results of the immunochromatographic assay were consistent with those of the PCR for bla_{IMP} and *aac(6′)-Iae* genes.

3.2. Genetic context of the IMP-type metallo-β-lactamases and AAC(6′)-Iae

DNA sequencing showed that the 145 isolates producing both IMP-type MBLs and AAC(6′)-Iae did not have a mutation in the *aac(6′)-Iae* gene. Of these, 125, isolates had bla_{IMP-1}, 6 had bla_{IMP-6} and 14 had bla_{IMP-10}.

PCR showed that of the 145 isolates producing IMP-type MBLs and AAC(6′)-Iae, 142 were positive for a class I integron. Of these 142 isolates, 124 had integron In*113*, which carried bla_{IMP-1} [9]; the remaining 18 isolates had In*113*-like integrons, which have the same structure as integron In*113* but the bla_{IMP-1} is replaced by IMP-6 (4 isolates) and IMP-10 (14 isolates).

3.3. Pulsed-field gel electrophoresis analysis, multilocus sequence typing and serotyping

The 145 isolates of MDR *P. aeruginosa* producing both IMP-type MBLs and AAC(6′)-Iae were analysed by PFGE. Analysis showed two clusters with >60% similarity (clusters I and II) (Fig. 1). Cluster I comprised 128 isolates and cluster II comprised 16 isolates; 1 isolate did not belong to any cluster. Cluster I included the NCGM2.S1 strain, which was the first reported MDR *P. aeruginosa* strain producing IMP-type MBLs and AAC(6′)-Iae [9]. The PFGE patterns of all of the isolates belonging to cluster II were identical (Fig. 1).

Of the 128 isolates belonging to cluster I, 127 were ST235 (STs: *acsA* 38, *aroE* 11, *guaA* 3, *mutL* 13, *nuoD* 1, *ppsA* 2 and *trpE* 4) and serotype O11, and 1 isolate was ST991 (STs, *acsA* 6, *aroE* 3, *guaA* 12, *mutL* 3, *nuoD* 3, *ppsA* 6 and *trpE* 7) and serotype O3. All 16 isolates belonging to cluster II were ST991 and serotype O18. ST991 does not appear to be related to ST235 because all the STs of the house keeping genes are different.

3.4. Antimicrobial susceptibility

All of the isolates belonging to clusters I and II were highly resistant to IPM, AMK and CIP; there was no difference in the MIC profiles of these two groups (Table 1). Of the 16 isolates belonging to cluster II, 15 were susceptible to colistin (MIC = 2 μg/mL) and 1 was intermediately susceptible (MIC = 4 μg/mL). One isolate belonging to cluster I and one isolate not belonging to any cluster were susceptible to colistin (MIC = 2 μg/mL).

3.5. Geographical distribution

MDR *P. aeruginosa* isolates producing IMP-type MBLs and AAC(6′)-Iae were obtained from 89 medical settings located in 22 prefectures in Japan (Fig. 2). Of these, isolates belonging to cluster I were obtained from 17 prefectures distributed along the northern to southern region of Japan. Isolates belonging to cluster II were obtained from nine prefectures that were also distributed along the northern to southern region of Japan (Fig. 2). The MDR *P. aeruginosa* isolates were obtained from relatively many medical settings in the Kanto area of Japan, e.g. 19 in Saitama, 15 in Tokyo and 9 in Chiba (Fig. 2). These findings suggest that MDR *P. aeruginosa* isolates belonging to both the clusters were spread throughout Japan.

4. Discussion

This study showed that IMP-type MBL- and AAC(6′)-Iae-producing MDR *P. aeruginosa* ST235, serotype O11, which belong to cluster I (Fig. 1), have undergone clonal expansion in medical settings in Japan. NCGM2.S1 strain, which belongs to cluster I, was

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Table 1
Minimum inhibitory concentrations (MICs) and percent antimicrobial resistance for IMP-type metallo-β-lactamase- and AAC(6′)-Iae-producing *Pseudomonas aeruginosa* isolates belonging to clusters I and II.

Antimicrobial agent	Breakpoint for resistance (μg/mL)	Cluster I (n = 128)				Cluster II (n = 16)			
		%R	MIC range (μg/mL)	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)	%R	MIC range (μg/mL)	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)
IPM	≥16	100	32 to >128	128	>128	100	128	128	128
AMK	≥32	100	32 to >128	128	>128	100	64	64	64
CIP	≥8	100	8 to >128	64	>128	100	>128	>128	>128

%R, percent resistance; $MIC_{50/90}$, MIC at which 50% and 90% of the isolates were inhibited, respectively; IPM, imipenem; AMK, amikacin; CIP, ciprofloxacin.

determined to be the cause of an outbreak of catheter-associated urinary tract infections in the neurosurgery ward of a hospital in Miyagi [8], Japan. Further epidemiological studies found that clonal expansion of this strain had also occurred in community hospitals in Kanto region [3] and Hiroshima [2]. Clonal expansion of MBL-producing *P. aeruginosa* ST235, serotype O11 has also been reported in South Korea [12] and Scandinavia [13].

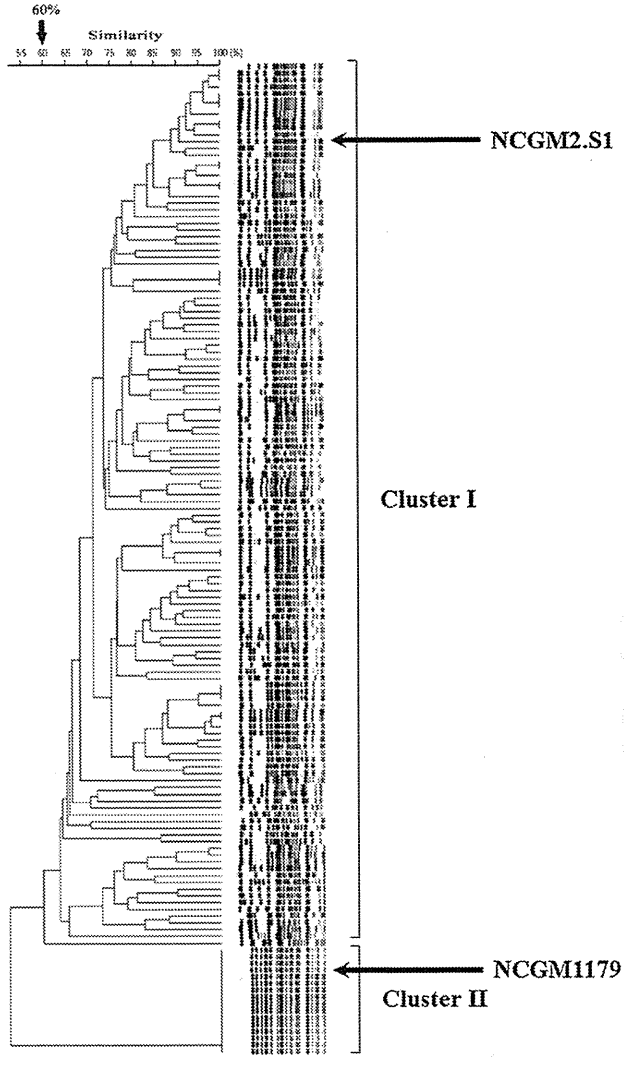

Fig. 1. Dendrogram of pulsed-field gel electrophoresis (PFGE) patterns of 145 multidrug-resistant *Pseudomonas aeruginosa* isolates producing IMP-type metallo-β-lactamases and AAC(6′)-Iae. Two clusters (I and II) were detected. Of the 128 isolates belonging to cluster I, 127 isolates were ST235 and serotype O11 and 1 isolate was ST991 and serotype O3. All of the 16 isolates belonging to cluster II were ST991 and serotype O18.

The isolates belonging to cluster I were mainly obtained from the urinary and respiratory tracts; the percentage of isolates from the urinary tract was markedly higher. A surveillance study of *P. aeruginosa* clinical isolates with and without multidrug resistance showed that MDR isolates were particularly increased in the urinary tract of Japanese individuals [1]. The increase in the number of MDR isolates in the urinary tract may be related to the epidemic of IMP-type MBL- and AAC(6′)-Iae-producing MDR *P. aeruginosa* in Japan.

This is the first report describing MDR *P. aeruginosa* ST991, serotype O18, which belonged to cluster II (Fig. 1) and is a recent emerging strain in medical settings in Japan. ST991 was originally registered by C. Giske at Karolinska University Hospital, Sweden in 2010 in the *P. aeruginosa* MLST Database (http://pubmlst.org/paeruginosa/). However, to the best of our knowledge, there are no reports on the association of ST991 and multidrug resistance in *P. aeruginosa*. All of the isolates belonging to cluster II were obtained from the respiratory tract. In contrast, 32.8% of the isolates belonging to cluster I (42/128) were obtained from the respiratory tract. MDR *P. aeruginosa* ST991 dominantly causes respiratory infections. MDR isolates of *P. aeruginosa* serotype O18 have not been previously reported. Most of the MDR clinical isolates of *P. aeruginosa* exhibit serotype O11 or O12 [11].

We have reported the complete genome sequences of NCGM2.S1 [5] and NCGM1179 [6]. Integron In*113* was inserted in the *oprD* gene and disrupted it in NCGM2.S1; integron In*113* was located downstream of the *tnpA* gene that codes for transposase

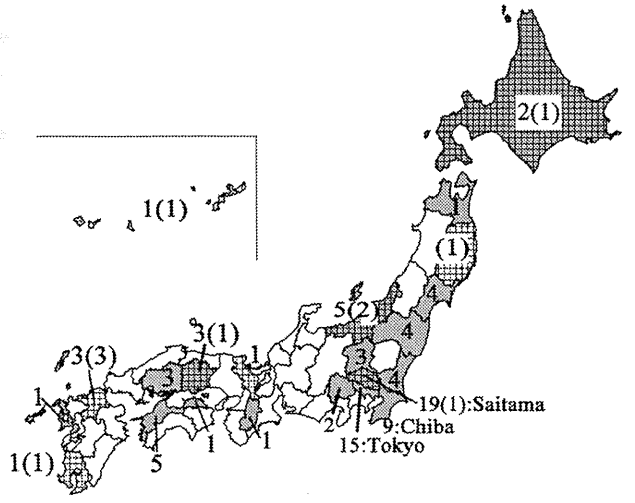

Fig. 2. Geographical distribution of multidrug-resistant (MDR) *Pseudomonas aeruginosa* isolates producing IMP-type metallo-β-lactamases and AAC(6′)-Iae in Japan. Isolates belonging to cluster I (Fig. 1) were obtained from prefectures marked in grey; isolates belonging to cluster II were obtained from prefectures marked in a checked pattern. Isolates belonging to both clusters were obtained from prefectures marked in a grey checked pattern. The number and the number in parenthesis represent the number of medical settings in the prefecture where MDR *P. aeruginosa* isolates belonging to cluster I and cluster II, respectively, were obtained.

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of Tn*4380* of the mercury transposon Tn*3* family and the *tnpR* gene that codes for serine-base site-specific recombinase of Tn*6050*. However, the *oprD* was found to be intact in the NCGM1179 strain. *oprD* codes for a specialised pore protein, OprD, which allows selective permeation of basic amino acids and their structural analogues such as carbapenems, including IPM and meropenem [14]. It is unclear whether OprD affects the MIC of carbapenems in IMP-type MBL- and AAC(6′)-Iae-producing MDR *P. aeruginosa*. The details of the comparative genome analysis of the two MDR strains will be reported elsewhere.

Of the 217 MDR *P. aeruginosa* isolates tested in this study, 72 did not produce IMP-type MBLs and/or AAC(6′)-Iae. At present, we are looking for genes conferring high resistance to all β-lactams, aminoglycosides and fluoroquinolones.

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Competing interests: None declared.

Ethical approval: This study was approved in 2010 by the Biosafety Committee, National Center for Global Health and Medicine (Tokyo, Japan) (approval no. 23-M-58).

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Isolation rates of multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter* spp. at medical facilities in Japan

Teruo Kirikae[1,*], Namiko Mori-Yoshikawa[2] and Yoshichika Arakawa[3]

[1]Department of Infectious Diseases, Research Institute, National Center of Global Health and Medicine, Tokyo, Japan

[2]National College of Nursing Japan, Tokyo, Japan

[3] Department of Bacteriology, Nagoya University Graduate School of Medicine, Nagoya, Japan

Email address: morin@adm.ncn.ac.jp, yarakawa@med.nagoya-u.ac.jp

Corresponding author:

*Teruo Kirikae, MD, PhD

Department of Infectious Diseases

Research Institute

National Center of Global Health and Medicine

Toyama 1-21-1, Shinjuku, Tokyo 162-8655, Japan

Tel: +81-3-3202-7181 (ext. 2838)

Fax: +81-3-3202-7364

E-mail: tkirikae@ri.ncgm.go.jp

Abstract

Background: The emergence of multidrug-resistant (MDR) *Pseudomonas aeruginosa* and *Acinetobacter baumannii* strains is a serious problem at medical facilities in Japan.

Methods: We distributed questionnaires to assess the isolation rates of multidrug-resistant (MDR) *P. aeruginosa* and *Acinetobacter* spp. at all medical facilities with more than 200 beds throughout Japan from 2007 to 2009.

Results: Completed questionnaires were received from 771 medical facilities. The total numbers of *P. aeruginosa* and MDR isolates obtained at the medical facilities were 684 982, and 19 911 (2.9% of *P. aeruginosa* isolates), respectively; MDR isolates were found nationwide. One or more MDR *P. aeruginosa* isolates were found at approximately 53% of the medical facilities each year. The percentages of MDR isolates decreased significantly from 2007 to 2009. MDR *P. aeruginosa* strains were obtained mainly from the urinary and respiratory tracts. The total numbers of *Acinetobacter* spp. and MDR isolates obtained at the medical facilities were 94 012 and 558 (0.6% of *Acinetobacter* spp. isolates), respectively. Of these MDR isolates, 82.1% were *Acinetobacter baumannii.* The percentages of MDR isolates increased significantly from 2007 to 2009. One or more MDR *Acinetobacter* spp. isolates were found at approximately 5% of the medical facilities each year. MDR *Acinetobacter* spp. strains were obtained mainly from the respiratory tract.

Conclusions: MDR *P. aeruginosa* was prevalent nationwide in Japan, but its incidence decreased significantly after 2007. MDR *Acinetobacter* spp. is an emerging problem in medical facilities in Japan.

Keywords: nationwide surveillance, retrospective questionnaire, laboratory-based surveillance

48 BACKGROUND

49 The emergence of multidrug-resistant (MDR) *Pseudomonas aeruginosa* and
50 *Acinetobacter baumannii* strains is a serious problem at medical facilities [1-5].
51 Outbreaks of MDR *P. aeruginosa* infection have become problematic in hospitals in
52 various countries [6-10], including Japan[2, 11, 12]. Nosocomial outbreaks of MDR *A.*
53 *baumannii* infection have been major issues in many countries [14], including the UK
54 [15], the USA [16], and Korea [3, 17]. There has been only one previous report of an
55 outbreak of MDR *Acinetobacter* spp. in Japan [18]. Recently, there was an outbreak of
56 *A. baumannii* infection at a university hospital in Fukuoka prefecture, Japan, in 2009.
57 The index case of the outbreak received medical treatment in another country (data not
58 shown). During the present study period, another large outbreak of *A. baumannii*
59 infection occurred at a university hospital in Tokyo, Japan, in 2010.

60 Previously, we reported that the isolation rate of MDR *P. aeruginosa* strains was
61 2.4% in medical facilities in Japan during the period January 2003 through June 2006
62 [19]. The percentages of MDR isolates increased significantly from 2003 to 2005 [19].
63 Here, we performed a surveillance study of clinically isolated MDR *P. aeruginosa* in
64 Japan to determine whether the rate of MDR isolates has increased or decreased at
65 medical facilities in Japan after the first surveillance [19]. We also investigated
66 clinically isolated strains of MDR *Acinetobacter* spp. in Japan. This is the first
67 surveillance study of clinically isolated MDR *Acinetobacter* spp. in Japan.
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74 METHODS

75 Information was gathered by a questionnaire survey. Questionnaires were sent on 16
76 March 2010 to all medical facilities with 200 or more beds in Japan (2 719 facilities). *P.*
77 *aeruginosa* isolates that were resistant to carbapenems (imipenem or meropenem),
78 amikacin, and fluoroquinolones (clinafloxacin, gatifloxacin, levofloxacin, lomefloxacin,
79 norfloxacin, or ofloxacin) were defined as MDR isolates. *P. aeruginosa* isolates that
80 were resistant to two of these drugs were defined as two-drug-resistant (TDR) isolates.
81 *Acinetobacter* spp. isolates that were resistant to carbapenems (imipenem or
82 meropenem), amikacin, and fluoroquinolones (levofloxacin or clinafloxacin) were
83 defined as MDR isolates. Drug resistance was assessed by determining the minimum
84 inhibitory concentration (MIC) in culture medium containing the drugs or by
85 determining the diameter of the growth inhibition zone (DGIZ) on culture agar with the
86 use of drug-sensitivity discs. Breakpoints were determined in accordance with the
87 criteria for MDR isolates specified by the Japanese Nosocomial Infection Surveillance
88 System (JANIS), Japanese Ministry of Health, Labour and Welfare. MIC breakpoints of
89 *P. aeruginosa* for carbapenems (imipenem or meropenem), amikacin, and
90 fluoroquinolones (clinafloxacin, gatifloxacin, levofloxacin, lomefloxacin, norfloxacin,
91 or ofloxacin) were ≥ 16, ≥ 16, ≥ 32, ≥ 4, ≥ 8, ≥ 8, ≥ 8, ≥ 16, and ≥ 8 mg/L, respectively;
92 DGIZ breakpoints for these drugs were ≤ 13, ≤ 13, ≤ 14, ≤ 15, ≤ 14, ≤ 13, ≤ 18, ≤ 12,
93 and ≤ 12 mm, respectively. MIC breakpoints of *Acinetobacter* spp. for carbapenems
94 (imipenem or meropenem), amikacin, and fluoroquinolones (levofloxacin or
95 clinafloxacin) were ≥ 16, ≥ 16, ≥ 64, ≥ 4 and ≥ 8 mg/L, respectively; DGIZ breakpoints
96 for these drugs were ≤ 13, ≤ 13, ≤ 14, ≤ 15, and ≤ 13 mm, respectively.

97 The questionnaire solicited information about: 1) the number of beds; 2) the total

number of *P. aeruginosa* isolates obtained each year with or without TDR or MDR; 3) the number of patients with TDR or MDR *P. aeruginosa* isolates; 4) the tissue sources of the *P. aeruginosa* isolates; 5) the total number of *Acinetobacter* spp. isolates obtained each year with or without MDR; 6) the number of patients with MDR *Acinetobacter* spp. isolates; 7) the tissue sources of *Acinetobacter* spp. isolates; and 8) the number of isolates of each *Acinetobacter* spp. each year when they were identified at the facilities.

Isolates were obtained from inpatients and outpatients with suspected *P. aeruginosa* or *Acinetobacter* spp. infection and subjected to drug susceptibility testing. Repeat testing of single patients was assumed when repeat examinations were ordered. Isolates for analysis in this study were not from the environment, carriers, nonsymptomatic patients, or healthy staff.

Chronological trends in the proportions of TDR and MDR isolates were assessed by Friedman's test. The numbers of isolates from various tissue sources and populations of drug-resistant isolates were analyzed by χ^2 test. In all analyses, $P < 0.0001$ was taken to indicate statistical significance.

The contents of the questionnaires are considered to be exempt from the ethical guidelines for epidemiological research, 5 December 2008, by Ministry of Education, Culture, Sports, Science and Technology and Ministry of Health, Labour and Welfare, Japan.

RESULTS

Completed questionnaires were returned by 771 medical facilities (collection rate, 28.4%) as of 30 April 2010. The average number of beds in these medical facilities was 410 ± 208 (median, 346; range, 50 – 1 494). The investigation was performed from fiscal year 2007 to 2009 (April 2007 through March 2010).

As shown in Table 1, during the study period, a total of 684 982 *P. aeruginosa* isolates were obtained at the 771 medical facilities. The numbers of TDR and MDR isolates were 41 392 (6.0% of the number of *P. aeruginosa* isolates) and 19 911 (2.9% of the number of *P. aeruginosa* isolates), respectively. The total numbers of isolates, as well as the adjusted numbers (number of isolates/1 000 beds), neither increased nor decreased between years during the study period.

The total numbers of TDR isolates and the adjusted numbers decreased gradually from 2007 to 2009 ($P = 0.0003$ and $P = 0.03$, respectively). The percentage of TDR isolates decreased significantly during the study period ($P < 0.0001$). The number of patients with TDR isolates and the number of patients per 1 000 beds/year decreased gradually from 2007 to 2009 ($P = 0.0002$ and $P = 0.03$, respectively).

TDR isolates were obtained at 583 of the 771 facilities (75.6%) during the study period: 521 (67.6%) in 2007, 537 (69.6%) in 2008, and 553 (71.7%) in 2009. The numbers of patients with TDR isolates at each facility from 2007 to 2009 (per 1 000 beds/year) were as follows: median values, 12.0, 12.5, and 11.4, respectively; maximum values, 255.4, 338.7, and 383.9, respectively; and 90th percentile, 53.3, 48.9, and 44.7, respectively.

Facilities with values higher than the 90th percentile were distributed throughout Japan but the distribution was uneven (data not shown). There were no facilities with

146 greater than the 90th percentile in 16 of all prefectures (47) in Japan.

147 The total numbers of MDR isolates and the adjusted numbers decreased gradually
148 from 2007 to 2009 ($P = 0.0001$ and $P = 0.02$, respectively). The percentage of MDR
149 isolates decreased significantly during the study period ($P < 0.0001$). The number of
150 patients with MDR isolates and the number of patients per 1 000 beds/year decreased
151 gradually from 2007 to 2009 ($P = 0.0001$ and $P = 0.003$, respectively). MDR isolates
152 were obtained at 545 of the 771 facilities (70.7%) during the study period: 411(53.3%)
153 in 2007, 405(52.5%) in 2008, and 409 (53.0%) in 2009.

154 The numbers of patients with MDR at each facility from 2007 to 2009 (per 1 000
155 beds/year) were as follows: median values, 1.9, 1.9, and 1.8, respectively; maximum
156 values, 502.5, 275.6, and 373.3, respectively; and 90th percentile values, 21.7, 19.6, and
157 18.7, respectively. The numbers of patients with MDR per 1,000 beds/year in each
158 group of medical facilities categorized by number of beds are shown in Figure 1. In
159 large-scale facilities with ≥ 600 beds and those of medium-scale with 300 – 599 beds,
160 the numbers decreased markedly from 2007 to 2009 ($P = 0.05$ for both groups), whereas
161 no changes were observed in small-scale facilities with < 300 beds ($P = 0.6$).

162 Facilities with values higher than the 90th percentile were distributed throughout
163 Japan but the distribution was uneven (data not shown). There were no facilities with
164 values greater than the 90th percentile in 18 of all 47 prefectures. There were no
165 significant differences in these values between geographic regions (data not shown).

166 The tissue sources and percentages of the total *P. aeruginosa* isolates obtained at the
167 771 medical facilities over the study period and those of TDR and MDR strains are
168 shown in Figure 2. The percentages for each year were similar to those for the entire
169 study period (data not shown). These results indicated that *P. aeruginosa*, including

TDR and MDR isolates, affected mainly the respiratory and urinary tracts. However, it is notable that the percentages of TDR and MDR isolates in the urinary tract were significantly greater than those of the total isolates ($P < 0.0001$) and that the percentages of MDR isolates in the urinary tract surpassed those in the respiratory tract.

A total of 94 012 *Acinetobacter* spp. isolates were obtained from 690 of the 771 medical facilities (89.5%) during the study period. As shown in Table 2, the total numbers of isolates, as well as the adjusted numbers (number of isolates/1 000 beds), decreased slightly during the study period ($P = 0.002$ and $P = 0.002$, respectively).

The total numbers of MDR isolates were 558 (0.6% of the numbers of isolated *Acinetobacter* spp.) during the study period. The total numbers of MDR and the adjusted numbers were small compared to those of *Acinetobacter* spp. in each year, but they increased markedly from 2007 to 2009 ($P = 0.06$ and $P = 0.06$, respectively) (Table 2). The percentage of MDR isolates increased significantly during the study period ($P < 0.0001$). The number of patients with MDR isolates and the number of patients per 1 000 beds/year increased markedly from 2007 to 2009 ($P = 0.2$ and $P = 0.2$, respectively).

MDR isolates were obtained at 92 of the 771 facilities (11.9%) during the study period: 39 (5.1%) facilities in 2007, 37 (4.8%) in 2008, and 49 (6.4%) in 2009. The numbers of patients with MDR isolates at each facility from 2007 to 2009 (per 1 000 beds/year) were as follows: median values, 0 in all of these years; maximum values, 10.7, 31.6, and 18.4, respectively; and 99th percentile values, 4.1, 4.1, and 5.5, respectively.

Facilities with *Acinetobacter* spp. were distributed throughout Japan but the distribution was uneven (data not shown). There were facilities without *Acinetobacter*

194 spp. in 14 of 47 prefectures.

195 Isolates obtained at the 771 medical facilities over the study period and those of
196 MDR isolates are shown in Fig. 2. The percentages for each year were similar to those
197 for the entire study period (data not shown). These results indicated that *Acinetobacter*
198 spp., including MDR isolates, mainly affected the respiratory tract.

199 *Acinetobacter* spp. isolates were identified to the spp. level at 558 medical facilities
200 in 2007, 571 in 2008, and 577 in 2009. A total of 86 834 *Acinetobacter* spp. isolates
201 were identified during the study period. As shown in the upper part of Table 3, 61 794
202 (71.2%) were *A. baumannii*, 8 983 (10.3%) were *A. lwoffii*, and 3 327 (3.8%) were *A.*
203 *calcoaceticus*. The percentages for each year were similar to those for the entire study
204 period.

205 MDR isolates were identified to the spp. level at 34 medical facilities in 2007, 33 in
206 2008, and 45 in 2009. A total of 515 MDR isolates included *A. baumannii* ($n = 423$,
207 82.1%), *A. lwoffii* ($n = 39$, 7.6%), and *A. calcoaceticus* ($n = 4$, 0.8%). As shown in the
208 lower part of Table 3, isolation rates of *A. baumannii* increased significantly during the
209 study period ($P < 0.0001$), and those of *A. lwoffii* and *A. calcoaceticus* decreased each
210 year during the study period.

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DISCUSSION

The results of the present survey showed that the percentages of MDR *P. aeruginosa* decreased significantly during the study period (April 2007 through March 2010). In addition, the number of patients with MDR isolates, as well as the number of patients per bed, decreased markedly. Our previous survey performed during the period January 2003 through 2006 showed that the percentages of MDR isolates increased significantly and the number of patients increased gradually [11]. The number of patients with MDR isolates seemed to decrease since 2007 in medical facilities in Japan, although these two surveys cannot be directly compared to each other because the hospitals surveyed were different between them. The hospitals surveyed in the present survey were institutions with over 200 beds, whereas medical facilities with 500 or more, and regional core hospitals with less than 500 beds were included in the previous study.

During the first survey, outbreaks of MDR *P. aeruginosa* had became a serious problem in medical facilities in Japan [19]. The Ministry of Health, Labour and Welfare of Japan, scientific societies on nosocomial infection controls and infectious diseases provided information about the current situation and infection control measures regarding MDR *P. aeruginosa* in medical settings in Japan. Most outbreaks were controlled by early involvement of management, including staff education, strict isolation of infected patients or carriers of MDR *P. aeruginosa*, active surveillance for drug-resistant *P. aeruginosa* and rigorous contact precautions [13]. Infection control measures especially focused on the handling of urine and urinary catheters, because the first survey suggested the importance of management of patients' urine in the prevention and control of nosocomial MDR *P. aeruginosa* infection in Japan [13].

It is essential to monitor MDR *A. baumannii* in medical facilities and to prepare

infection control measurements for patients with MDR *A. baumannii* in Japan. The present study revealed that the majority of MDR *Acinetobacter* spp. isolated from patients in Japan were *A. baumannii.* Although the number of the MDR isolates was still small, these findings agreed with those in other countries [5].

CONCLUSIONS

A large-scale investigation of multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter* spp. was performed at medical facilities in Japan. MDR *P. aeruginosa* was prevalent nationwide in Japan, but its incidence decreased significantly after 2007. MDR *Acinetobacter* spp. is an emerging problem in medical facilities in Japan.

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ABBREVIATION

JANIS: Japanese Nosocomial Infection Surveillance System; DGIZ: Diameter of the Growth Inhibition Zone; MDR: MultiDrug-Resistant; MIC: Minimum Inhibitory Concentration; TDR: Two-Drug-Resistant

COMPETING INTERESTS

All authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

TK and NMY carried out this study. All authors participated in the design of the study and they read and approved the final manuscript.

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384 **Figure legends**

385 **Figure 1.** Numbers of patients with MDR *P. aeruginosa* per 1,000 beds/year during the
386 study period in each group of medical facilities categorized according to the number of
387 beds.
388 Four sets of three box plots represent the numbers of patients in all facilities, large-scale
389 facilities with ≥ 600 beds, medium-scale facilities with 300 – 599 beds, and small-scale
390 facilities with < 300 beds during the study period. The top and bottom of the boxes
391 indicate the 75th and 50th percentiles, respectively. The ends of the whiskers indicate 90th
392 and 25th percentiles.
393 §§§: $p \leqq 0.01$, §§: $p \leqq 0.05$ for Freedman's test; ***: $p \leqq 0.01$, **: $p \leqq 0.05$ for
394 Wilcoxon's test.
395
396 **Figure 2.** Tissue sources of total *Pseudomonas aeruginosa* isolates, TDR isolates, and
397 MDR isolates obtained during the study period at medical facilities.
398
399 **Figure 3.** Tissue sources of total *Acinetobacter* spp.. isolates and MDR isolates obtained
400 during the study period at medical facilities.
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