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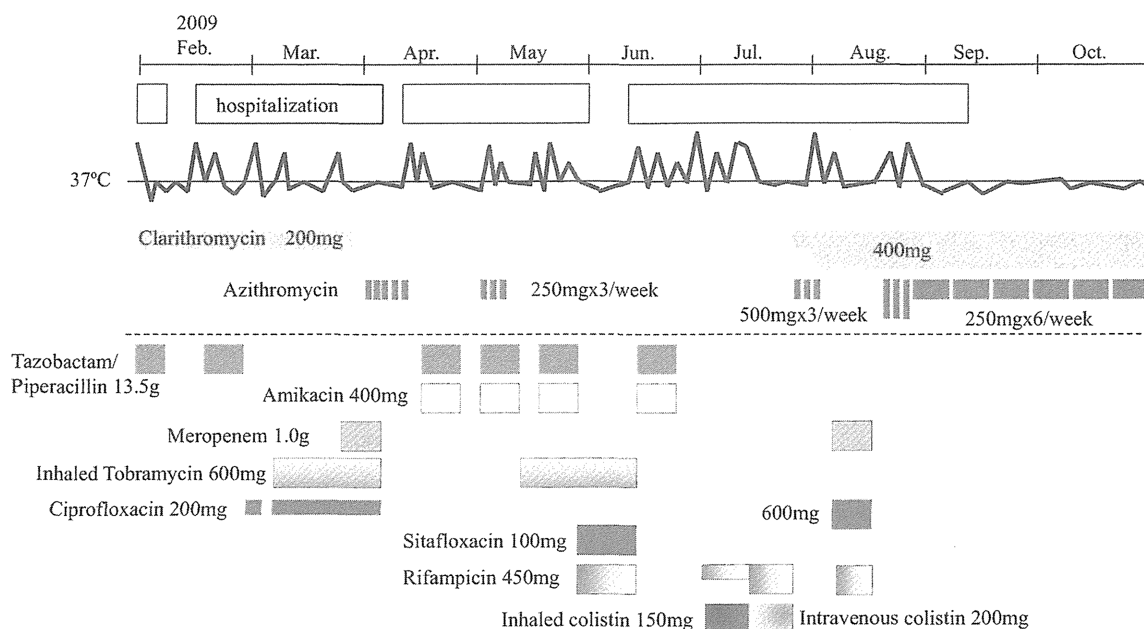

Figure 2. **The clinical course of the patient. Fever was not reduced by either single low-dose clarithromycin or azithromycin, but the patient did markedly respond to the combination of both macrolides.**

Table 1. **Minimum Inhibitory Concentration (MIC) of Antimicrobials against *Pseudomonas aeruginosa***

	2008 Dec.	2009 Jan.	Feb.	May	2010 Jan.	Aug.	2011 Jan.	Apr.	Sep.	Dec.
P. aeruginosa (CFU/mL)	2×10^6	1×10^5	1×10^7	6×10^7	1×10^7	1×10^6	2×10^7	5×10^7	2×10^7	5×10^6
PIPC	≤0.5	1.0	≥32	≥32	≥32	≥32	≥32	16.0	≥32	≥32
CAZ	1.0	1.0	≥32	≥32	≥32	16.0	8.0	4.0	≥32	16.0
CFPM	≤0.5	8.0	≥32	≥32	≥32	16.0	8.0	4.0	16.0	8.0
AZT	≤0.5	≤0.5	≥32	≥32	≥32	16.0	≤0.5	4.0	16.0	8.0
MEPM	≤0.5	≤0.5	1.0	8.0	4.0	2.0	2.0	2.0	2.0	1.0
GM	2.0	2.0	4.0	4.0	2.0	2.0	2.0	1.0	1.0	2.0
CPFX	≤ 0.5	≤ 0.5	≤ 0.5	1.0	2.0	2.0	2.0	2.0	2.0	2.0

PIPC: piperacillin, CAZ: ceftazidime, CFPM: cefepime, AZT: aztreonam, MEPM: meropenem, GM: gentamicin, CPFX:ciprofloxacin, MIC: μg/mL

integrally and lead to a better outcome in patients with chronic bacterial pulmonary infection.

It is also well established that 14- and 15-membered macrolides possess these immunomodulatory effects but not 16-membered macrolides (10). We investigated the differences in these effects between CAM, a 14-membered macrolide, and AZM, a 15-membered macrolide. We discovered that AZM and CAM exerted different immunomodulatory effects in murine dendritic cells (11). AZM increased interleukin (IL)-1 production and inhibited the excess immune response, whereas CAM inhibited IL-2 and IL-6 production; thus, these macrolides possess anti-inflammatory activities. Fukuda et al. reported that pneumolysin activity was inhibited by CAM rather than by AZM, although both the macrolides inhibited hemolytic activity (12). Moreover, Morinaga et al. reported the presence of a correlation between macrolides and MUC5AC production in bronchial epithelial cells *in vitro* (13). CAM, AZM, and telithromycin (TEL) inhibited the production of MUC5AC *in vitro*; however, CAM and TEL, but not AZM, significantly inhibited the activity of nuclear factor-κB (NF-κB). On the other hand, Araki et al. showed that AZM exerted stronger effects on the inhibition of MUC5AC expression induced by *Haemophilus influenzae* than did CAM (14).

Since two major macrolides, CAM and AZM indicated various and excellent immunomodulating effects, treatment with both AZM and CAM may be considered in cases of refractory chronic pulmonary airway infections. A review of the clinical course of the present patient indicated the apparent inhibition of exacerbation by the combined treatment with AZM and CAM, although a single administration of CAM and AZM did not reduce exacerbation. The dose of CAM was decreased from 400 mg/day to 200 mg/day 1 year after the initiation of the combination therapy with

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AZM and CAM, because the condition of the patient improved. However, fever, cough, and sputum production were observed again, and the dose of CAM was again increased to 400 mg/day. The reason we increased the amount of CAM was that immunomodulating effects of macrolides have been proven to be increased depending on their amount *in vivo* experiment (12, 13). The clinical symptoms gradually improved. Thus, administration of low-dose CAM was not effective in the present case. Additionally, the administration of AZM was also considered given as 500 mg/day for every other day at first, however, we switched to 250 mg/day six days a week due to the hepatotoxicity and fever that occurred during administration of 500 mg/day every other day. Many of the studies cited above indicated that the immunomodulatory effects of macrolides are dose dependent, and this may have been reflected in the present case as well.

Through *in vivo* study, we have proved the efficacy of the combination use of AZM and CAM in choric pulmonary *P. aeruginosa* infection mice model previously established by Yanagihara et al. (15). We compared the bacterial burden in lung tissues between 5 treatment groups: (a) low-dose CAM (20 $mg{\cdot}kg^{-1}{\cdot}day^{-1}$), (b) high-dose CAM (200 $mg{\cdot}kg^{-1}{\cdot}day^{-1}$), (c) low-dose AZM (20 $mg{\cdot}kg^{-1}{\cdot}day^{-1}$), (d) high-dose AZM (200 $mg{\cdot}kg^{-1}{\cdot}day^{-1}$), and (e) low-dose of CAM and AZM (20 $mg{\cdot}kg^{-1}{\cdot}day^{-1}$). The bacterial burden in the lung tissues was apparently lower in the 2 groups that received high-dose macrolides and in the group that received a combination of AZM and CAM compared to the groups that received low-dose macrolides (data not shown). Further *in vivo* or *in vitro* experiments are warranted.

Drug susceptibility of *P. aeruginosa* in the present case became more resistant during the repeated usage of antimicrobial agents for repeated exacerbation events. *P. aeruginosa* tended to be more sensitive to antimicrobial agents after the initiation of combined therapy with CAM and AZM. This finding might be explained by the significant reduction in exacerbation with a decrease in the use of antimicrobial agents, and it highlights another benefit of the novel combination of macrolides.

Conclusion

We encountered a severe refractory chronic pulmonary infection case that was successfully controlled by the combination use of CAM and AZM. Our findings indicate that combined therapy with macrolides can be considered as a treatment option for refractory chronic pulmonary infection.

Author's disclosure of potential Conflicts of Interest (COI).

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Antimicrobial susceptibility and molecular characteristics of 857 methicillin-resistant *Staphylococcus aureus* isolates from 16 medical centers in Japan (2008–2009): nationwide survey of community-acquired and nosocomial MRSA

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Abstract

This study is a nationwide survey of all clinical methicillin-resistant *Staphylococcus aureus* (MRSA) isolates, including community-acquired MRSA (CA-MRSA), in Japan. A total of 857 MRSA clinical isolates were collected from the 16 institutions throughout Japan that participated in the survey (2008–2009). The drug susceptibility and staphylococcal cassette chromosome *mec* (SCC*mec*) typing and the presence of specific pathogenic genes were evaluated. The isolates comprised SCC*mec* type II (73.6%), type IV (20%), and type I (6%). The percentage of SCC*mec* type IV isolates was significantly higher in outpatients than in inpatients. Most of the isolated strains were sensitive to vancomycin (VCM, MIC ≤2 μg/mL), linezolid (MIC ≤4 μg/mL), and teicoplanin (MIC ≤8 μg/mL). Although most strains were sensitive to VCM, the MIC value of VCM for SCC*mec* type II strains was higher than that for SCC*mec* type IV strains. Only 4 (2.3%) of 171 SCC*mec* type IV strains were Panton–Valentine leukocidin (*lukS/F-PV*)–positive. Thus, this result indicates a unique feature of SCC*mec* type IV

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strains in Japan. The information in this study not only is important in terms of local public health but will also contribute to an understanding of epidemic clones of CA-MRSA.
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Keywords: MRSA; CA-MRSA; Nationwide survey; Antimicrobial susceptibility; Molecular characteristic

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) causes a wide range of infections in health care settings and community environments. The prevalence of MRSA in Japan is one of the highest (about 60%) among developed countries (Niki et al., 2011). In the past, MRSA was known as a nosocomial pathogen, and most MRSA cases were categorized as hospital-acquired MRSA (HA-MRSA) infection. However, community-acquired MRSA (CA-MRSA) infection has been reported frequently in the United States and occasionally in Japan (Bonnstetter et al., 2007; Ito et al., 2008).

Staphylococcal cassette chromosome *mec* (SCC*mec*) typing is a method for analysis of SCC*mec*, a mobile genetic element containing the *mecA* gene, which encodes methicillin resistance. Eight major SCC*mec* types have been described to date. SCC*mec* types I, II, and III are usually associated with HA-MRSA, whereas types IV, V, and VI are generally characteristic of CA-MRSA. In the United States, the USA300 clone of CA-MRSA, which was reported by the Centers for Disease Control and Prevention, represents a major health problem. Most of the USA300 clones carry the Panton–Valentine leukocidin (*lukS/F-PV*) genes, which produce cytotoxins that cause leukocyte destruction and tissue necrosis (Bonnstetter et al., 2007; Genestier et al., 2005). We previously showed that there was a transition in SCC*mec* types of MRSA isolated from pus over a 10-year period from 2000 to 2009 at Nagasaki University Hospital (Motoshima et al., 2010). The number of isolates of SCC*mec* type II that is usually associated with HA-MRSA decreased and that of SCC*mec* type IV that is associated with CA-MRSA increased.

There has not been any nationwide Japanese MRSA survey that covers all clinical isolates including both HA-MRSA and CA-MRSA. In particular, the detection frequency of CA-MRSA is still unknown. To determine the status of MRSA infections in Japan, we evaluated their SCC*mec* type, which we classified into 4 major SCC*mec* types, their pathogenic genes, and their antimicrobial susceptibility.

2. Materials and methods

2.1. Bacterial isolates

MRSA were collected from 16 institutions throughout Japan that participated in the survey. A total of 857 MRSA clinical isolates were collected from 2 groups. One group was composed of infection control/infectious disease departments (infection control/infectious disease group) that were mainly responsible for collecting HA-MRSA isolates, and the second group was composed of dermatologists (dermatology group) who were mainly responsible for collecting CA-MRSA isolates during the period between 2008 and 2009 (805 strains from 10 sites in the infection control/infectious disease group, 52 strains from 6 sites in the dermatology group).

2.2. Antimicrobial susceptibility testing

All isolates were tested for antimicrobial susceptibility using the broth microdilution method as described by the Clinical and Laboratory Standards Institute's (CLSI) *Performance Standards for Antimicrobial Susceptibility Testing: Eighteenth Informational Supplement M100-S18* (CLSI, Wayne, PA, USA, 2008). As arbekacin was not referenced by CLSI, it was determined according to standard Japanese methods (MICs of ≤4 μg/mL were susceptible, MICs = 8 were intermediate resistant, and MICs of ≥16 μg/mL were resistant). MRSA was elevated in terms of the MIC value for oxacillin (MPIPC), imipenem (IPM), gentamicin (GM), erythromycin (EM), levofloxacin (LVFX), clindamycin (CLDM), trimethoprim/sulfamethoxazole (S/T), minocycline (MINO), linezolid (LZD), vancomycin (VCM), teicoplanin (TEIC), and arbekacin (ABK). Strains with an MPIPC MIC of ≥4 μg/mL were considered to be MRSA strains.

2.3. Detection of toxin genes and genotyping of HA-MRSA and CA-MRSA

We used a previously reported multiplex real-time polymerase chain reaction (PCR) method to distinguish SCC*mec* types and to detect toxin genes (Motoshima et al.,

Table 1
Patient characteristics and isolation sites

Age, mean ± SD (range)	59.6 ± 24.8 (0–99)
Male (%)	66.3
Female (%)	33.6
Inpatient (%)	86.3
Outpatient (%)	13.7
Isolation sites (%)	
Pus	33.1
Blood	27.4
Sputum	23.9
Urine	4.6
Pharyngeal swab	2.9
Abdominal area	2.8
Otorrhea	2.1
Others	3.2

Table 2
Analysis of pathogenic genes of isolates

Pathogenic genes	All strains (%)	SCC*mec* II (%)	SCC*mec* IV (%)	*P* value (χ^2)
tst	465/857 (54.3)	384/631 (60.9)	76/171 (44.4)	0.0001
sec	464/857 (54.1)	386/631 (61.2)	73/171 (42.7)	<0.0001
tst + *sec*	450/857 (52.5)	374/631 (59.3)	71/171 (41.5)	<0.0001
etb	10/857 (1.2)	6/631 (1.0)	4/171 (2.3)	0.1467
lukS/F-PV	6/857 (0.7)	0/631 (0)	4/171 (2.3)	0.0020

2010). In brief, a PCR assay was performed to amplify a total of 10 genes in the same run. Target genes assayed were SCC*mec* type I–IV, *nuc*, *mecA*, *vanA*, *tst*, *sec*, exfoliative toxin type b (*etb*), and *lukS/F-PV*. *Nuc* is a nuclease gene that is used for identification of *S. aureus* and *vanA* is a gene that encodes resistance to VCM. Strains in which the *mecA* gene were detected were considered to be MRSA strains.

3. Results

3.1. Clinical background

A total of 857 clinical isolates were investigated in this study, including 740 isolates from hospital patients and 117 isolates from outpatients. The major specimen types from which MRSA was isolated were pus (33.1%), blood (27.4%), and sputum (23.9%) (Table 1).

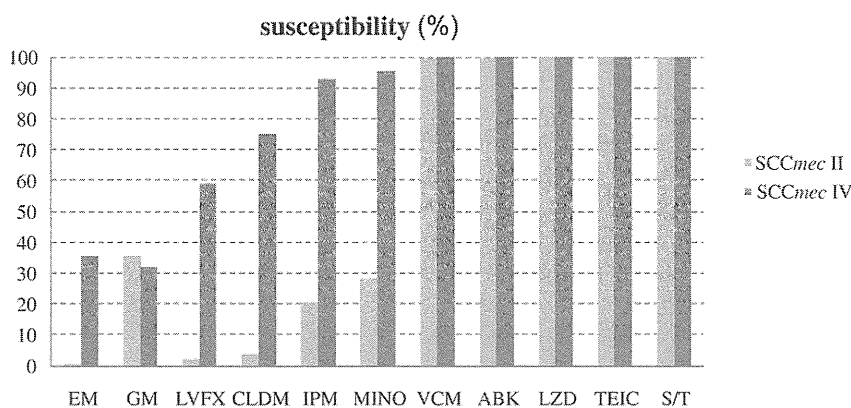
3.2. Distribution of SCCmec types and pathogenic genes

The majority of Japanese MRSA strains were SCC*mec* type II. The combined isolates from all sources comprised SCC*mec* type II (73.6%), type IV (20%), and type I (6%) strains. The percentage of SCC*mec* type IV strains was significantly higher in outpatients than in inpatients (P = 0.0003). Thus, 17.8% of the strains from inpatients were SCC*mec* type IV strains and 75.8% were SCC*mec* type II strains, whereas 33.3% of the strains from outpatients were SCC*mec* type IV strains and 59.8% were SCC*mec* type II strains.

More than half of the total MRSA strains had *tst* and *sec* genes, and a few strains had the *etb* and/or *lukS/F-PV* genes. A higher percentage of SCC*mec* type II than type IV strains were positive for *tst* and/or *sec*. Only 4 of 171 SCC*mec* type IV strains were *lukS/F-PV*-positive (Table 2). No isolates were *vanA*-positive in this study.

3.3. Antimicrobial susceptibility testing

The antimicrobial susceptibility of the strains is shown in Fig. 1. All strains were sensitive to LZD, TEIC, and S/T. Although most strains were sensitive to VCM, 1 strain showed a decreased susceptibility to VCM (MIC = 4 μg/mL). The percentages of SCC*mec* type II and type IV



	Susceptibility (%)										
	EM	**GM**	**LVFX**	**CLDM**	**IPM**	**MINO**	**VCM**	**ABK**	**LZD**	**TEIC**	**S/T**
SCC*mec* II	0.3	35.7	2.2	3.8	20.4	28.2	99.8	99.8	100	100	100
SCC*mec* IV	35.7	31.6	59.1	75.4	93.0	95.3	100	100	100	100	100

P-value(χ^2): < 0.0001= EM, LVFX, CLDM, IPM, MINO

Fig. 1. Antimicrobial susceptibility testing. All strains were sensitive to LZD, TEIC, and S/T. Although most strains were sensitive to VCM, 1 strain showed a decreased susceptibility to VCM (MIC = 4 μg/mL). MICs of several antibiotics were different for SCC*mec* type II and type IV strains.

strains for which the MIC of VCM was ≤0.5 μg/mL were 15.7% and 32.2%, respectively; the percentages for which this MIC was 1 μg/mL were 79.6% and 64.9%, respectively; and the percentages for which this MIC was 2 μg/mL were 4.6% and 2.9%, respectively.

The MIC value of IPM was also different for SCC*mec* type II and type IV strains. Thus, 93% of SCC*mec* type IV but only 8.2% of type II isolates displayed MICs of <2 μg/mL for IPM.

The MIC value of MPIPC was ≥128 for 76.5% of the SCC*mec* type II strains. In contrast, MICs of MPIPC ≥128 were detected for only 3.5% of the type IV strains. Similarly, SCC*mec* type II strains had higher resistance rates to EM, LVFX, CLDM, and MINO than SCC*mec* type IV strains.

4. Discussion

CA-MRSA isolates generally carry the SCC*mec* type IV element, which is much smaller than the SCC*mec* type I, II, and III elements (Francois et al., 2004). Therefore, the SCC*mec* type IV element is thought to be prone to insertion into the MSSA genome in a community setting. In previous Japanese studies, about 4% of MRSA clones were classified as SCC*mec* type IV (Chongtrakool et al., 2006; Zaraket et al., 2007).

However, in our study, the percentage of SCC*mec* type IV was 20.0%. This percentage was slightly higher in outpatients than in inpatients. These data may suggest that CA-MRSA has increased in Japan in recent years.

A previous study of HA-MRSA strains in Japan showed that many of these strains were SCC*mec* type II and that a large number of these strains exhibited coexistence of *tst*, *sec,* and *sel* genes (Ohkura et al., 2009). These results were similar to the results of our study in that a high number of strains displayed coexisting *tst* and *sec* genes. However, Ohkura et al. (2009) reported that *tst* and *sec* coexisted in more than 80% of these strains, whereas in our study only approximately 60% of SCC*mec* type II strains coexpressed these genes. This difference is presumably caused by differences in the collection period and in the institutions in which the samples were collected.

There have been few surveys of CA-MRSA in Japan, especially surveys that include adults. The CA-MRSA strains that were analyzed from children in 2003 and 2004 were mostly *lukS/F-PV*–negative strains, which expressed combinations of *eta* and *etb,* or *tst* and *etb,* or expressed new types of the *spa* (staphylococcal protein A) gene (Takizawa et al., 2005). In the United States and Europe, the USA300 strain, which carries SCC*mec* type IV and the *lukS/F-PV* gene, is the most common CA-MRSA clone. A recent report from Canada indicated that *lukS/F-PV* was detected in 201 (89.7%) of 224 CA-MRSA strains assayed (Nichol et al., 2011). In contrast, our study revealed that Japanese SCC*mec* type IV CA-MRSA strains mostly carry *tst* and *sec* pathogenic genes, and only a few carry the *lukS/F-PV* gene. Thus, we believe that most of the CA-MRSA isolates in this study are not USA300. CA-MRSA strains in Japan were significantly different from the isolates in the United States.

The SCC*mec* type IV strain in Japan was sensitive to CLDM and MINO, similar to the USA300 clone, and was slightly more susceptible to EM. CLDM has good antimicrobial activity against SCC*mec* IV in this study. However, we have to pay attention to clinical use because this resistance may be inducible.

This antibiotic susceptibility of SCC*mec* type IV strains is very different from that of SCC*mec* type II strains. Although most of the strains that we assayed were sensitive to VCM, the MIC values of VCM for sensitive strains have been reported to be increasing (Wang et al., 2006). Therefore, future studies should focus on potential changes in the MIC of VCM for MRSA. In our study, the MIC value of VCM for SCC*mec* type II was higher than that for SCC*mec* type IV, which is consistent with an increase in the MIC of VCM for type II strains.

In conclusion, the combined isolates comprised 73.6% SCC*mec* type II, 20% type IV, and 6% type I strains. The percentage of SCC*mec* type IV isolates was significantly higher in outpatients than in inpatients. Most strains were sensitive to VCM, LZD, TEIC, and S/T. Although most strains were sensitive to VCM, the MIC value of VCM for SCC*mec* type II was higher than that for SCC*mec* type IV. Only 4 (2.3%) of 171 SCC*mec* type IV strains were *lukS/F-PV*–positive. This low frequency of the *lukS/F-PV* gene appears to be a unique feature of SCC*mec* IV type strains in Japan. The information provided here not only is important in terms of local public health but will also enhance our understanding of epidemic clones of CA-MRSA.

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Original Article

Active Surveillance of Methicillin-Resistant *Staphylococcus aureus* with the BD GeneOhm MRSA™ Assay in a Respiratory Ward in Nagasaki, Japan

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SUMMARY: The utility of active surveillance cultures (ASCs) in respiratory wards, that do not have an associated intensive care unit (ICU), and the usefulness of the BD GeneOhm MRSA™ system for rapid detection of methicillin-resistant *Staphylococcus aureus* (MRSA) have not been previously evaluated in Japan. ASCs using conventional culture methods and the BD GeneOhm MRSA™ assay were conducted in adult inpatients between May 11, 2009 and November 10, 2009 in a respiratory ward, without an associated ICU, in Nagasaki University Hospital. The infection and colonization rates of MRSA acquired in this respiratory ward were both investigated. A total of 159 patients were investigated. Of these, 12 (7.5%) were found positive for MRSA by the BD GeneOhm MRSA™ assay and 9 (5.7%) were found positive by a conventional culture test upon admission. All cases were MRSA-colonized cases and cross-transmission was not found to occur during hospitalization. The BD GeneOhm MRSA™ assay had a sensitivity of 100% and a specificity of 98%. ASCs in our respiratory ward revealed that MRSA was brought in from other sites in some cases, and that current infection control measures in Nagasaki University Hospital are effective. The BD GeneOhm MRSA™ assay was proven to be a useful and rapid detection tool for MRSA.

INTRODUCTION

Hospital-acquired infections are a major clinical concern and their management and control are strictly required in order to improve hospital-related mortality and morbidity rates (1). Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most prominent drug-resistant pathogens responsible for nosocomial infection and the incidence of MRSA is almost 60% of overall *S. aureus* infections in Japan (2,3).

Active surveillance cultures (ASCs) were first recommended by the Society for Healthcare Epidemiology of America for preventing nosocomial transmission of multidrug-resistant *S. aureus* and *Enterococcus* (4). Although the primary objective of ASCs is to control increasing number of MRSA infection cases, their efficacy and cost-effectiveness have been questioned. The utility of ASCs has been evaluated in many studies with different designs, evaluation methods, and interventions, and their effectiveness is still controversial (5,6). Many ASC studies of MRSA were conducted in intensive care facilities and surgery wards in Europe and the United States (6–10), since the major risk factors for MRSA infection, including recent surgical procedures, exposure to broad-spectrum antibiotics, hemodialysis, and indwelling percutaneous medical devices and catheters (11–13), are well recognized in such facilities. However, there are other medical settings, such as hospitals in which intensive care unit (ICU) is not equipped and long-term care facilities, where MRSA can be endemic. To date, no ASC studies have been conducted in common respiratory wards without ICU, or in Japanese medical facilities, even though such hospitals and wards are not less common in Japan than elsewhere.

Conventional methods for the detection and identification of MRSA are Gram staining and bacterial culture. Such methods require at least 1 day for detecting MRSA and additional days to determine drug-susceptibility. The BD GeneOhm MRSA™ assay (Nippon Becton Dickinson Co., Tokyo, Japan) has been recently developed as a rapid detection system for MRSA that gives results in 2 h (14). The BD GeneOhm MRSA™ assay

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uses multiplex real-time polymerase chain reaction (PCR) to detect the staphylococcal cassette chromosome (SCC) *mec* insertion site in MRSA and the chromosomal *orfX* of *S. aureus*. Its high performance for detecting MRSA isolates in clinical samples from Japanese patients has been reported previously (15).

In this study, we conducted the first ASCs in a respiratory ward without an associated ICU in Japan using the BD GeneOhm MRSA™ assay and compared the results obtained with those of a standard detection technique to evaluate its usefulness.

MATERIALS AND METHODS

Setting: This observational study was conducted between May 11, 2009 and November 10, 2009 in Nagasaki University Hospital (NUH). All adult inpatients (age, $\geq$16 years) in the respiratory ward were eligible for inclusion in this study. Approximately 350–500 patients, including 150–200 new patients per year, are admitted to this respiratory department ward, with the most common lung disease being cancer. There is no ICU associated with this respiratory ward. This study was approved by the ethical committee of NUH and informed consent from each patient was acquired prior to performing the ASCs.

Microbiological surveillance: Microbiological surveillance of colonization with methicillin-sensitive *S. aureus* (MSSA) and MRSA was performed. Nasal swab specimens from all registered patients were obtained within 48 h after admission, or transfer, to the respiratory ward in NUH. All specimens were plated directly on MRSA selective agar with oxacillin (Nippon Becton Dickinson) and blood agar (Nippon Becton Dickinson) and were tested by the BD GeneOhm MRSA™ assay. The BD GeneOhm MRSA™ assay was performed according to the manufacturer's instructions, and the PCR step was performed within 36 h after sample acquisition. The same nasal swab specimen was also incubated in trypticase soy broth (Nippon Becton Dickinson) as a backup culture and microbial identification was subsequently performed if the BD GeneOhm MRSA™ assay showed a positive result but no microorganisms were initially detected from the MRSA-selective agar or blood agar. The Clinical and Laboratory Standards Institute definition was used for confirmation of MRSA (16). If both conventional culture and the BD GeneOhm MRSA™ assay indicated negative results at the initial screening upon entry to the respiratory ward, subsequent screening for MRSA by culture and BD GeneOhm MRSA™ assay was continued once per week until the time of patient discharge (maximum 7 weeks from admission). When MRSA was identified within the first 48 h after respiratory ward admission, subsequent culture and BD GeneOhm MRSA™ assay in following weeks were discontinued. In cases where MRSA was identified at the initial screening of first admission with active symptoms, such as fever and elevation of inflammatory markers, including leukocyte counts, C-reactive protein, and procalcitonin, MRSA was considered as having been introduced into the respiratory ward with active infection. In the absence of such symptoms and signs, it was considered as having been introduced into the respiratory ward without active infection. When MRSA was found with active symptoms, such as fever and elevation of inflammatory markers, after the first 48 h after admission to the respiratory ward, it was considered to be a hospital-acquired infection. It was considered to be a hospital-acquired colonization if there were no such symptoms and signs.

Data analysis of infection rate: Patient information was acquired at the time of registration for this study. Sex, age, and status of admission to the respiratory ward were recorded. The route of admission, i.e., if the patient was (i) transferred from another ward of NUH, (ii) transferred from another medical facility, (iii) transferred from a nursing home facility, or (iv) admitted directly from home, was noted. History of admission to other medical facilities or nursing homes within the previous year was also recorded. Pulmonary diseases in patients with MRSA positive results, whether by PCR assay or culture methods, were recorded. The endpoints of this study were (i) infection rates of MRSA acquired in a respiratory ward without ICUs and having been introduced from other sites, (ii) colonization rates of MRSA acquired in a respiratory ward and having been introduced from other sites, and (iii) evaluation of the performance of the BD GeneOhm MRSA™ assay compared to conventional cultures as the gold standard.

Statistical analysis: Categorical variables were studied using McNemar's test. A P-value of <0.05 was considered to be statistically significant.

RESULTS

Characteristics of recruited patients: A total of 159 patients (81 men and 78 women) were enrolled in this study. The mean patient age was 66 years, with most being over 50 years. A total of 147 patients (92.4%) were directly admitted to the respiratory ward from their homes, 9 patients were transferred from other medical facilities, and 3 patients were transferred from other wards in NUH. A total of 71 patients (44.7%) had no history of prior admission to medical facilities within the previous year, while 82 patients (51.6%) had been admitted to a medical facility in the previous year (unknown for remaining 6 patients).

Positive rate of MRSA by BD GeneOhm MRSA™ assay and culture: At the initial screening, 12 (7.5%) patients had positive results when using the BD GeneOhm MRSA™ assay and 9 (5.7%) had positive results when using a conventional culture test. Table 1 shows the numbers of samples positive for MRSA, either by conventional culture or by BD GeneOhm MRSA™ assay, from the 1st to 7th weeks after admission. There were no cases in which either culture or BD GeneOhm MRSA™ assays became MRSA-positive from the 2nd week after admission until discharge (maximum 7 weeks) in patients who were MRSA-negative at the time of admission. Table 2 indicates the characteristics of patients who were MRSA-positive by the BD GeneOhm MRSA™ assay or by conventional culture at the first screening. In 3 PCR-positive cases, no evidence of MRSA was found by conventional culture (Case nos. 9, 10, and 12), and these cases were considered to be false-positives. On the other hand, there were no cases in which PCR-negative but culture-positive results were obtained. None of the MRSA-positive patients indicat-

Table 1. Numbers of nasal swab samples taken and positive results with the BD GeneOhm MRSA™ assay or conventional culture method

Timing of sampling nasal swab weeks after admission	No. of samples	No. of PCR positive	No. of culture positive	No. of patients discharged during week
0	159	12	9	80
1	67	0	0	30
2	37	0	0	15
3	22	0	0	12
4	10	0	0	5
5	5	0	0	0
6	5	0	0	4
7	1	0	0	—

Table 2. Characteristics of patients with MRSA-positive results either by BD GeneOhm MRSA™ assay or conventional culture method

Case no.	Age	Sex	PCR	Blood agar	MRSA selective agar	Subsequent identification of MRSA from TSB culture	Colonization or infection	Underlying disease	Admission from	History of admission within a year
1	56	F	positive	positive	positive	positive	colonization	lung cancer	home	positive
2	73	M	positive	positive	positive	positive	colonization	lung cancer	home	positive
3	84	M	positive	positive	positive	positive	colonization	pneumonia	other ward in NUH	negative
4	56	F	positive	positive	positive	positive	colonization	lung cancer	home	positive
5	86	M	positive	negative	negative	positive	colonization	lung cancer	home	negative
6	53	F	positive	positive	positive	positive	colonization	lung cancer	home	positive
7	75	M	positive	negative	negative	positive	colonization	COPD	home	negative
8	69	M	positive	positive	positive	positive	colonization	lung cancer	home	positive
9	66	F	positive	negative	negative	negative	colonization	NTM	home	negative
10	69	F	positive	positive	negative	negative	colonization	NTM	other medical facility	positive
11	75	M	positive	positive	positive	positive	colonization	lung cancer	home	positive
12	48	M	positive	positive	negative	negative	colonization	IPF	home	positive

TSB, trypticase soy broth; NTM, non-tuberculosis mycobacterium infection; COPD, chronic obstructive pulmonary disease; IPF, idiopathic pulmonary fibrosis.

ed active signs of infection, and therefore, they were considered as being MRSA colonized. There was only 1 MRSA-positive patient with pneumonia, who was transferred from another ward of NUH. However, the causative pathogen in this case was *Pseudomonas aeruginosa* and the patient was MRSA colonized.

Performance evaluation of the BD GeneOhm MRSA™ assay: In this study, the BD GeneOhm MRSA™ assay had a sensitivity of 100%, a specificity of 98.0%, a positive predictive value of 75.0%, and a negative predictive value of 100.0%.

DISCUSSION

S. aureus is a major causative agent in hospital-acquired pneumonia (HAP) (17,18) and almost 50% of *S. aureus* infections involve MRSA. Depending on its severity, patients with HAP may be first admitted to a respiratory ward before being transferred to an ICU. Therefore, ASCs in the primary respiratory ward, as the initial admission place, are worth evaluating. Additionally, to date, no ASC studies from respiratory wards that do not have an associated ICU have been published. Our results indicated that few cases of MRSA were introduced into the respiratory ward from other sites, but all were colonized cases and no definite cases of MRSA infection were identified. Subsequent screening until the time of patient discharge also revealed that there were no cases of cross-transmission of MRSA between patients while in the respiratory ward. Approximately 1,000 *S. aureus* isolates per year are detected from all clinical specimens in NUH, approximately 200 isolates are obtained from sputum, and almost 60% of *S. aureus* isolates are positive for MRSA. Despite this high incidence of MRSA in clinical specimens at NUH, infection control and management by the infection control team appears to be quite effective and cross-transmission of MRSA is well controlled in the respiratory ward. Additionally, patients with MRSA pneumonia are critically ill and tend to be admitted to the ICU, which in NUH is managed by a completely separate medical team to that of the respiratory ward. Further assessment of ASCs in the ICU of NUH will be required to determine if infection control across the whole of the NUH facility is appropriately effective. Furthermore, since methods of infection control in respiratory wards differ between different medical facilities, further surveillances at other facilities in Japan are also required.

The prevalence of community-acquired (CA)-MRSA is quite low in Japan, and few cases have been reported to date (19,20). In this study, we encountered 2 cases in which MRSA was introduced directly from the patient's home. These patients had neither a history of admission to other medical facilities, nor of prior usage of an-

tibiotics. Molecular analysis revealed that these isolates did not possess the type-IV SCC *mec*, or the Panton-Valentine leucocidin genes that are unique to CA-MRSA (21–23). Although no apparent transmission route was identified for these 2 cases, we have to note that such cases exist.

Several rapid tests to detect MRSA have been recently developed, and in particular new real-time quantitative PCR assays that enable its detection within 2 h could provide an alternative to conventional Gram stain or culture technique. The sensitivities of newly available commercial PCR-based assays range from 68% to 100%, and their specificities range from 64% to 99% (24–27). This is the first prospective study to evaluate the performance of the BD GeneOhm MRSA™ assay in Japan. Our data indicate that this assay possesses high sensitivity (100%) and specificity (98%) for detecting MRSA. Although we have not performed a study of its cost-effectiveness, we found the BD GeneOhm MRSA™ assay to be highly useful for detecting MRSA within a very short time.

In conclusion, it might not be necessary to screen all patients in our respiratory ward without an ICU for MRSA as long as infection control management is well-executed. The BD GeneOhm MRSA™ assay was proven to be a useful and rapid detection system for MRSA.

Conflict of interest Nippon Becton Dickinson Co., Ltd. (Tokyo, Japan) supported the study with a grant; the sponsor was not involved in the enrollment of patients, collection, analysis, interpretation of the data, or preparation of the manuscript.

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Antifungal Susceptibilities of *Aspergillus fumigatus* Clinical Isolates Obtained in Nagasaki, Japan

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We investigated the triazole, amphotericin B, and micafungin susceptibilities of 196 *A. fumigatus* clinical isolates in Nagasaki, Japan. The percentages of non-wild-type (non-WT) isolates for which MICs of itraconazole, posaconazole, and voriconazole were above the ECV were 7.1%, 2.6%, and 4.1%, respectively. A G54 mutation in *cyp51A* was detected in 64.2% (9/14 isolates) and 100% (5/5 isolates) of non-WT isolates for itraconazole and posaconazole, respectively. Amphotericin B MICs of ≥2 μg/ml and micafungin minimum effective concentrations (MECs) of ≥16 μg/ml were recorded for two and one isolates, respectively.

The clinical importance of *Aspergillus* infection has increased as the number of immunocompromised patients has risen (16). Antifungals recommended for treatment of patients with invasive pulmonary aspergillosis (IPA) or chronic pulmonary aspergillosis (CPA) are triazoles, amphotericin B, and echinocandins (13, 15, 37). Patients with CPA often need years of treatment (13, 37). Although oral therapy is important for carrying out long courses of treatment, azoles (with the exception of fluconazole) are the only class of oral drugs licensed for the treatment of aspergillosis (14, 37).

Aspergillus fumigatus is the most common and pathogenic species of *Aspergillus* (34, 37). Antifungal resistance of *A. fumigatus*, especially to azoles, is one of the concerns in treatment of aspergillosis. During the last decade, many cases of treatment failure due to azole-resistant *Aspergillus* infection have been reported, and in the past few years a growing body of papers about antifungal susceptibilities of *A. fumigatus* has been accumulating (1, 3–6, 9, 10, 12, 18, 23–27, 31–33, 35, 36). Even though an increased rate of azole resistance has been reported recently in the Netherlands and the United Kingdom, the prevalence of azole resistance reportedly remains low in other countries (1, 3, 6, 9, 12, 23, 25, 33).

The azole target protein lanosterol 14α-demethylase of *Aspergillus* is encoded by the *cyp51A* gene, and mutations of *cyp51A* are a major mechanism of azole resistance (8, 17, 19, 20, 22, 32). Some mutational hotspots, such as G54, M220, and TR/L98H, have been identified as causes of azole resistance (2, 21, 22). Of these mutations, TR/L98H is especially prevalent in the Netherlands. An environmental origin (resulting from agricultural antifungal drug usage) is suspected, in spite of the fact that the mechanism(s) of mutation induction has not been shown definitively (24, 31, 32).

We studied the antifungal susceptibility of 196 *A. fumigatus* clinical isolates obtained in the Pneumology Department of Nagasaki University Hospital, Nagasaki, Japan. The isolates were collected between February 1994 and April 2010. All of the isolates were subjected to susceptibility testing and *cyp51A* sequence analysis. All isolates were identified as *A. fumigatus* by macroscopic colony morphology, micromorphological characteristics, and the ability to grow at 48°C. Non-wild-type (non-WT) isolates were subjected to additional molecular identification by amplification of ribosomal internal transcribed spacers (ITSs) and ribosomal large-subunit D1-D2 sequencing as described previously (11). MICs of itraconazole, posaconazole, voriconazole, and amphotericin B and minimum effective concentrations (MECs) of micafungin were determined using the Clinical and Laboratory Standards Institute (CLSI) M38-A2 broth microdilution method. Assays were performed using RPMI 1640 broth (0.2% dextrose), final inoculum concentrations ranging from 0.4×10^4 to 5×10^4 CFU/ml, and 48 h of incubation at 35°C (7). The MIC was defined as the lowest drug concentration that produced complete growth inhibition; the MEC was read as the lowest concentration of drug that led to the growth of small, rounded, compact hyphal forms compared to the hyphal growth seen in the control well. Susceptibility tests of non-WT isolates were performed at least three times for each isolate; each test was performed on different days. Because clinical breakpoints have not been established yet, we used epidemiological cutoff values (ECVs) to evaluate azole susceptibility (9, 25, 29). Wild-type (WT) isolates of *A. fumigatus* (MIC ≤ ECV) were distinguished from non-WT isolates (MIC > ECV), which may exhibit acquired low-susceptibility mechanisms. ECVs used in this study were as follows: itraconazole, 1 μg/ml; posaconazole, 0.5 μg/ml; voriconazole, 1 μg/ml, all as previously suggested (9, 25).

For sequence analyses, genomic DNA was extracted from non-WT isolates using a MasterPure yeast DNA purification kit (Epicentre Biotechnologies, Madison, WI). The full coding region of the *cyp51A* gene was amplified as previously described (32). DNA sequences were determined using a BigDye Terminator ver-

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TABLE 1 MIC and MEC distributions of five antifungals

Antifungal agent	No. of isolates[a]											% of non-WT isolates
	Total	With a MIC or MEC[b] (μg/ml) of:										
		≤0.03	0.06	0.125	0.25	0.5	1.0	2.0	4.0	8.0	≥16	
Triazoles												
Itraconazole	182 (14)			1	28	107	46	(8)	(4)	(1)	(1)	7.1
Posaconazole	182 (14)	14	108	35	24 (2)	(8)	(1)	1 (2)			(1)	2.6
Voriconazole	182 (14)			1 (3)	20 (7)	101 (2)	53 (1)	7 (1)				4.1
Polyene												
Amphotericin B	182 (14)		6	8 (1)	19 (1)	90 (7)	57 (5)	2				
Echinocandin												
Micafungin	182 (14)	177 (14)	2	1							2	

[a] MIC distributions for each agent were obtained by subtracting from the total isolates tested the 14 non-WT isolates resistant to itraconazole. The MIC distribution for the 14 non-WT isolates is in parentheses.
[b] MICs are shown for amphotericin B, itraconazole, posaconazole, and voriconazole; MECs are shown for micafungin.

sion 1.1 cycle sequencing kit (ABI) and an ABI 3100xl DNA analyzer. Sequence alignments were performed against the sequence from an azole-susceptible strain (GenBank accession no. AF338659) using MacVector10.0 software (MacVector, Inc., Cary, NC) (20). Mutations were confirmed three times by repeating the PCR and sequencing the relevant region using the closest primer.

In this study, using the ECVs, the percentages of non-WT isolates for which MICs of itraconazole, posaconazole, and voriconazole were above the ECV were 7.1%, 2.6%, and 4.1%, respectively (Table 1). To exclude the possibility of increased proportions of non-WT isolates due to clonal spread (notably, the potential repeated isolation of a drug-resistant strain originating from one patient), we confirmed those proportions on a per-case basis, which (for non-WT isolates) were 7.5%, 4.3%, and 6.5% for itraconazole, posaconazole, and voriconazole, respectively. These proportions of non-WT isolates were not much different from previous data from other regions, with the exception of data for the Netherlands and the United Kingdom (3, 9, 12, 23, 25, 33). All the itraconazole-resistant isolates (MIC ≥ 4 μg/ml) were obtained from 1998 to 2001. No consistent trend of increased proportion of non-WT isolates was observed. Amphotericin B MICs of ≥2 μg/ml were recorded for 1.0% of the isolates (2/196); micafungin MECs of ≥16 μg/ml were recorded for 1.0% of the isolates (2/196) (Table 1). For these antifungals, the proportions of resistant isolates were low and similar to those in previous reports (3, 10, 23, 26).

In Japan, posaconazole has not been approved for clinical use; nonetheless, non-WT isolates for posaconazole already existed (Table 1). Resistance in these isolates might reflect native biological variability. Alternatively, this phenomenon could be associated with cross-resistance between itraconazole and posaconazole, because 80% (4/5) of non-WT isolates for posaconazole were also non-WT isolates for itraconazole (Table 2). In addition, non-WT isolates for itraconazole tended to be more resistant to posaconazole, though not to voriconazole (Table 1). Cross-resistance between itraconazole and posaconazole, but not with voriconazole, may result from the G54 mutation of *cyp51A*, which was present in 64.2% (9/14) of the non-WT isolates for itraconazole and also present in 100% (5/5) of the non-WT isolates for posaconazole (Table 2). There is a known structural basis for the association of the G54 mutation with this pattern of cross-resistance among the azoles: unlike voriconazole, itraconazole and posaconazole have long side chains that clash with the amino acid side chain of the residue replacing G54 in the mutated CYP51A protein (8, 27, 32, 38).

Among mutations of the *cyp51A* gene, TR/L98H has received the most attention, notably because this mutation was seen in a specific country and found in *A. fumigatus* isolated from the environment (17, 22, 24, 31–33). Similarly, TR/L98H was recently detected in a multi-azole resistant isolate in China (17), suggesting that the TR/L98H mutation could be selected in Asia as well as in Europe. Of all 22 non-WT isolates in our study of Japanese isolates, CYP51A mutations were detected as follows: G54W, two isolates; G54R, one isolate; I266N, two isolates; G54E plus I266N,

TABLE 2 MICs and Cyp51A substitutions in 22 non-WT *Aspergillus fumigatus* isolates

Isolate	MIC (μg/ml)			Cyp51A substitution
	Itraconazole	Posaconazole	Voriconazole	
MF-452	>8	0.5	0.5	I266N
MF-469	8	1	0.25	G54E, I266N
MF-460	4	2	0.25	G54E, I266N
MF-357	4	0.5	0.5	I266N
MF-468	4	0.5	0.25	G54E, I266N
MF-329	4	0.5	0.25	None
MF-331	2	>16	0.25	G54W
MF-327	2	2	0.12	G54R
MF-439	2	0.5	0.25	G54E, I266N
MF-473	2	0.5	0.25	G54E, I266N
MF-454	2	0.5	0.12	G54E, I266N
MF-472	2	0.5	0.12	G54E, I266N
MF-843	2	0.25	2	None
MF-748	2	0.25	1	ND[a]
MF-1011	1	2	0.12	G54W
MF-855	1	0.25	2	None
MF-336	1	0.25	2	None
MF-486	1	0.25	2	None
MF-520	1	0.25	2	None
MF-1091	0.5	0.25	2	None
MF-474	0.5	0.25	2	None
MF-303	0.5	0.12	2	None

[a] ND, not determined.

seven isolates (Table 2). No TR/L98H-bearing isolates were detected. The I266N mutation, which has (to our knowledge) not been reported previously, was also seen in other azole-susceptible isolates; therefore, it might not be directly related to azole resistance. Of 21 non-WT isolates, 9 had no CYP51A substitution (Table 2). Interestingly, most non-WT isolates for voriconazole did not possess a *cyp51A* mutation. Although Bueid et al. reported an increase of frequency of azole-resistant isolates without *cyp51A* mutations, other possible resistant mechanisms (e.g., upregulation of efflux pump) have not yet been fully identified (6, 28, 30), and further analysis is warranted.

Only a few previous analyses have examined changes in susceptibility over time; therefore, it is not clear that the frequency of azole-resistant *A. fumigatus* is increasing worldwide (12, 25, 33). Nevertheless, mechanisms of resistance induction in clinical settings or the environment (e.g., selection following agricultural antifungal exposure) remain poorly understood. Given that azole usage varies from one country to another, the mechanism of azole resistance may differ between regions.

In this study, we found a low prevalence of resistance to triazoles in Japanese isolates of *A. fumigatus*, a clinically important fungus of increasing concern in respiratory medicine. The proportions of non-WT isolates were similar to those previously reported for other countries. In the future, Japanese *A. fumigatus* isolates may develop azole resistance by different mechanisms (such as TR/L98H); therefore, we urge the continued monitoring of azole susceptibility in this species.

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II. 抗ウイルス薬の特性と適応・使い分け

抗サイトメガロウイルス薬

安岡 彰

Anti-cytomegaloviral drugs

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Abstract

Cytomegalovirus infection is often life threatening in immunocompromised patients. Three anti-CMV drugs, ganciclovir, valganciclovir and foscarnet are available in commercial basis in Japan. In addition to them, cidofovir is provided for patients with HIV by a research group supported by Ministry of Health, Labour and Welfare. Every anti-CMV drug has serious side effects, as bone marrow suppression with ganciclovir and valganciclovir, renal toxicity with foscarnet and both with cidofovir. Strict dose adjustment to the renal function and close monitoring with laboratory examinations are essential when using those drugs.

Key words: herpesvirus, ganciclovir, valganciclovir, foscarnet, cidofovir

はじめに

主に細胞性免疫不全者に発症する重症感染症であるサイトメガロウイルス(cytomegalovirus: CMV)にも，適応疾患に限定はあるものの有効な抗ウイルス薬がある．

1. CMVとその感染症

CMVはエンベロープをもつDNAウイルスであるβヘルペスウイルスに分類され，ヒトのみを宿主とし，ヒトに感染するウイルスとしては最大の大きさ(直径約180nm)である．このほかヘルペス属β群にはHHV-6，7ウイルスがある．唾液などの体液や母乳によっても感染するためヒトに普遍的に感染しているウイルスで，国や地域によって感染率は異なるものの，成人の60-95％が既感染となっている．他のヘルペス属ウイルスと同様，いったん感染すると体内で潜伏感染状態となる．

CMVの病型としては初感染時の伝染性単核症，潜伏感染後に宿主が細胞性免疫不全となった場合に潜伏感染が再活性化されて生ずる全身臓器感染症，妊娠中に初感染を受けることによって胎児が感染する先天感染の3つの病型がある．

CMVの診断は感染細胞の細胞質内に巨大封入体を形成することから，病変部の組織・細胞診で封入体細胞を検出することが確定診断となる．ウイルスの活性化の指標として，血液のサイトメガロウイルス抗原血症(アンチゲネミア)や血漿を用いたPCRによるウイルス定量も診断の補助として用いられる．また血清のCMV

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0047-1852/12/¥60/頁/JCOPY

表1　サイトメガロウイルス治療薬

薬　剤	商品名	投与経路	剤　形	治療量投与量 (維持量)	主たる副作用
ガンシクロビル	デノシン	点滴静注	500 mg/V	5 mg/kg×2回 (5 mg/kg×1回)	骨髄抑制
バルガンシクロビル	バリキサ	経口	450 mg錠	900 mg×2回 (900 mg×1回)	骨髄抑制
ホスカルネット	ホスカビル	点滴静注	6 g/V (24 mg/mL)	90 mg/kg×2回 (90-120 mg/kg×1回)	腎機能障害 電解質異常
シドフォビル	Vistide	点滴静注	375 mg/V	5 mg/kg/週1回を2回 以後2週に1回	腎機能障害 骨髄抑制

に対するIgMとIgG抗体の測定も感染状態の判定に用いられる.

2. 抗ウイルス薬(表1)

1) ガンシクロビル

ガンシクロビルはCMVの遺伝子複製時にデオキシグアノシン三リン酸(dGTP)と競合して取り込まれることにより，遺伝子の伸延を阻害する核酸アナログである．剤形としてガンシクロビル注射剤と，このプロドラッグであるバルガンシクロビルの経口剤がある．ガンシクロビルがウイルスのプロテインキナーゼでガンシクロビル一リン酸となり，更に細胞の酵素でガンシクロビル三リン酸となって抗ウイルス活性を発揮する．バルガンシクロビルはバリンエステルを結合したプロドラッグで，腸管からの吸収過程でガンシクロビルとなる.

本剤は大部分が未変化体のまま主に尿路から排出されるため，腎障害がある患者では用量の調節が必要である.

重要な副作用として骨髄抑制があり，このため好中球数500/mm³未満または血小板数25,000/mm³未満の場合は使用禁忌となっている．骨髄抑制は投与開始1週後くらいから顕性化する．このため本剤投与開始後は頻回の臨床検査が必須であり，週2-3回の頻度でチェックし，早期発見に努める必要がある．骨髄抑制が認められた場合は減量または治療の中断が必要である．また腎障害や肝障害にも注意が必要である．動物実験により催奇形性が示されており，妊婦への投与は禁忌となっている.

前述のように本剤は活性体となるのにウイルス酵素を必要とするため，ウイルス遺伝子の変異により耐性を生じることがある．特に本剤による長期治療を受けた履歴がある場合に耐性化のリスクがある．この場合ガンシクロビルの効果は期待できないので，他剤による治療が必要となる．ホスカルネット，シドフォビルとの交差耐性は認められない(ウイルスが両剤に対するそれぞれの耐性変異を獲得することはありうる).

a. ガンシクロビル(注射剤)

ガンシクロビルは点滴静注で使用される．経口剤形もあったが，腸管からの吸収率が極めて低いため治療効果は期待できず，維持・発症予防としてのみ使用されていた．後述のバルガンシクロビルの登場によって経口剤はその役割を終えた.

体重によって投与量の調整が必要で，5 mg/kgを1日2回投与するのが標準投与量である．腎機能によって用量調節が必要で，クレアチニンクリアランスが70 mL/min未満では減量する．2週間程度の投与によって効果を確認し，治療効果や副作用発現の度合いをみて，治療の延長または維持量への減量，中止を検討する．維持量は5 mg/kgを1日1回投与するものであり，治療が有効で病状が安定しているが，基礎疾患の状態から再燃が懸念される場合にこの量を維持する．維持量であっても治療長期化に伴って骨髄抑制のリスクがあるため，定期検査は必須である.

b. バルガンシクロビル

バルガンシクロビルは450 mgの錠剤であり，

1回900mgを1日2回投与するのが治療量としての標準量である．この投与量で血中のガンシクロビル濃度のピーク(Cmax)は5-6μg/mLに達すると報告されており，ガンシクロビルを点滴静注した場合と遜色ない移行を示している．ただCMV感染症は多くの場合致死的リスクのある感染症であるため，治療開始はガンシクロビルの経静脈投与で開始して，治療効果がみられた段階で経口のバルガンシクロビルに切り替えるという使用法が主流である．CMV網膜炎の初期や軽症の食道病変などでは，最初からバルガンシクロビルによる治療を行うことも可能である．

治療維持量は900mg 1日1回投与で，効果が安定した場合にはこの量への減量も可能である．

ガンシクロビルよりはマイルドではあるものの本剤でも骨髄抑制は必至であり，定期的な血液検査が不可欠である．

2) ホスカルネット

ホスカルネットはウイルスDNAポリメラーゼの阻害薬で，そのままで活性体である．ガンシクロビルと異なりウイルス酵素による代謝を必要としないため，薬剤耐性を生じにくいとされている．

本剤はHIV感染者のCMV感染症では90mg/kgを1日2回投与する方法と60mg/kgを1日3回投与する方法があるが，抗CMV効果に目立った違いがみられないため，投与の簡便さから90mg/kg 1日2回投与が主に行われている．造血幹細胞移植患者のCMV感染症では60mg/kgを1日2回が用量となっている．治療効果が現れた場合は，ガンシクロビルと同様投与回数を1回に減らす(投与量は90-120mg/kg)ことで維持量とする．

本剤は腎機能障害，血中のMg，Ca，K濃度低下といった電解質異常をきたしやすい．このためガンシクロビルと同様定期的な血液検査が必須であり，また投与に際しては1回あたり生理食塩液500-1,000mLの輸液を追加することが推奨される．また腎機能に応じたきめ細かい用量調節が必須であり，特に腎機能低下が出現した場合は速やかな減量・中止を行うことが重要である．

3) シドフォビル

日本では認可されていないが，第3のCMV治療薬としてシドフォビルがある．ガンシクロビルとホスカルネットの両剤が無効なCMVに対しても効果がある．効果と副作用の持続が長いため，治療開始時は週に1回の投与，3回目以降は2週に1回の投与で臨床効果がある．本剤は骨髄抑制と腎機能障害の両方の副作用が発現しやすく，腎機能障害を低減するためにプロベネシドの併用と投与時の輸液負荷が必須である．いったん副作用が発現すると遷延し，また1回の投与でも腎障害が発生することがあり，コントロールに難渋する薬剤である．腎障害は不可逆的であるとされている．HIV感染者のCMV感染症に対してのみ，厚生労働省エイズ治療薬研究班から入手することができる．使用が認可されている米国でも適応はエイズ患者のCMV網膜炎に限定されている．

3. 抗ウイルス薬の使い分けと注意点

CMV感染症で治療を考慮する場合は，基礎疾患の状態，患者の腎機能，骨髄機能をはじめとした生体・代謝機能と，合併する感染症への治療内容などを考慮して治療薬を選択する．腎障害がある場合やニューモシスチス肺炎治療のペンタミジンを使用しているときなどはガンシクロビルを，骨髄抑制がみられる場合はホスカルネットを選択する．いずれの問題もない場合は治療効果と副作用の調節のしやすさからガンシクロビルが第1選択となる．

いずれの薬剤も体重と腎機能による補正を厳密に行って投与量を決定し，原則として治療量で開始する．いずれの薬剤の副作用も1週間投与した頃から顕性化するので，特にこの頃の臨床検査値には十分注意を払う必要がある．薬剤を中止した後も副作用はしばらく持続/進行するという印象があるため，機能障害が出現したら早め早めの減量・中止の判断が必要である．副作用出現時に臨床効果が不十分であった場合は，可能であれば他剤(ガンシクロビル治療中であればホスカルネット)に変更して治療を継

続する.

CMVの病変にもよるが，原則として2週間程度治療量を投与した後，臨床効果を判定し治療量を維持するか，維持量に減量するかを検討する．ガンシクロビル治療例であれば重症でないCMV網膜炎などでは治療効果が明らかになった段階でバルガンシクロビルに切り替えて，外来で治療することも可能である．

CMV治療薬の効果は生体の免疫機能によって大きく影響を受ける．免疫不全程度が高ければ治療薬単独での効果は限定的である．基礎疾患の改善を図ったり，CMV抗体高力価のガンマグロブリン製剤や，場合によっては両ウイルス薬の併用などが必要な場合もある．

おわりに

CMVは免疫不全状態に発症する重篤な感染症であるが，治療薬は他のウイルス薬と比べて重篤な副作用が起こりやすく，一方抗ウイルス効果は十分とはいえない．十分なモニタリングと適時適切な臨床判断が必要な治療薬であることを，十分理解したうえでの使用が求められる．

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Answer

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背　景

抗HIV療法(anti-retroviral therapy：ART)が標準的に行われるようになった今日，HIV感染による日和見感染症の85%以上はHIV感染症が判明していない新規の患者に発症している。しかし，本例のような通院の中断など適切なHIV感染症治療を受けずに発症する例もHIV感染者の増加に伴い少しずつ増加している。

治療方針

AIDSのクリプトコックス症は頭痛・嘔気といった初期症状が軽微で，本例のように意識障害で発見されることが少なくないが，病状の進行を意味し，予後不良である。

HIV感染症無治療の日和見感染症発症例では，まず適切な日和見感染症の治療を導入することが最優先である。進行したクリプトコックス症では脳内の占拠性病変や全身播種を起こすリスクがあるため，頭部MRIやCT，真菌対応の血液ボトルを用いた血液培養，尿培養などを速やかに行い病状を評価する。また，免疫不全状態にある患者ではニューモシスチス肺炎，トキソプラズマ症，サイトメガロウイルス感染症，結核・非結核抗酸菌症やB型肝炎，C型肝炎，アメーバ症などの合併感染がないかについても検索を進める必要がある。

治療薬

クリプトコックス髄膜炎／全身播種に対する治療は，アムホテリシンB(AMPH-B)とフルシトシン(5-FC)の併用が標準治療である。米国感染症学会(IDSA)のガイドラインでは，エビデンスの集積とコストの観点からAMPH-B 0.7～1.0mg/kg/日の経静脈投与を第一選択として推奨しているが，効果と副作用の観点からはリポソーム化AMPH-B 3～4mg/kg/日の経静脈投与が勧められる。AMPH-Bでは腎機能障害，高カリウム(K)血症が高頻度にみられ，また投与後の発熱の頻度も高い。十分な輸液負荷や血液生化学検査を週に数回行うなど，注意深い対応が必要である。

5-FC 100mg/kg/日を併用するのが原則であるが，HIV感染者では造血(骨髄)機能が疲弊しており，容易に副作用の好中球減少症が出現する。経口のみの剤形であるため，本例のような意識障害例では経管投与をするなどの工夫を要する。また，血液細胞数検査を頻回に行い副作用の早期検出を図る。AMPH-B+5-FC治療の主役はAMPH-Bであり，5-FCの併用についてはあまり深追いしないほうがよいようである。

AMPH-B+5-FCによる治療を2週間以上継続し臨床的改善が得られれば，フルコナゾール(FLCZ)400mg/日による経口投与に移行する。なお，非AIDS例でのAMPH-B標準治療期間は従来6週間である。AIDS例で2週間以上とされているのは副作用の観点が主であるので，AMPH-Bが耐用できていればなるべく長く使用することを推奨する。FLCZ 400mg/日による治療期間は，8週間以上が推奨されている。臨床症状の改善と培養陰性が確認されれば，FLCZ 200mg/日による維持治療に移行する。末梢血CD4数が100/μL以上でかつHIV-RNA量が検出限界以下となってから3ヵ月以上経過した場合，治療の中止を考慮できる。

補助治療

クリプトコックス髄膜炎では，脳圧亢進が予後悪化因子である。本例の22cmCSFはさほど高い値ではないが，治療中に上昇することもあるため定期的に髄液圧と髄液中のクリプトコックスの塗沫・培養所見を観察し，圧の上昇(目安として25cmCSF)や頭痛などの脳圧亢進症状がみられる場合は排液による減圧を図る必要がある。この目的でのステロイドの投与は推奨されない。

ARTと免疫再構築症候群

AIDSの日和見感染症は免疫不全を背景として発症