Table 2
The combination of mAbs used to construct the 9 prototypes and their performance.

Prototype no.	mAb immobilized to membrane	mAb labbeled with colloidal gold	Detection result[a]		
			Extraction buffer	IMP-1 (100 ng/test)	IMP-1 (1 ng/test)
No.1	1H11-C/F5	1H11-C/F5	−	−	−
No.2	1H11-C/F5	4C9-C/F6	−	+ +	−
No.3	1H11-C/F5	4E7-C/F6	−	+ +	−
No.4	4C9-C/F6	1H11-C/F5	−	+ +	−
No.5	4C9-C/F6	4C9-C/F6	−	+ + +	−
No.6	4C9-C/F6	4E7-C/F6	−	+ + +	+ +
No.7	4E7-C/F6	1H11-C/F5	−	+ +	−
No.8	4E7-C/F6	4C9-C/F6	−	+ + +	+
No.9	4E7-C/F6	4E7-C/F6	−	+ + +	+ +

[a] The intensity of test line was evaluated by visual inspection on four scale of − to +++.

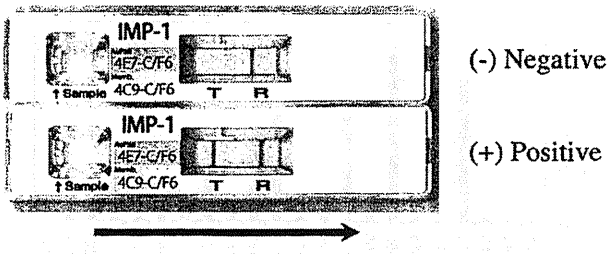


Fig. 1. Immunochromatography developed using mAbs 4E7-C/F6 and 4C9-C/F6. In the case of negative results, a single line appears at the position of the reference line (R) only. In the case of positive results, another line also appears at the position of the test line (T) in addition to the reference line. The arrow indicates the direction of lateral flow.

To investigate the reliability of the assay, all strains were analyzed by PCR detection of bla_{IMP} using specific primer sets as previously described (Poirel et al., 2011).

2.9. Analytical sensitivity testing of the assay

The detection limit of the assay was determined using *P. aeruginosa* NCGM2.S1 strain and purified recombinant IMP-1 protein. The procedure was same as previously described (Kitao et al., 2010).

3. Results

3.1. Development of the assay

In the screening of mAbs generated by hybridoma clones, we found 3 mAbs, 1H11-C/F5, 4E7-C/F6, and 4C9-C/F6, having high reactivity with recombinant IMP-1 from *P. aeruginosa* NCGM2.S1 in ELISA.

To determine the best combination of mAbs to detect of IMP-1, the 9 prototypes of the immunochromatographic assay were constructed using the 3 identified mAbs (Table 2). In the test using 100 ng of IMP-1 per plate, the test line appeared in the prototypes except for prototype no. 1, which consisted of mAb 1H11-C/F6. In the test using 1 ng of IMP-1 per plate, the test line appeared in prototype nos. 6, 8, and 9. The intensity of the test line was highest in prototype no. 6, consisting of 4C9-C/F6 immobilized to the membrane and 4E7-C/F6 labeled with colloidal gold in both tests. Therefore, these mAbs were utilized in the development of a novel immunochromatographic assay for the rapid detection of IMP-1 (Fig. 1).

3.2. Identification of epitopes recognized by mAbs

To determine the region in IMP-1 recognized by 4E7-C/F6 and 4C9-C/F6, we synthesized 22 different 15-mer peptides (≥70% purity) covering the 21 to the 246-end region of IMP-1 without the N-terminal signal sequences (Table 3). In ELISA using these peptides, both mAbs bound to peptides 51–65, 81–95, 131–145, 141–155, and 161–175, as well as rat IgG, prior to immunization, indicating that these peptides are not epitopes (Fig. 2A). The 4C9-C/F6 bound to peptides 101–115, 191–205, and 201–215. The 4E7-C/F6 bound to peptides 111–125, 191–205, and 201–215.

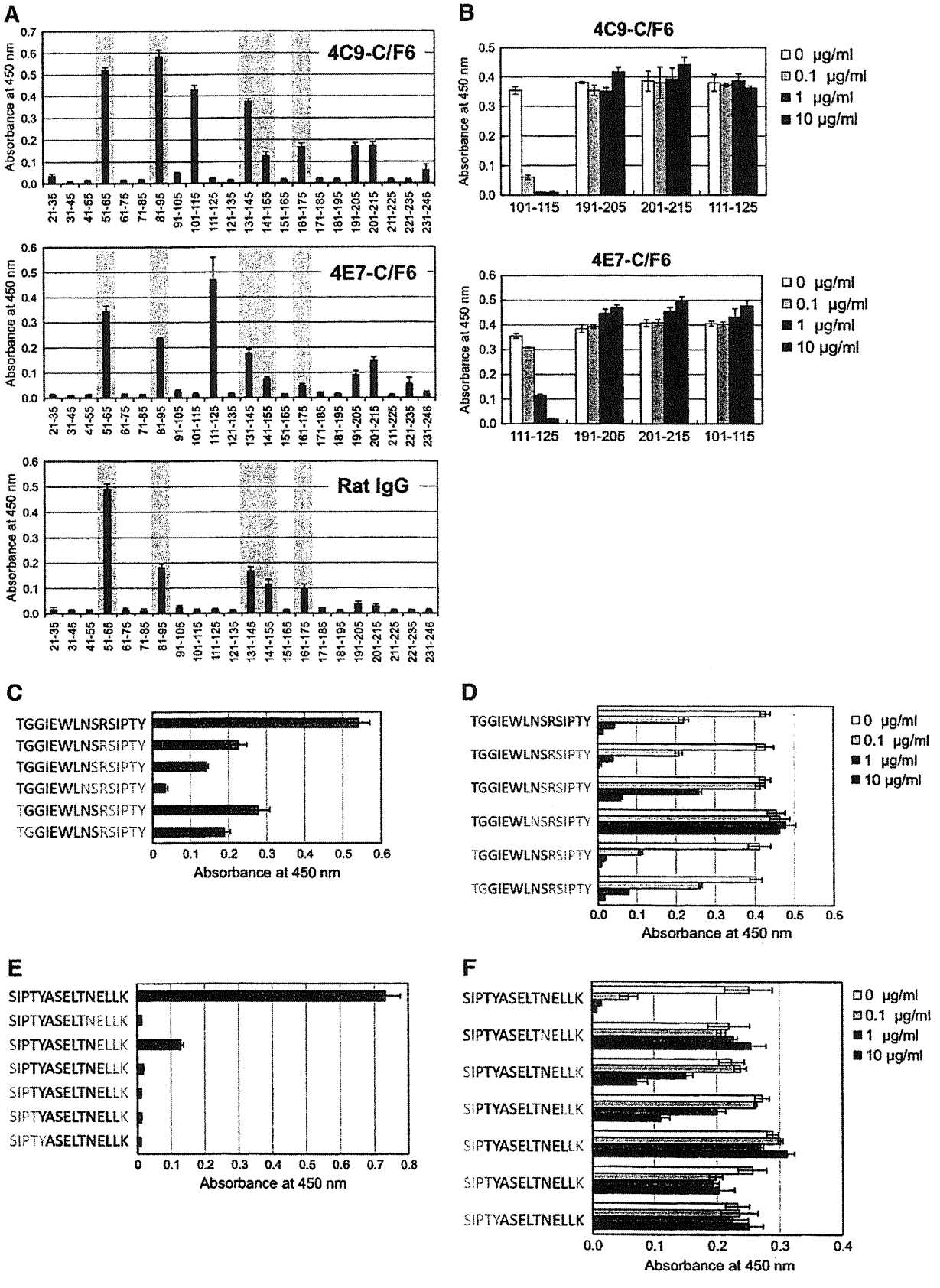
A competitive assay using the candidate peptides was also performed (Fig. 2B). The recognition of IMP-1 by 4C9-C/F6 was significantly inhibited by only peptide 101–115 inhibited in a dose-dependent manner. The recognition of IMP-1 by 4E7-C/F6 was significantly inhibited by only peptide 115–125 in a dose-dependent manner. Due to a sharing a 5-mer amino acid sequence, SIPTY, in both peptide 111–125 and 101–115, the epitope region recognized by both mAbs was assumed to overlap. Peptide 111–125 recognized by 4E7-C/F6 did not inhibit the recognition of IMP-1 by 4C9-C/F6, while peptide 101–115, recognized by 4C9-C/F6 also did not inhibit the recognition of IMP-1 by 4E7-C/F6. These results indicate that the epitopes of 4C9-C/F6 and 4E7-C/F6 were independently included in peptides 101–115 and 111–125, respectively.

To narrow the range of peptide 101–115 or 111–125 required for mAb recognition, 10-mer truncated peptides 101–115 and 111–125 were also synthesized. The 4C9-C/F6 captured 4 kinds of peptide except for TGGIEWL, in addition to peptide 101–115 consisting of TGGIEWLNSRSIPTY (Fig. 2C). These 4 kinds of peptide also inhibited the recognition of IMP-1 by 4C9-C/F6 in a dose-dependent manner (Fig. 2D). These results indicate that TGGIEWLN is a region required for IMP-1 recognition by 4C9-C/F6, and that the C-terminal end of the asparagine residue (N) in the TGGIEWLN peptide plays a key role in antigen recognition. In contrast, the 4E7-C/F6 captured only

Table 3
Peptides used in epitope mapping.

Number	Region in IMP-1	Sequence
1	21–35	SLPDLKIEKLDEGVY
2	31–45	DEGVYVHTSFEEVNG
3	41–55	EEVNGWGVVPKHGLV
4	51–65	KHGLVVLVNAEAYLI
5	61–75	EAYLIDTPFTAKDTE
6	71–85	AKDTEKLVTWFVERG
7	81–95	FVERGYKIKGSISSH
8	91–105	SISSHFHSDSTGGIE
9	101–115	TGGIEWLNSRSIPTY
10	111–125	SIPTYASELTNELLK
11	121–135	NELLKKDGKVQATNS
12	131–145	QATNSFSGVNYWLVK
13	141–155	YWLVKNKIEVFYPGP
14	151–165	FYPGPGHTPDNVVVW
15	161–175	NVVVWLPERKILFGG
16	171–185	ILFGGCFIKPYGLGN
17	181–195	YGLGNLGDANIEAWP
18	191–205	IEAWPKSAKLLKSKY
19	201–215	LKSKYGKAKLVVPSH
20	211–225	VVPSHSEVGDASLLK
21	221–235	ASLLKLTLEQAVKGL
22	231–246	AVKGLNESKKPSKPSN

Fig. 2. Determination of epitope by ELISA. (A, C, and E) The peptides were immobilized onto an EIA plate, and the interaction between peptides and mAb was detected with anti-rat IgG goat antibody. The shaded regions in panel A show the nonspecific regions captured by rat IgG prior to immunization of IMP-1. (B, D, and F) Recombinant IMP-1 was immobilized onto an EIA plate, and the interaction between IMP-1 and mAb was inhibited using serially diluted peptides. In the peptide sequences presented in C, D, E, and F, the truncated amino acids are indicated by gray letters.



by peptide IPTYASELTN of the 6 peptides in addition to peptide 111–125 consisting of SIPTYASELTNELLK (Fig. 2E). The recognition of IMP-1 by 4E7–C/F6 was inhibited by peptides IPTYASELTN and PTYASELTNE in a dose-dependent manner (Fig. 2F). These results indicate that IPTYASELTNE is a region required for IMP-1 recognition by 4E7–C/F6. The peptide PTYASELTNE that inhibited the recognition of IMP-1 by 4E7–C/F6 in Fig. 2F was not captured by 4E7–C/F6 in Fig. 2E, probably due to differences in peptide condition between the 2 assays because the N-terminal end of the peptide was not free in the solution in the experiment in Fig. 2E.

We also analyzed the location of the region recognized by 4C9–C/F6 and 4E7–C/F6 in the three-dimensional structure of IMP-1 (PDB ID: 2DOO) (Kurosaki et al., 2006). The region recognized by both mAbs was found to be located around the active center of IMP-1 consisting of α-β-α motif (Wang et al., 1999) (Fig. 3A). The epitope region showed considerable sequence diversity in other types of MBLs, despite the α–β–α motif was shared (Fig. 3B).

3.3. Potential of the assay for broad reactivity with IMP-type MBLs

To date, 24 subtypes of IMP-type MBL have been submitted to GenBank. The amino acid sequence alignment indicated that the 101–125 region on IMP-1 including the epitopes of 4E7–C/F6 and 4C9–C/F6 shows high similarity among these known IMP-type MBLs (Fig. 4). Moreover, the region among IMP-type MBLs corresponding to the 101–125 region on IMP-1 is of 8 types according to sequence alignment.

To examine whether the 4E7–C/F6 and 4C9–C/F6 could capture the 7 types of 101–125 regions on IMP-1, as well as type 1 epitope (WT), we created IMP mutants. The interactions between IMP mutants and mAbs were analyzed by ELISA (Fig. 5A). The binding ability of 4C9–C/F6 to IMP mutant with type 6 epitope (G102A–R110Q–P113S) decreased by about 30% compared with WT, whereas the binding abilities of 4E7–C/F6 to IMP mutants with type 2 epitope (R110Q), type 3 epitope (E105G), type 4 epitope (R110Q–E122D), type 5 epitope (T101S–R110Q), type 7 epitope (G102A–R110Q), and type 8 epitope (R110Q–E118V) were similar to those of WT. The binding ability of 4E7–C/F6 to IMP mutant with type 6 epitope (G102A–R110Q–P113S) also decreased by about 60% compared with WT, whereas the binding abilities of 4E7–C/F6 to other 6 kinds of IMP mutant were similar to those of WT. Considering that both mAb bound to IMP mutant with type 7 epitope (G102A–R110Q) and that the epitope of 4C9–C/F6 does not include the proline residue at position 113 in IMP-1, the amino acid substitution from proline to serine at position 113 in IMP-1 might affect the stability of the three-dimensional conformation of IMP-1, resulting in a decrease in the binding between the IMP mutant with type 6 epitope (G102A–R110Q–P113S) and mAbs.

The IMP mutants were further tested using the immunochromatographic assay (Fig. 5B). The positive line clearly appeared when 100 ng of protein was used per test. This result indicated that the assay could detect all the IMP mutants as well as WT IMP-1, suggesting that the assay constructed using 4C9–C/F6 and 4E7–C/F6 has the potential to capture all subtypes of IMP-type MBLs.

3.4. Evaluation of the assay

To investigate the reliability of the assay, the developed assay was evaluated using 248 clinical isolates of *P. aeruginosa*. Bacterial colonies grown on BHI (brain heart infusion) agar plates were assessed using the developed assay, and the results were compared with those of PCR detection of bla_{IMP} genes.

As shown in Table 4, the immunochromatographic assay identified the production of IMP-type MBLs in 191 (77%) of the 248 strains tested. These results were fully consistent with those of PCR analysis for bla_{IMP} gene, showing 100% specificity and 100% sensitivity. The developed

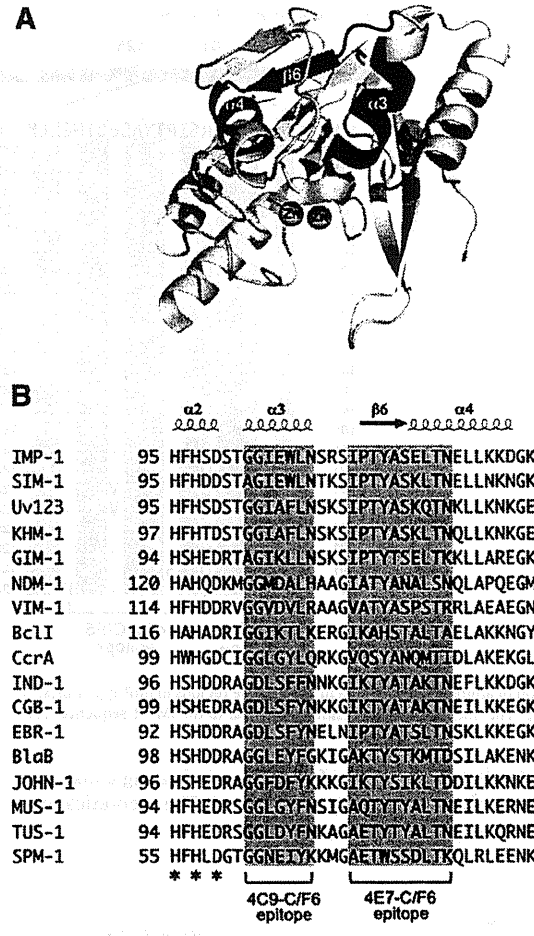


Fig. 3. Sequence alignment of the epitope region among MBLs. (A) Epitope region on the three-dimensional structure of IMP-1. The figure was created with MacPymol software according to the IMP-1 structure (PDB ID: 2DOO) determined by Dr. Kurosaki (Kurosaki et al., 2006). The black part presents the region recognized by 4C9–C/F6 and 4E7–C/F6. The 2 zinc molecules indicate the location of active center. (B) Sequence alignment of the epitope region among MBLs. The GenBank accession numbers of MBLs used in the alignment analysis are as follows: IMP-1, AAB30289; SIM-1, AAX76774; Uvs123, AAP70377; KHM-1, BAH16555; GIM-1, CAF05908; NDM-1, ADP20459; VIM-1, CAB46686; BclI, P04190; CcrA, P25910; IND-1, AAD20273; CGB-1, AAL55263; EBR-1, AAN32638; BlaB, CAA65601; JOHN-1, AAK38324; MUS-1, AAN63647; TUS-1, AAN63648; and SPM-1, CAD37801. Sequence alignment was performed using the ClustalW2 program (Larkin et al., 2007). The two-dimensional information was analyzed with ESPript (Gouet et al., 2003). The parts corresponding to the regions recognized by 4C9–C/F6 and 4E7–C/F6 are shaded.

assay therefore yielded no false-positives or false-negative results, indicating that is reliable. All positive strains showed carbapenem resistance (MIC ≥ 16 μg/ml). The sequencing analyses of DNA amplicons indicated that the 101–125 amino acid region in the IMP-type MBLs produced by the positive strains are type 1, 3, and 4 epitopes (data not shown). Additionally, the assay also identified the production of IMP-type MBLs in 2 strains of *P. putida*, 2 strains of *A. baumannii*, and 4 strains of *A. xylosoxidans*, which were carrying bla_{IMP} genes.

The detection limit of the assay was determined by using diluted culture of *P. aeruginosa* NCGM2.S1 strain and diluted recombinant IMP-1 protein. The intensity of the test line was correlated with the number of NCGM2.S1 in the range of 5.2×10^4 to 5.2×10^5 cfu, indicating a high degree of linearity ($r^2 = 0.9992$) (data not shown). The theoretical detection limit for bacteria was 5.7×10^4 cfu per test. At protein level, the intensity of the test line was correlated with

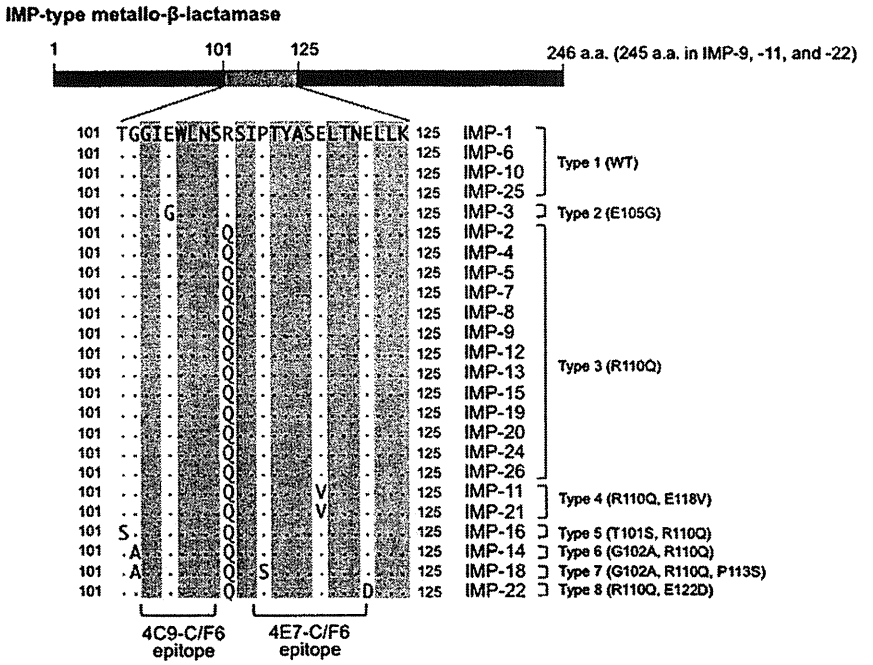


Fig. 4. Comparison of amino acid sequences in the epitope regions of IMP-type MBLs. The regions in the 24 subtypes of IMP-type MBLs corresponding to the 101–125 region in IMP-1 were compared. The dots indicate the residues identical to the IMP-1 sequence. The completely conserved amino acids are shaded. The amino acids are shown as single letters.

the amount of IMP-1 in the range of 0.1–10 ng, indicating a high degree of linearity ($r^2 = 0.9407$) (data not shown). The theoretical detection limit for antigens was 0.3 ng per test.

Taken together, the data strongly suggest that the developed assay meets the requirements for the rapid detection of IMP-type MBLs produced by Gram-negative bacteria in the clinical laboratory.

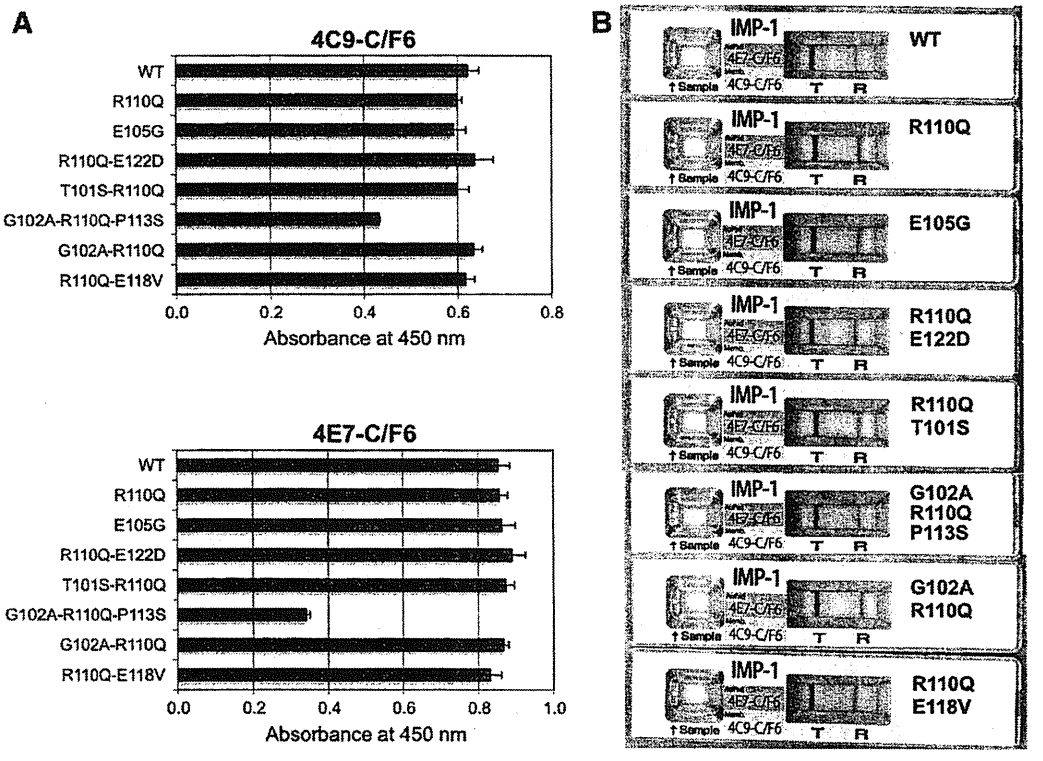


Fig. 5. Evaluation of mAbs and the assay using IMP mutants. (A) Interaction between mAbs and IMP mutants by ELISA. The purified IMP mutant proteins were immobilized onto an EIA plate, and the interaction between mutants and mAb was detected with anti-rat IgG goat antibody. (B) Immunochromatographic detection of IMP mutants. One hundred nanograms of protein of mutant per test were analyzed using the developed assay.

Table 4
Diagnostic performance of the IMP-immunochromatographic assay compared with PCR detection of bla_{IMP}.

Result of PCR detection	No. of isolates ($N=248$)	Result of IMP-immunochromatographic assay		Specificity (%)	Sensitivity (%)
		Positive	Negative		
Positive	191	191	0	100	100
Negative	57	0	57		

4. Discussion

Immunodiagnostic assays rely on specific antigen–antibody interactions for accurate results. ELISA, immunofluorescent antibodies, and immunochromatographic assay are often employed in the diagnosis of several infectious diseases. Of these, immunochromatographic assay is the most commonly used diagnostic method owing to its ease of handling.

In this study, we focused on the production of exogenous enzymes that mediate antibiotic resistance in bacteria, and designed a novel immunochromatographic assay to detect IMP-type MBLs produced by nosocomial pathogens such as *P. aeruginosa*. To our knowledge, this is the first report on an immunological detection assay using antibodies specific for IMP-type MBLs.

The assessment of the assay using clinical isolates of *P. aeruginosa* showed that the assay yielded no false positives and negatives. Additionally, all positive strains were resistant to imipenem (MIC≥16 μg/mL). These results indicate that the immunochromatographic assay developed in this study is a reliable technique for rapid detection of IMP-type MBLs. Although PCR is a reliable detection method, it requires special equipment such as micropipettes, thermal cycler, and agarose gel electrophoresis apparatus. Given that immunochromatographic detection does not require the aforementioned equipment, it may be more suitable for clinical laboratory use compared with PCR detection. Nonetheless, the PCR method will remain to be a powerful technique for the identification of novel mutations in target genes.

The tested samples were prepared using a buffer containing nonionic detergent from bacterial colonies. Given that the developed assay was able to detect the IMP-type MBLs produced by *P. putida*, *A. baumannii*, and *A. xylosoxidans* in addition to *P. aeruginosa*, the sample extraction strategy with detergent is considered efficient for lysing Gram-negative bacterial cells. The antibiotic resistance gene can be transferred across bacteria through plasmids, transposons, and integrons. Indeed, many reports have shown the carriage of bla_{IMP} genes in Gram-negative bacteria. Thus, the data presented in this study support that the assay could be widely adopted in routine work in the screening for carbapenem-resistant bacteria producing IMP-type MBLs.

The analyses using IMP mutants suggested that the assay constructed using 4E7–C/F6 and 4C9–C/F6 has a potential to detect all known IMP-type MBLs. However, it could not detect all carbapenem-resistant bacteria because carbapenem resistance in bacteria is mediated by several classes of MBLs. Although the amino acid sequence analyses have shown that the IMP epitope region recognized by 4E7–C/F6 and 4C9–C/F6 is antigenically distinct from other classes of MBLs such as NDM-1, GIM-1, and SIM-1, further evaluation including clinical trials will be required to elucidate the cross-reactivity of the assay with other types of MBLs.

In recent years, nosocomial infections caused by antibiotic-resistant bacteria have become more complex. The occurrence of MDR nosocomial pathogens, in particular, is currently regarded as a serious medical problem. The determinant of antibiotic resistance in nosocomial bacteria varies with geographical location. The immunochromatographic assay can adopt multiple test lines using various antibodies against different antigens of interest; it therefore has potential for wide use in infection-control measures and epidemiological researches. In Japan, *P. aeruginosa* isolate showing resistance to carbapenem (MIC≥16 μg/ml), amikacin (MIC≥32 μg/ml), and fluoroquinolone (MIC≥4 μg/ml) is defined as MDR *P. aeruginosa* according to the criteria established by the Japanese Ministry of Health, Labor, and Welfare (Kirikae et al., 2008). In our laboratory, further work is in progress to design an immunochromatographic assay recognizing both AAC(6′)-Iae and IMP-type MBLs to survey the prevalence of these enzymes among the MDR clinical strains in Japan.

5. Conclusion

The findings presented in this study indicate that the newly developed assay is a highly sensitive, reliable, easy-to-use, and rapid immunological method for diagnosing the production of IMP-type MBLs in *Pseudomonas*. It will be useful for infection-control measure and a crucial aspect of antimicrobial chemotherapy, and may also useful to investigate epidemiological information about carbapenem-resistant Gram-negative bacteria.

Supplementary materials related to this article can be found online at doi:10.1016/j.mimet.2011.09.011.

Acknowledgment

This study was supported by grants (H21-Shinko-ippan-008) from the Ministry of Health, Labor, and Welfare of Japan. Tohru Miyoshi-Akiyama was supported by a Grant for International Health Research (21A-6) from the Ministry of Health, Labor and Welfare. We thank Mrs. Mayumi Komiya and Mrs. Nobuko Saito for preparation of the antibodies. We are grateful to Dr. Yoshichika Arakawa for providing *A. baumannii* NCB0211-439.

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International Journal of Antimicrobial Agents xxx (2012) xxx–xxx

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Short communication

Emergence of a novel multidrug-resistant *Pseudomonas aeruginosa* strain producing IMP-type metallo-β-lactamases and AAC(6′)-Iae in Japan

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ARTICLE INFO

Article history:
Received 22 November 2011
Accepted 31 January 2012

Keywords:
Metallo-β-lactamase
Aminoglycoside 6′-*N*-acetyltransferase
Multilocus sequence typing
Pseudomonas aeruginosa
Pulsed-field gel electrophoresis

ABSTRACT

The emergence of multidrug-resistant (MDR) *Pseudomonas aeruginosa* isolates producing IMP-type metallo-β-lactamases (MBLs) and aminoglycoside 6′-*N*-acetyltransferase [AAC(6′)-Iae] has become a serious problem in medical settings in Japan. A total of 217 MDR *P. aeruginosa* isolates were obtained from August 2009 to April 2010 from patients at 144 hospitals in Japan, of which 145 (66.8%) were positive for IMP-type MBLs and AAC(6′)-Iae when tested with an immunochromatographic assay. Polymerase chain reaction (PCR) showed that these isolates were also positive for *bla*IMP and *aac(6′)-Iae* genes. When these IMP-type MBL- and AAC(6′)-Iae-producing isolates were analysed by pulsed-field gel electrophoresis (PFGE), two clusters (I and II) were detected. Most of the isolates (88.3%; 128/145) were grouped under cluster I and had multilocus sequence type ST235 and serotype O11, except for one isolate that was ST991 and serotype O3. The isolates were mainly isolated from the urinary tract (82/145; 56.6%) and respiratory tract (58/145; 40.0%). The epidemiological properties of the isolates belonging to cluster I were similar to those of MDR *P. aeruginosa* isolates that have been previously reported in Japan. The remaining 16 isolates belonged to cluster II, had identical PFGE patterns and were multilocus sequence type ST991 and serotype O18; all of these isolates were isolated from the respiratory tract. The properties of isolates belonging to cluster II have not been previously described, indicating that a novel IMP-type MBL- and AAC(6′)-Iae producing *P. aeruginosa* strain is emerging in Japan. Isolates belonging to both clusters were isolated from different parts of the country.

1. Introduction

The emergence of multidrug-resistant (MDR) *Pseudomonas aeruginosa* isolates, which are resistant to all β-lactams, aminoglycosides and fluoroquinolones, is a serious medical problem in Japan. MDR *P. aeruginosa* are defined as strains showing resistance to carbapenems [minimum inhibitory concentration (MIC) ≥ 16 μg/mL], amikacin (AMK) (MIC ≥ 32 μg/mL) and fluoroquinolones (MIC ≥ 4 μg/mL) based on the criteria specified by the Ministry of Health, Labour, and Welfare of Japan [1].

MDR *P. aeruginosa* isolates in Japan frequently produce IMP-type metallo-β-lactamases (MBLs) and aminoglycoside 6′-*N*-acetyltransferase [AAC(6′)-Iae] [2–4]. Therefore, we recently designed immunochromatographic assay kits for the detection of IMP-type MBL- [4] and AAC(6′)-Iae producing *P. aeruginosa* [3].

In this study, 145 isolates of MDR *P. aeruginosa* were randomly obtained from 89 medical settings to perform a nationwide epidemiological study on IMP-type MBL- and AAC(6′)-Iae producing MDR *P. aeruginosa* in Japan.

2. Materials and methods

2.1. Bacterial strains

A total of 217 clinical isolates of *P. aeruginosa* resistant to imipenem (IPM) (MIC ≥ 16 μg/mL), AMK (MIC ≥ 32 μg/mL) and ciprofloxacin (CIP) (MIC ≥ 4 μg/mL) were obtained from 144 hospitals located in 31 of the 47 prefectures in Japan from August 2009 to April 2010. The strains were isolated from the urinary tract (n = 111), respiratory tract (n = 94) and other systems of patients (n = 12). MDR *P. aeruginosa* strains NCGM2.S1 [5] and NCGM1179 were used as reference strains. NCGM1179 [6] strain was one of the 217 isolates.

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0924-8579/$ – see front matter © 2012 Published by Elsevier B.V.
doi:10.1016/j.ijantimicag.2012.01.020

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].

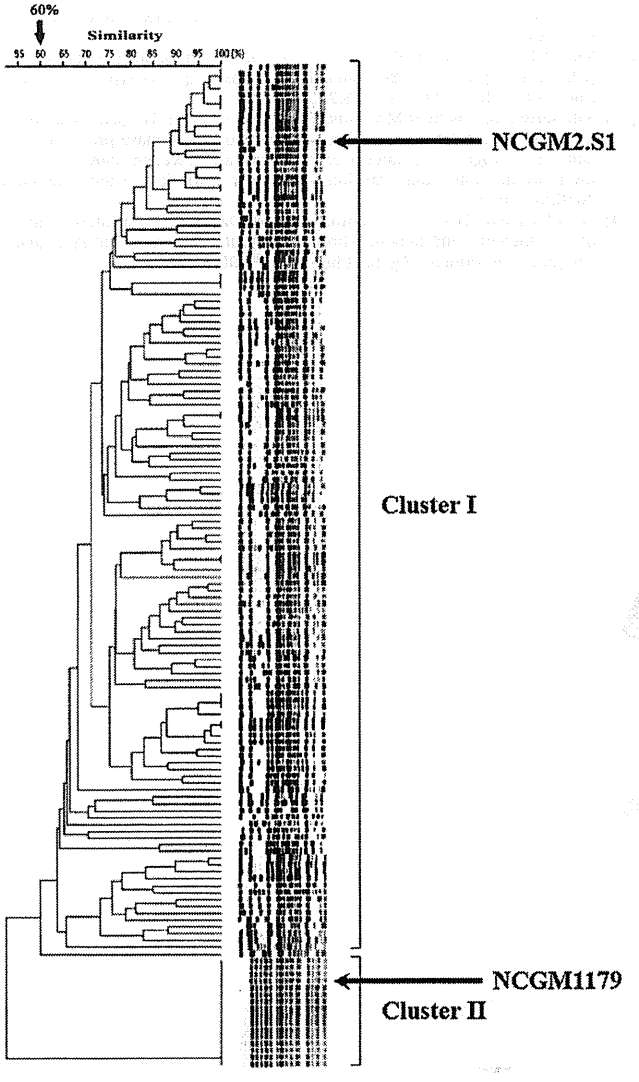

Fig. 1. Dendrogram of pulsed-field gel electrophoresis (PFGE) patterns of 145 multidrug-resistant *Pseudomonas aeruginosa* isolates producing IMP-type metallo-β-lactamases and AAC(6′)-Iae. Two clusters (I and II) were detected. Of the 128 isolates belonging to cluster I, 127 isolates were ST235 and serotype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

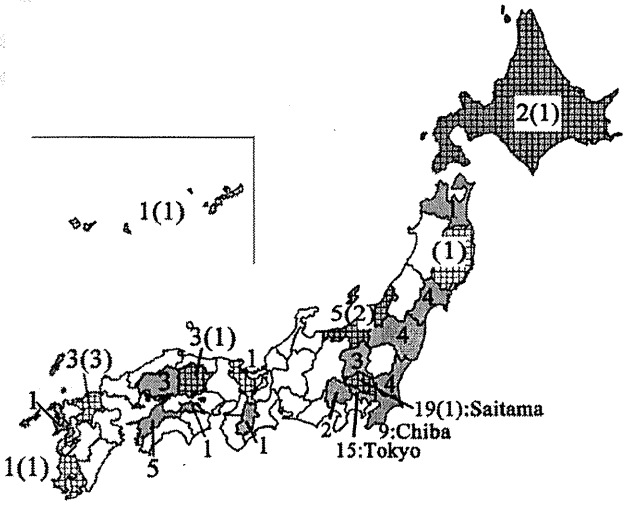

Fig. 2. Geographical distribution of multidrug-resistant (MDR) *Pseudomonas aeruginosa* isolates producing IMP-type metallo-β-lactamases and AAC(6′)-Iae in Japan. Isolates belonging to cluster I (Fig. 1) were obtained from prefectures marked in grey; isolates belonging to cluster II were obtained from 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.

Funding: This study was supported by grants (H21-Shinko-ippan-008 and H22-Shinko-ippan-003) from the Ministry of Health, Labour, and Welfare of Japan. TM-A was supported by a Grant for International Health Research (21A-6) from the Ministry of Health, Labour, and Welfare.

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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Journal Hospital Infection submitted

Organization of nosocomial infection control activities and local networks on infectious disease control in middle-scale hospitals in Japan

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Running title: Infection Control at hospitals in Japan

SUMMARY

Background: In Japan, middle-scale hospitals with 100 to 299 beds account for 40.8% of all medical facilities and for 39.5% of beds at all medical facilities. To date, however, infection control activities at middle-scale hospitals have not been explored.
Aim: To assess nosocomial infection control measures at middle-scale hospitals (100 to 299 beds) throughout Japan, including their infrastructure, activities, and partnership with other local organizations for exchange of information on infections.
Methods: Questionnaires were sent to middle-scale hospitals throughout Japan. Open-ended questions were analyzed qualitatively.
Findings: More than half of the middle-scale hospitals have implemented nosocomial infection control activities, including infection surveillance or infection control rounds, while acknowledging a shortage of infection control staff. In partnering with the general community, public health centers were the most frequently consulted organizations, from which middle-scale hospitals sought information and advice. Middle-scale hospitals expect that public health centers will be more actively involved in nosocomial infection control and local networking.
Conclusion: Improved nosocomial infection control in middle-scale hospitals requires sufficient staffing and a local network, with active participation by public health centers.

Keywords: Middle-scale hospitals, infection control activities

Introduction

All hospitals require nosocomial infection control, with smooth implementation provided by appropriate infrastructure and staffing. Furthermore, local hospitals must share information on infectious diseases[1], particularly during outbreaks, such as during outbreaks of pandemic influenza A and severe acute respiratory syndrome (SARS) viruses.[2,3] Drug-resistant pathogens, such as methicilln-resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant *Pseudomonas aeruginosa*, have been transmitted within and between hospitals,[4,5] and there have been reports describing the spread of community-acquired drug-resistant metallo-β-lactamase producers,[6,7] the spread of MRSA within communities[8] and its introduction into hospitals.[9], and trans-border spread of NDM-1 metallo-β-lactamase producers.[10]

According to information supplied by the Ministry of Health, Labour and Welfare (MHLW) in Japan, most hospitals throughout the country are of medium size, with 100 to 299 beds.[11] Of the 7,655 medical facilities registered as general hospitals in Japan in 2009, 5.5% had ≥500 beds, 11.5% had 300 to 499 beds, 40.8% had 100 to 299 beds, and 42.3% had <100 beds. Moreover, of the 1,343,065 beds in general hospitals in Japan, 21.6% were in hospitals with ≥500 beds; 24.2% in hospitals with 300~499 beds; 39.5% in hospitals with 100~299 beds; and 14.8% in hospitals with < 100 beds. Thus, hospitals with 100 to 299 beds account for 40.8% of medical facilities throughout Japan for 39.5% of beds at all medical facilities. Despite their large share, the infection control activities in middle-scale hospitals have not been fully investigated. We report here the results of a questionnaire-based survey of these hospitals on their nosocomial infection control and information sharing.

Methods

Self-administered questionnaires were sent by poste restante to 2,000 facilities, including 1521 with 100 to 299 beds, and 489 with 200 to 299 beds, randomly selected from the 3,120 general hospitals of these sizes throughout Japan, including 2,336 facilities with 100 to 199 beds and 784 facilities with 200 to 299 beds. The questionnaires were posted on July 30, 2011, and the submission deadline was August 31, 2011.

The questionnaire explored two areas: (1) infection control infrastructure and infection control activities, and (2) sharing of infection information with other organizations. The former area included the availability of information control infrastructure, the presence of information control specialists (and, if present, their profession), the presence of part-time/full-time information control practitioners (ICPs), and the details of any information control activities (e.g. training, infection control rounds, surveillance, monitoring of pathogens, and consultation). The latter area included the availability of organizations from which the hospitals can request guidance, information sources about local infectious diseases, and information gathering and sharing activities. In addition, the questionnaire included items asking about the need for any additional institutional structure and for partnerships with other external organizations that could improve nosocomial infection control. Basic information on each hospital was obtained separately.

Quantitative data were analyzed using IBM SPSS Statistics version 19 (Japan IBM, Tokyo) and compared using the χ^2-test. Open-ended questions were analyzed qualitatively.

In Japan, personnel in the following job categories are considered Infection Control Practitioners (ICP): Certified Infection Control Doctors (CICD), including certified members of the Japanese College of Infection Control Doctors; Certified Nurses in Infection Control (CNIC) and Certified Nurse Specialists in Infection Control Nursing (CNSICN) as certified by the Japanese Nursing Association; Board Certified Pharmacists in Infection Control (BCPIC) and Board Certified Infection Control Pharmacy Specialists (BCICPS), including pharmacists certified by the Japanese Society of Hospital Pharmacists; Infection Control Microbiological

Technologists (ICMT), defined as clinical laboratory technicians certified by the Japanese Society for Clinical Microbiology; Certified Dentists in Infection Control/Certified Infection Control Dentists and Certified Dental Hygienists in Infection Control, consisting of dentists and dental hygienists certified by the Japanese Association for Oral Infectious Diseases; and Advisers on Infection Control and Managers of the Medical Environment, as certified by the Japan Medical Environment Care Association (Advisers on Infection Control are individuals with knowledge of infection control and Managers of Medical Environment are individuals with knowledge of infection control who manage and/or establish a medical environment infrastructure). A "full-time ICP" was defined as a professional assigned to work exclusively on nosocomial infection control, with no other duties, whereas a "part-time ICP" is assigned to nosocomial infection control but also has other duties.

Results

1. Summary of the answers from respondent facilities

Of the 2000 facilities sent questionnaires, 823 returned responses usable for analysis (response rate, 41.2%), including 621 responses (response rate, 40.8%) from facilities with 100 to 199 beds and 202 responses (response rate, 41.3%) from facilities with 200 to 299 beds. The response rates did not differ significantly by facility size. Of the 823 responding facilities, 571 (69.4%) were private medical facilities, 168 (20.4%) were government-run/public medical facilities, 7 (0.8%) were university hospitals, and 73 (8.9%) were facilities of other types.

2. Infection control infrastructure and personnel (Table 1)

Most facilities had an Infection Control Committee (ICC), more than half had an Infection Control Team (ICT), and 40% had infection control specialists. When facilities with 200~299 and 100-199 beds were compared, more of the former had ICT and specialists. The infection control specialists were medical doctors in 31.2% of the facilities and nurses in 14%. Half of the facilities (50.8%) had either full- or part-time ICPs. We found that 8.6% of facilities had full-time ICPs, with the proportion having full-time ICPs higher for facilities with 200-299 beds than with 100-199 beds.

In the 335 facilities with infection control specialists, the average number of specialists was 1.8 (SD=1.4) and was higher in 200-299 than in 100-199 bed facilities (2.0 vs. 1.6 persons). The average number of ICPs in the 391 facilities with full- or part-time ICPs was 3.1 persons (SD=4.7) and was similar in 200-299 and 100-199 bed (3.0 vs. 3.2 persons; data not shown). The average number of full-time ICPs in the 71 medical facilities with full-time ICPs was 1.5 (SD=1.8) and was higher in 100-199 than in 200-299 bed facilities (2.1 vs. 1.1 persons; data not shown).

3. Nosocomial infection control activities

Most facilities had nosocomial infection control training programs, and conducted infection control rounds, surveillance, and monitoring of pathogens. More than half of the facilities conducted infection control consultations (Table 2). Infection control rounds, surveillance, monitoring of pathogens and infection control consultations were implemented at higher rates in larger than in smaller facilities and in those with than without specialists. Rates of implementation of infection control rounds and surveillance were higher in facilities with full-time than with part-time ICPs (Table 2).

More than half of the facilities conducted surveillance of infectious agents, with 58.2% conducting surveillance for drug-resistant bacteria and 29% for specific infectious agents. Rates of surveillance for bloodstream infections (34%), urinary catheterization-related infections (28.7%), hand hygiene (27.1%) and surgical site infections (22.2%) were moderate,

whereas rates of syndromic surveillance for fever (13.8%), respiratory infections (8.7%), and digestive tract infections (5.8%) were lower (data not shown).

4. Community partnership for infection control

We found that 74.8% of these facilities consulted external organizations about nosocomial infection control, including 73.8% of the 100-199 bed and 78.2% of the 200-299 bed facilities. Of the facilities that consulted external organizations, 80.4% reported consulting public health centers (Figure) and 22.9% consulted with university hospitals. Facilities tended to receive information on infectious diseases from public health center, followed by the Medical Association. These patterns remained unchanged when the facilities were asked about their sources for general information or for information on specific infectious diseases or pathogens. The overwhelming majority of these medical facilities reported that public health centers were the recipients of information on infectious diseases. We found that 80.8% of these facilities stated that public health centers should serve as the gateway to the local network, while 46.4% chose the Medical Association.

5. Improvements in nosocomial infection control in responding facilities

The questionnaire included were open-response questions, asking the respondents to comment on the kinds of "internal system" and "partnership with external organization" they considered most necessary to improve nosocomial infection control measures in their own facilities.

(1) Internal system

Responses were obtained from 531 facilities (64.5%). From the responses, 804 codes were extracted and divided into 8 core categories and 19 sub-categories (data not shown). The core category with the largest number of codes (215) was "infection control staffing" and more than half of the comments were related to "infection control specialist", one of its sub-categories. The most frequent comments were "we need a specialist" (95 codes), "we are training a specialist" (40 codes), and "we want to train a specialist but we are having difficulties" (7 codes). The core category with the second largest number of codes was "infection prevention education" (120 codes). There were many codes in the core category "shortages" (116 codes) including the sub-categories of "shortages of human resources" (39 codes) and "shortages of funds" (48 codes).

(2) Partnership with external organizations

Responses were obtained from 349 facilities (42.4%). From the responses, 548 codes were extracted and divided into 3 core categories and 11 sub-categories (data not shown). The core category with the largest number of codes (235) was "working in partnership (networking)" and more than half of the comments were related to the "information-sharing" sub-category (126 code). Other sub-categories included "infection consultation" (62 codes), "education" (31 codes), and "infection control rounds" (16 codes). There were a large number of codes in the core category "partner organization" (158 codes), including 64 related to "public health centers" and 58 to "medical facilities in the neighboring area or the same community". The core category "partnership (including current situation, future plans, and expectations)" also had a large number of codes (155).

Discussion

Our findings indicate that many middle-scale medical facilities in Japan are implementing nosocomial infection control activities. However, the appointment of infection control specialists or full-time ICPs may be necessary for further improvements in implementation rate. The facilities with specialists showed higher implementation rate of these activities,

especially of surveillance. The facilities with full-time ICPs showed extremely high implementation rates of all infection control activities, especially infection control rounds. Previous studies of Japanese teaching hospitals[12] and Japanese teaching and non-teaching hospitals[13] showed that the infection control infrastructure was significantly associated with infection control performance.

Considering the number of beds, it may be appropriate for each middle-scale hospital to appoint one full-time ICP. We found that, although 42.2% of the facilities had part-time ICPs, only 5.7% had full-time ICPs. Results of the Study on the Efficacy of Nosocomial Infection Control (SENIC), performed in the 1970s, indicated that 1 full-time ICP be appointed per 250 beds.[14] A second SENIC performed in the 1990s[15] suggested a standard for acute care hospitals of 3 ICPs per 500 beds.[16] Similarly, the Panel of ICP in the United States has recommended that 1 ICP be appointed for every 100 acutely-ill patients.[17]

In Japan, it will be necessary to establish a system that promotes specialists' involvement in nosocomial infection control activities of middle-scale medical facilities. Our survey revealed that the ICPs in middle-scale medical facilities strongly desired the assignment of specialists to their own facilities. Since, however, many small to middle-scale facilities may be economically unable to appoint any specialist at this stage, it will be necessary to develop community networks allowing these facilities to consult directly with specialists. Beginning in 2012, the MHLW will launch a medical service fee system to support local nosocomial infection control activities, with hospitals staffed with infection control specialists playing the central role. Since Japan has a universal health insurance system, using this system to support nosocomial infection control activities would be effective.

Although, ideally, all middle-scale facilities in Japan should have infection control teams (ICTs), we found that only about half of these facilities had ICTs, made up of physicians, nurses, clinical laboratory technologists, and pharmacists. These ICTs were similar to those in the U.K.[18] A survey of teaching hospitals in Japan showed that 83% had similar ICTs in 2005, suggesting that almost all relatively large-scale acute care hospitals in Japan have ICTs at present.[12]

Middle-scale medical facilities should conduct surveillance for infectious agents. It will therefore be necessary to develop surveillance methods appropriate for these facilities and to increase the participation of these hospitals in nationwide surveillance programs. This is especially important, since we found that the medical facilities without a full-time ICP or infection control specialist have low surveillance implementation rates.

At present, there is no nationwide surveillance system reflecting the situation of nosocomial infections in small to middle-scale medical facilities. The existing continuous surveillance system in Japan, the Japanese Nosocomial Infection Surveillance System (JANIS), suggests that facilities with 200 or more beds participate. It will therefore be necessary to establish a surveillance system that includes small-scale medical facilities.

Community partnership is necessary to prevent nosocomial infections, and public health centers should actively join these networks. The Infectious Disease Law in Japan requires facilities to notify public health centers upon confirmation of the onset of a designated infectious disease in an individual or group of people. Moreover, public health centers inspect medical facilities. These may be the reasons that the largest number of medical facilities reported that public health centers were their local partners in medical cooperation, consulting organizations, sources of information on infectious diseases, and recipients of information on nosocomial infections.

This study has several limitations. First, the survey response rate was not high (41.2%), suggesting that the data we collected was not necessarily representative of small to middle-scale medical facilities in Japan. Second, nosocomial infection control activities remain unclear at medical facilities with fewer than 100 beds. Continued monitoring of

infection control activities at medical facilities in Japan is needed to improve them.

Acknowledgments

We thank Dr. Hiroshi Yoshikura (National Institute for Infectious Diseases, Tokyo, Japan) for discussion and advice, and Ms. Kayo Shimada (Research Institute, National Center of Global Health and Medicine, Tokyo, Japan) for technical assistance.

Conflict of interest

None declared.

Funding

This study was supported by Health Sciences Research Grants from the Ministry of Health, Labor, and Welfare Japan (H22-SINKO-003).

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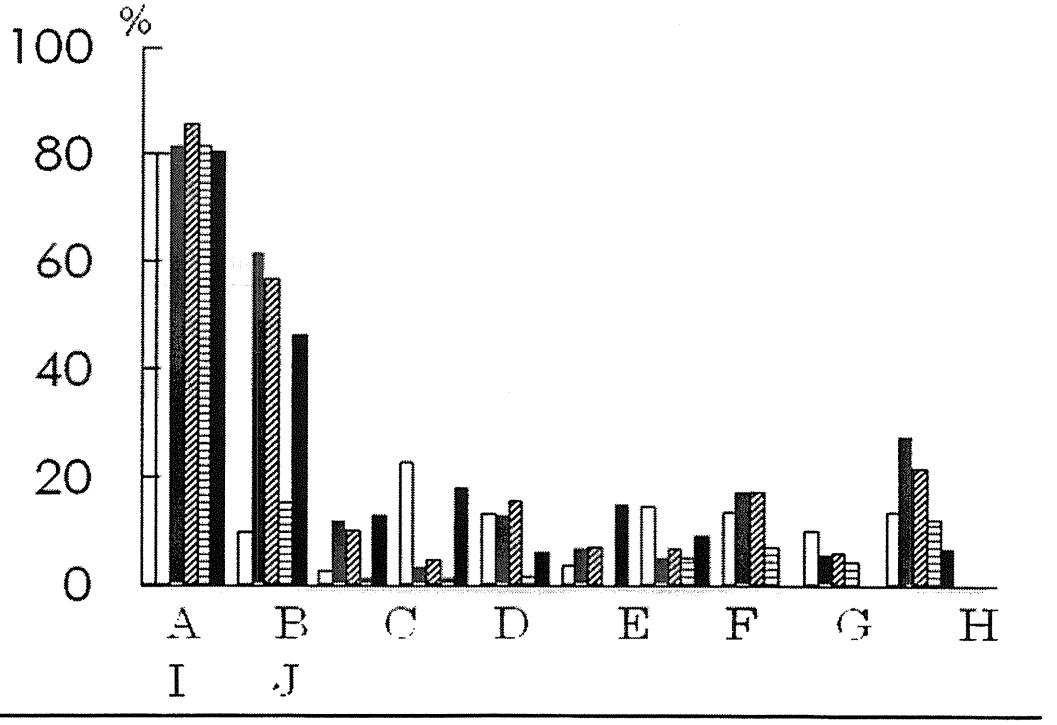

Legends

Figure. Community partnership for infection control

Hospitals (%) consulted external organizations about nosocomial infections (open bars: n=616), received information about infectious diseases in general from external organizations (gray bars: n=752) and information on specific infectious diseases and pathogens (diagonal line bars: n=749), released information about infectious diseases occurring at their own facilities (cross bars: n=275), and replied to public inquiries about which organizations should serve as gateways to local networks (closed bars: n=823). A, public health centers; B, the Medical Association; C, the Hospital Association; D, university hospitals; E, clinical laboratories; F, the Nursing Association; and G, affiliated hospitals.

Table1. Infrastructure and relevant human resources (%)

Hospital sizes		total (n=823)	100-199 beds (n=621)	200-299 beds (n=202)	*p* value
Organization	ICC	99.5	99.5	99.5	
(multiple answers allowed)	ICT	56.1	50.2	74.3	***
Infection control specialist		40.8	33.5	63.4	***
Title (multiple answers allowed)	CICD	31.2	26.1	47	***
	CNIC/CNSICN	14	8.9	29.7	***
	BCPIC/BCICPS	3.3	1.9	7.4	***
	ICMT	1.3	1	2.5	
	CDIC/CDHIC	0.2	0.2	0.5	
	AIC/MME	4.1	3.9	5	
Infection Control Practitioner (ICP)	full/part-time	50.8	47.5	60.4	***
(multiple answers allowed)	full time	8.6	5	19.8	***
	part time	45.1	44.1	48	

Table 2. Infection control activities (%)

Hospital sizes	total (n=823)	100-199 beds (n=621)	200-299 beds (n=202)	*p* value
Training program (at same facility)	99.2	99.2	99.1	
Infection control rounds	76.2	73.9	83.2	***
Surveillance	71.2	67	84.2	***
Monitoring of pathogens	79.2	77	86.1	***
Consultations	63.4	59.9	74.3	***
With/without specialist		with specialist (n=338)	without specialist (n=485)	*p* value
Training program (at same facility)		99.1	98.1	
Infection control rounds		79.6	73.8	*
Surveillance		76.9	67.2	***
Monitoring of pathogens		80.2	77.3	
Consultations		66.9	61	
With/without full/part time ICP	full time (n=47)	part time (n=347)	non (n=405)	*p* value
Training program (at same facility)	100	98.6	96.5	
Infection control rounds	95.7	76.9	72.6	***
Surveillance	85.1	68.9	71.1	
Monitoring of pathogens	83	77.2	77.8	
Consultations	78.7	64.8	59	**

地域ネットワークによる感染対策

Regional Infection Control Network in Hospitals

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1. はじめに

我が国では従来，医療施設における MRSA，ESBLs 産生菌，ときに MDRP や VRE などの薬剤耐性菌による感染症が問題となっている．基幹病院では，医師や看護師，薬剤師，検査技師などによる ICT (Infection Control Team) の活動により，これらの施設では近年，急速に感染対策が確立されつつあるということができる．しかしながら，医療施設によっては，必ずしもこれらの人的ならびに物的リソースを有していない施設もあることから，基幹病院と同様の対策を行うことは困難であることも多い．感染症は伝播するという特殊性により，基幹病院だけでなく地域全体における感染対策の充実が必要不可欠であり，近年，様々な地域で院内感染地域ネットワークが構築されている．平成 23 年 6 月 17 日に発出された厚労省医政局指導課長通知「医療機関等における院内感染対策について」においても，“医療機関間の連携について”，“地方自治体の役割”，“アウトブレイク時の対応”といった更なる地域ネットワークの構築が記載されており，今後より一層の活動が期待されている．

2. 我が国における医療背景

我が国には現在，約 9,000 の病院，約 90,000 の診療所があり，日々多くの診療を行い，地域における欠かせない社会基盤となっている．そのいずれも良質な医療の提供が求められており，感染対策もそのなかで重要な位置を占めるとともに，その充実が求められている．現在，医師ではインフェクションコントロールドクター (ICD)，感染症専門医・指導医，抗菌化学療法指導医・専門医，薬剤師では感染制御認定薬剤師や抗菌化学療法認定薬剤師，看護師では感染管理認定看護師 (ICN)，検査技師では感染制御認定検査技師 (ICMT) など，様々な資格認定や教育制度がある．しかしながら実際には，我が国では 50~100 床の病床数の病院が最も多く，自施設で研修を行うことは困難で，いずれも専門知識を有する職員として感染対策に関わる人的リソースは大きく不足しているのが現状である．

加えて，感染対策には多くの物的リソースも必要とされる．すなわち，速乾性アルコール手指消毒薬を含めた各種消毒薬や清掃物品，手袋・マスク・ガウンなどの個人防護具，職業感染予防のための各種ワクチン，手洗い場や空調などの施設整備，医療廃棄物の処理費用や教育研修費など様々な経費が必要であり，また採算部門となることが困難な院内の微生物検査室や抗菌薬の TDM(薬物血中濃度モニタリング) などにも，多くの資源が必要である．求められる感染対策が年々多岐にわたり高度化するにもかかわらず，医業収益が医療施設それぞれで大きく異なる現状においては，施設によっては感染対策が大きな負担となるとともに，施設による感染対策の現状に差異がみられることも考えられる．

3. 医療施設に応じた感染対策

感染症の発生は，病原体と宿主の関係が重要であることから，医療施設や宿主状態によって対応する感染症は大き