表7. 就業停止期間

	麻疹	水痘	風疹	ムンプス
感染者	発疹出現後 7 日間か急性疾患が続く期間のいずれか長い方の期間	全ての発疹が乾燥し痂皮化するまで	発疹出現後5日間	耳下腺炎発症後9日間
ウイルス曝露感受性者	曝露後5日目から最後の曝露後21日目まで	曝露後10日目から最後の曝露後 21 日目まで（グロブリン投与後は 28 日目まで）	曝露後 7 日目から最後の曝露後21日目まで	曝露後12日から最後の曝露後26日目まで

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Correlation between Triazole Treatment History and Susceptibility in Clinically Isolated *Aspergillus fumigatus*

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This is the first report of a detailed relationship between triazole treatment history and triazole MICs for 154 *Aspergillus fumigatus* clinical isolates. The duration of itraconazole dosage increased as the itraconazole MIC increased, and a positive correlation was observed ($r = 0.5700$, $P < 0.0001$). The number of itraconazole-naïve isolates dramatically decreased as the itraconazole MIC increased, particularly for MICs exceeding 2 μg/ml (0.5 μg/ml versus 2 μg/ml, $P = 0.03$). We also examined the relationship between cumulative itraconazole usage and the MICs of other azoles. A positive correlation existed between itraconazole dosage period and posaconazole MIC ($r = 0.5237$, $P < 0.0001$). The number of itraconazole-naïve isolates also decreased as the posaconazole MIC increased, particularly for MICs exceeding 0.5 μg/ml (0.25 μg/ml versus 0.5 μg/ml, $P = 0.004$). Conversely, the correlation coefficient obtained from the scattergram of itraconazole usage and voriconazole MICs was small ($r = -0.2627$, $P = 0.001$). Susceptibility to three triazole agents did not change as the duration of voriconazole exposure changed. In addition, we carried out detailed analysis, including microsatellite genotyping, for isolates obtained from patients infected with azole-resistant *A. fumigatus*. We confirmed the presence of acquired resistance to itraconazole and posaconazole due to a G54 substitution in the *cyp51A* gene for a patient with chronic pulmonary aspergillosis after oral itraconazole therapy. We should consider the possible appearance of azole-resistant *A. fumigatus* if itraconazole is used for extended periods.

Aspergillosis has become an increasingly important fungal infection, because the number of immunocompromised patients has increased (21, 29). However, antifungal drugs for treating different types of aspergillosis such as invasive pulmonary aspergillosis or chronic pulmonary aspergillosis have insufficient efficacy (18–20, 32). Among the few types of drugs with anti-*Aspergillus* activity, triazoles hold a prominent position because they are the only licensed class of oral drugs for treating aspergillosis (32).

Recently, the appearance of azole-resistant *Aspergillus fumigatus* has come under scrutiny in several countries (1, 2, 7, 14, 17, 23–27, 30). Reports from some countries have raised concerns over the increased prevalence of azole-resistant *A. fumigatus* (7, 17, 27). Therefore, it is important to elucidate the mechanism of resistance to prevent the spread of azole-resistant *A. fumigatus* and subsequent outbreaks. The possible origins of these azole-resistant isolates include the environment and the patient's own body (31). Some cases of acquired resistance in *A. fumigatus* have been reported in patients with aspergilloma during treatment with azoles (3, 6, 8, 9, 11, 22). Environments such as farms are especially suspected of promoting the production of azole-resistant isolates harboring the TR/L98H mutation in the *cyp51A* gene, which encodes cytochrome P450 14-α sterol demethylase, the primary target for azole compounds (23, 31).

Despite the presence of case reports on the development of azole resistance during azole therapy, little information is available on the amount of azole needed for the development of azole resistance (8, 17, 22). Howard et al. reported that the first azole-resistant isolate was identified after using azole for 1 to 30 months (17). A recent study by Camps et al. warned of a rapid induction of resistance for which the median time between isolation of the last cultured wild-type isolate and isolation of the first azole-resistant isolate was 4 months (8). Such data are important, because long-term, perhaps lifelong, antifungal treatment is required for some chronic pulmonary aspergillosis cases (32).

Recently, we reported the antifungal MIC distribution of 196 *A. fumigatus* clinical isolates with a *cyp51A* gene mutation in Nagasaki, Japan (28). Of those, we analyzed 154 isolates from 64 patients retrospectively in this study, and we evaluated the cumulative amount of azoles administered to patients at the time of isolation of each *A. fumigatus* clinical isolate. Moreover, we investigated the backgrounds of patients from whom azole-resistant *A. fumigatus* was isolated and conducted microsatellite genotyping of the isolates to analyze their genetic relationships. This is the first report to analyze the correlation between azole usage and azole susceptibility of *A. fumigatus* clinical isolates.

MATERIALS AND METHODS

***A. fumigatus* isolates.** The isolates were collected in the Pneumology Department of Nagasaki University Hospital, Nagasaki, Japan, between February 1994 and April 2010. We identified all isolates as *A. fumigatus*

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TABLE 1 Characteristics of patients and isolates

Parameter	Value(s)[a]
No. of isolates	154
No. of patients	64
Sample origin	
Sputum	96/154 (62)
Bronchoalveolar lavage fluid	36/154 (23)
Lung tissue	9/154 (5.8)
Other[b]	2/154 (1.3)
Unknown	11/154 (7.1)
Clinical diagnoses[c]	
Invasive pulmonary aspergillosis[d]	9/64 (14)
Chronic pulmonary aspergillosis except simple aspergilloma	27/64 (42)
Simple aspergilloma	12/64 (19)
Allergic bronchopulmonary aspergillosis	4/64 (6.3)
Colonization	12/64 (19)

[a] Other than patient and isolate data, all values represent number of positive results/total number of results (percent).
[b] "Other" includes lung abscess and bone marrow.
[c] Diagnoses of 23 other patients were unknown.
[d] All diagnoses were classified as "probable."

according to the macroscopic colony morphological and micromorphological characteristics and the ability to grow at 48°C (4). Azole-resistant isolates were subjected to additional molecular identification by amplification of ribosomal internal transcribed spacers and ribosomal large-subunit D1-D2 sequencing as described previously (16).

Patients. Clinical information was extracted from the clinical records on the type of aspergillosis and history of azole antifungal use. The periods of triazole administration were cumulatively determined until the time of *A. fumigatus* isolation; therefore, the periods were different for each isolate and even for isolates obtained from the same patient. In patients infected with azole-resistant *A. fumigatus*, we examined the underlying diseases and characteristics of therapeutic failure. Patient 1 (a 48-year-old man) had chronic cavitary pulmonary aspergillosis (CCPA) (see Table 2). Both his lungs were damaged by multiple partial lobectomies because of repeated refractory pneumothorax, and multiple cavities and bullas with pleural thickness were observed in both the lungs. *A. fumigatus* was frequently cultured from his sputum despite oral itraconazole treatment (200 to 400 mg/day). After the isolation of itraconazole-resistant *A. fumigatus*, the patient was treated with oral voriconazole. Since then, his symptoms such as productive cough or hemosputum have improved, and no fungus has been subsequently isolated from his sputum. Patient 2 (a 70-year-old woman) was clinically diagnosed as having aspergilloma in the upper lobes of both the lungs (see Table 2). She had a history of pulmonary tuberculosis and had several cavities in both the lungs. Patients 3 (an 80-year-old woman) and 5 (a 63-year-old man) were diagnosed with simple aspergilloma. Patient 4 (a 56-year-old woman) was diagnosed with CCPA (see Table 2).

Antifungal susceptibility testing and *cyp51A* sequencing. We previously reported results for antifungal susceptibility analysis and *cyp51A* sequencing (28). The breakpoints used for resistance were ≥4 μg/ml for itraconazole and voriconazole and ≥1 μg/ml for posaconazole (30).

Genotyping. Sixteen isolates (including both azole-susceptible and azole-resistant isolates) were obtained from 5 patients infected with azole-resistant *A. fumigatus*. DNA was extracted from these isolates by using a MasterPure yeast DNA purification kit (Epicentre Biotechnologies, Madison, WI), and 9 short tandem-repeat regions (2A, 2B, 2C, 3A, 3B, 3C, 4A, 4B, and 4C) were amplified by PCR as described previously (12). The repeat numbers were determined by sequencing analysis, and we compared the patterns of repeat numbers. DNA sequences were determined using a BigDye Terminator version 1.1 cycle sequencing kit (ABI) and an ABI 3100xl DNA analyzer.

Statistics. Statistical analyses of azole usage and azole susceptibility were performed using Pearson's correlation and Fisher's exact tests with Prism version 5.0 (GraphPad). Differences were considered significant when $P < 0.05$.

RESULTS

Correlation between azole usage (duration and amount) and azole susceptibility. A total of 154 *A. fumigatus* clinical isolates obtained from 64 patients were analyzed. Most of the specimens were isolated from the lungs (Table 1). Chronic pulmonary aspergillosis (including simple aspergilloma) accounted for 61% of the clinical diagnoses (Table 1).

The scatter plot of the itraconazole dosage period and itraconazole MICs is shown in Fig. 1A. Patients infected by *A. fumigatus* with itraconazole MICs < 2 μg/ml had been treated with itraconazole for <1 year. All isolates with itraconazole MICs ≥ 4 μg/ml (MF-452, MF-460, MF-468, MF-469, MF-329, and MF-357) had been exposed to itraconazole for >115 days (Table 2). The itraconazole dosage duration increased as the itraconazole MIC increased, and the dosage duration was positively correlated with the itraconazole MIC ($r = 0.5700$, $P < 0.0001$) (Fig. 1A). The number of itraconazole-naïve isolates dramatically decreased as the MIC increased, particularly for MICs exceeding 2 μg/ml (0.5 μg/ml versus 2 μg/ml, $P = 0.03$) (Fig. 1B). These results indicated that long-term itraconazole treatment could induce azole-resistant *A. fumigatus*.

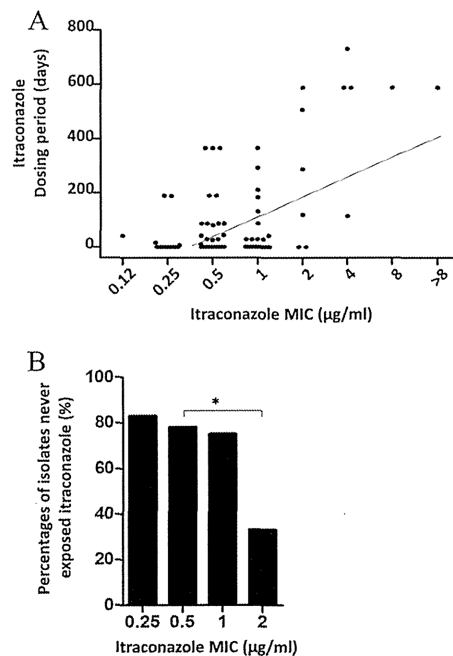

FIG 1 Relationship between itraconazole MICs and the history of itraconazole usage for 154 *A. fumigatus* clinical isolates. (A) The itraconazole dosage duration increased as the itraconazole MIC increased, and a positive correlation was observed between the itraconazole dosage duration and the itraconazole MIC ($r = 0.5700$, $P < 0.0001$). (B) The number of itraconazole-naïve isolates dramatically decreased as the itraconazole MIC increased, particularly for itraconazole MICs exceeding 2 μg/ml (0.5 μg/ml versus 2 μg/ml, $P = 0.03$). *, $P < 0.05$ (Fisher's exact test).

TABLE 2 Characteristics of the 16 isolates obtained from patients infected with azole-resistant *A. fumigatus*[a]

Patient no.	Isolate no.	Date of isolation (day-mo-yr)	ITC exposure[b]		Time from end of ITC therapy (days)	MIC (μg/ml)[c]			Cyp51A substitution[d]
			Period (days)	Amt (mg)		ITC	POS	VRC	
1	MF-368	16-08-2000	189	37,800	252	0.5	0.06	0.5	No substitution
	MF-367	16-08-2000	189	37,800	252	0.5	0.06	0.25	No substitution
	MF-370	07-09-2000	189	37,800	274	0.25	0.06	0.25	No substitution
	MF-439	19-10-2001	507	144,850	0	2	0.5	0.25	G54E
	MF-452	03-04-2002	589	161,650	84	>8	0.5	0.5	No substitution
	MF-454	17-04-2002	589	161,650	98	2	0.5	0.125	G54E
	MF-460	08-05-2002	589	161,650	119	4	2	0.25	G54E
	MF-468	22-05-2002	589	161,650	133	4	0.5	0.25	G54E
	MF-469	29-05-2002	589	161,650	140	8	1	0.25	G54E
2	MF-329	24-08-1998	115	23,000	0	4	0.5	0.25	No substitution
	MF-331	29-08-1998	120	24,000	0	2	>8	0.25	G54W
	MF-336	10-09-1998	132	26,400	0	1	0.25	2	No substitution
3	MF-357	09-02-2000	731	146,200	1223	4	0.5	0.5	No substitution
4	MF-933	11-03-2008	0	0		0.5	0.25	0.25	No substitution
	MF-1011	09-10-2008	210	42,000	0	1	2	0.125	G54W
5	MF-327	16-07-1998	287	43,050	435	2	2	0.125	G54R

[a] Azole-resistant *A. fumigatus* had itraconazole MIC ≥ 4μg/ml or posaconazole MIC ≥ 1μg/ml. Voriconazole resistant isolates (voriconazole MIC ≥ 4μg/ml) were not found.
[b] Accumulated periods and amounts before isolation.
[c] ITC, itraconazole; POS, posaconazole; VRC, voriconazole.
[d] Only substitution associated with azole resistance.

A positive correlation was also observed between the itraconazole dosage period and the posaconazole MIC ($r = 0.5237$, $P < 0.0001$) (Fig. 2A). The number of itraconazole-naïve isolates decreased as the posaconazole MIC increased, particularly for posaconazole MICs exceeding 0.5 μg/ml (0.25 μg/ml versus 0.5 μg/ml, $P = 0.004$) (Fig. 2C). The correlation coefficient obtained from the scattergram of itraconazole usage and voriconazole MICs was small ($r = -0.2627$, $P = 0.001$) (Fig. 2B). The voriconazole MIC did not increase with increasing itraconazole usage. In addition, the numbers of itraconazole-naïve isolates were not correlated with the voriconazole MIC (Fig. 2D). These results suggested the possibility of inducing resistance to posaconazole but not to voriconazole by long-term itraconazole therapy.

A. fumigatus was isolated after voriconazole treatment from only a few patients; therefore, analysis of the relationship between voriconazole usage histories before *A. fumigatus* isolation and azole susceptibilities was limited. Only 10 isolates were exposed to voriconazole therapy before isolation, and the average duration of the therapy was 8.3 ± 6.3 days. Voriconazole exposure did not alter the susceptibility to the 3 triazole agents.

In this study, we counted the duration of azole exposure as the cumulative time of treatment. *A. fumigatus* was not always clinically isolated from patients during therapy; it was also isolated after the cessation of azole therapy. Because the selection pressure on azole-resistant *A. fumigatus* might be at its highest during the treatment, azole resistance might dissipate over time after therapy. Hence, we examined the relationship between the itraconazole MIC and the time from the end of itraconazole therapy to isolation. Of the 154 isolates, 42 had been exposed to itraconazole therapy before isolation. The time from the end of itraconazole treatment to isolation had no relationship with itraconazole susceptibility ($r = -0.1302$, $P = 0.4110$) (Fig. 3). Azole-resistant *A. fumigatus* was isolated even after azole treatment had been discontinued.

Clinical analysis of patients infected with azole-resistant *A. fumigatus*. Five patients were infected with azole-resistant *A. fumigatus*, and 16 isolates (including susceptible isolates) were obtained from these patients (Table 2). To analyze the genetic relationships among these 16 isolates, a panel of nine short tandem repeats for exact and high-resolution fingerprinting of *A. fumigatus* isolates was examined in this study. The 16 isolates obtained from the 5 patients were divided into 6 genotypes via microsatellite typing (Table 3).

Nine isolates were cultured from patient 1 (Table 2). *A. fumigatus* isolated in earlier periods was susceptible to azole, and it harbored the I266N mutation in the *cyp51A* gene; however, later isolates showed itraconazole or posaconazole resistance and new mutations such as G54E. Despite the discontinuation of itraconazole treatment, azole-resistant isolates were cultured from sputum of the patient 140 days after the end of the treatment (Table 2). All isolates were confirmed to be genetically homogeneous (Table 3).

From patient 2, three *A. fumigatus* isolates were cultured during days 115 to 132 of the itraconazole dosage period. The isolates were homogeneous; however, the itraconazole or posaconazole MICs and *cyp51A* mutations in the three isolates were significantly different (Tables 2 and 3). *A. fumigatus* isolates from patient 4 were heterogeneous.

DISCUSSION

In this study, we showed a correlation between the duration of clinical itraconazole exposure and the MICs of triazoles for *A. fumigatus*. It has already been reported that itraconazole exposure can induce the formation of azole-resistant *A. fumigatus* carrying a G54 mutation in the *cyp51A* gene *in vitro* (13). As expected,

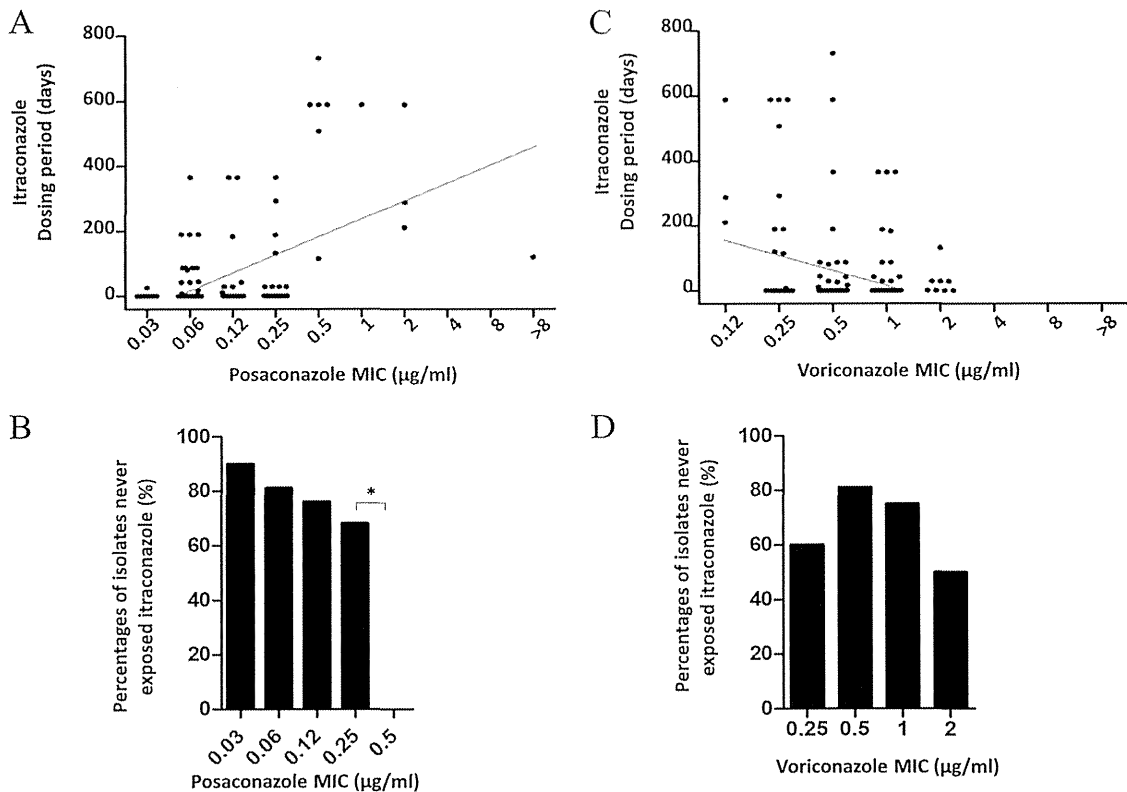

FIG 2 Relationship between the MICs of other triazoles and the history of itraconazole usage for the 154 *A. fumigatus* clinical isolates. (A) A positive correlation was observed between the itraconazole dosage period and the posaconazole MIC ($r = 0.5237$, $P < 0.0001$). (B) The number of itraconazole-naïve isolates decreased as the posaconazole MIC increased, particularly for posaconazole MICs exceeding 0.5 μg/ml (0.25 μg/ml versus 0.5 μg/ml, $P = 0.004$). (C) The correlation coefficient obtained from the scattergram of itraconazole usage and voriconazole MICs was small ($r = -0.2627$, $P = 0.001$). (D) No significant difference was observed in the percentages of itraconazole-naïve isolates and the individual MICs of voriconazole. *, $P < 0.05$ (Fisher's exact test).

increased use of itraconazole was associated with decreased itraconazole susceptibility among the *A. fumigatus* clinical isolates. The posaconazole susceptibility of the isolates was also decreased, presumably because of the appearance of G54 substitution in the *cyp51A* gene, indicating that clinicians should be careful when selecting posaconazole as an antifungal agent for the treatment of patients who had previously received long-term itraconazole therapy. If long-term itraconazole therapy induces voriconazole resistance in *A. fumigatus*, then this will have a significant impact on the treatment of aspergillosis. Our study indicated that itraconazole treatment did not induce voriconazole cross-resistance. These results were consistent with previous reports (15, 25). The reason for the lack of cross-resistance between itraconazole and voriconazole in this study was that the G54 mutation in azole-resistant isolates resulted in a resistance to itraconazole and posaconazole but not to voriconazole.

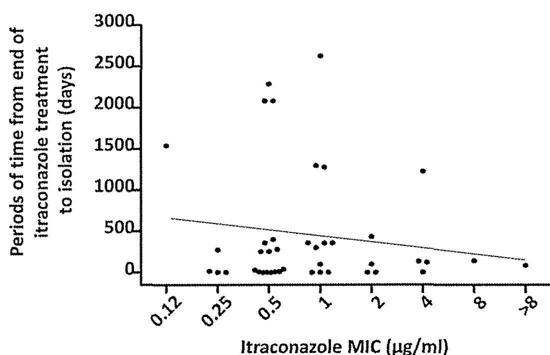

FIG 3 We examined the relationship between itraconazole MICs and the time from the end of itraconazole therapy to *A. fumigatus* isolation. Of the 154 isolates, 42 had been exposed to itraconazole before isolation. These isolates were analyzed for the relationship; however, the relationship could not be confirmed by the scatter plot ($r = -0.1302$, $P = 0.4110$).

The most important limitation of this study was that no data could be obtained regarding the serum concentration of itraconazole during its usage. Itraconazole has a relatively low bioavailability after oral administration, especially when given in capsule form (33). Of the 42 isolates exposed to itraconazole before isolation, 39 had been exposed to itraconazole capsules, and the remaining 3 isolates had been exposed to the oral solution, which has a greater bioavailability than the capsule form (5). Most patients who were administered the capsule form of itraconazole were prescribed a dose of 200 mg/day, which is the approved dose in Japan. Despite the lack of a report examining the presence of a mutation selection window for itraconazole by *A. fumigatus*, both the low bioavailability and blood concentration of itraconazole in capsule form might be risk factors for azole resistance. The solution form may overcome these disadvantages; however, patient 4, who was infected with posaconazole-resistant *A. fumigatus* carrying the G54W *cyp51A* mutation, had been administered the itraconazole oral solution at a dose of 200 mg/day for 210 days.

TABLE 3 Genotypes of the 16 *A. fumigatus* isolates by STR*Af*

Patient no.	Isolate no.	No. of tandem repeats at indicated microsatellite by STR*Af*[a]								
		2A	2B	2C	3A	3B	3C	4A	4B	4C
1	MF-368	23	15	10	25	11	32	8	10	7
	MF-367	23	15	10	25	11	32	8	10	7
	MF-370	23	15	10	25	11	32	8	10	7
	MF-439	23	15	10	25	11	32	8	10	7
	MF-452	23	15	10	25	11	32	8	10	7
	MF-454	23	15	10	25	11	32	8	10	7
	MF-460	23	15	10	25	11	32	8	10	7
	MF-468	23	15	10	25	11	32	8	10	7
	MF-469	23	15	10	25	11	32	8	10	7
2	MF-329	19	21	14	18	10	16	7	13	5
	MF-331	19	21	14	18	10	16	7	13	5
	MF-336	19	21	14	18	10	16	7	13	5
3	MF-357	18	19	23	34	13	20	18	9	8
4	MF-933	20	12	20	24	22	36	13	9	5
	MF-1011	11	21	11	28	12	31	18	9	10
5	MF-327	21	21	10	23	11	27	8	9	8

[a] Data represent the number of tandem repeats at the given microsatellite number.

Itraconazole oral therapy is often administered long-term for the treatment of chronic pulmonary aspergillosis (32). The judgment of treatment failure is still difficult; therefore, we need more information to decide whether itraconazole treatment should be continued. Despite the importance of the duration of itraconazole treatment with respect to the induction of azole resistance, few studies have investigated the relationship between azole resistance and azole exposure. Howard et al. reported that the duration of azole exposure before the identification of the first resistant isolate was 1 to 30 months, and the most commonly administered azole was itraconazole (17). Mortensen et al. also reported that patients with azole-resistant *A. fumigatus* isolates had received mold-active azoles for 11.5 to 69.5 months before the detection of resistant isolates (22). In our study, patients with azole-resistant *A. fumigatus* had been administered itraconazole for 3.8 to 24.3 months. These data are similar to those described above. Moreover, patients infected by *A. fumigatus* with itraconazole MICs < 2 μg/ml had been administered itraconazole for <1 year. Clinicians should be careful of the potential appearance of itraconazole-resistant isolates during long-term sequential itraconazole therapy administered for several months to more than 1 year.

Recently, Camps et al. reported that median time between collection of the last cultured wild-type isolate and the first azole-resistant isolate was 4 months (range, 3 weeks to 23 months) (8). In our study, the times between the last isolation of an azole-sensitive strain and the first appearance of an azole-resistant strain were about 10 and 7 months in patients 1 and 4, respectively (Table 2). These periods were longer than the median time reported by Camps et al. but fell within the reported range (3 weeks to 23 months).

We confirmed that long-term itraconazole therapy induced azole resistance in *A. fumigatus*. Even if azole-resistant mutants were dominant during treatment, their dominance could dissipate after cessation of the therapy because of the differences in the growth rates of the resistant and susceptible specimens (3). However, resistant isolates were still cultured 140 days after the cessation of azole therapy in patient 1. In patients 3 and 5, the times from the end of treatment to isolation were 1,223 and 435 days, respectively, which might indicate the possibility of the presence of resistant isolates for years after the end of azole therapy or the possibility of new infection. There were no differences in the growth rates of azole-resistant and azole-susceptible *A. fumigatus* isolates *in vitro* (data not shown). When patients receive long-term itraconazole therapy, clinicians should aggressively culture *A. fumigatus* from the patients and perform susceptibility tests even long after the cessation of itraconazole therapy.

We isolated azole-resistant *A. fumigatus* from clinical samples, such as sputum, but we did not isolate *A. fumigatus* from the environment or detect a TR/L98H mutant (28). It is interesting that the most common mechanism of resistance detected in this study was G54 substitution, because the selection pressure of itraconazole induces G54 mutation (13). Moreover, most resistant isolates detected in the environments around the world carry the TR/L98H substitution and no other mutation such as an G54 substitution (10, 23). These facts suggest that different azoles select different mutations. Itraconazole might selectively induce mutations such as G54 substitution, whereas some azoles used in agriculture may tend to select the TR/L98H mutation. The mechanisms of these differences remain to be completely elucidated. Further investigation is needed to clarify these mechanisms, and this knowledge may enable us to prevent the induction of the TR/L98H mutation in the environment.

In conclusion, this is the first report to show a detailed relationship between azole usage and azole MICs for *A. fumigatus*. Furthermore, we confirmed the presence of acquired resistance to itraconazole and posaconazole in a patient with chronic pulmonary aspergillosis after consecutive oral itraconazole treatments in Japan. The possibility of the presence of azole-resistant *A. fumigatus* should be considered during long-term itraconazole therapy in patients with chronic pulmonary aspergillosis.

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□ CASE REPORT □

A Case of Refractory Chronic Respiratory Tract Infection due to *Pseudomonas aeruginosa* Successfully Controlled by Combination of Clarithromycin and Azithromycin

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Abstract

The prognosis of patients with chronic respiratory tract infections, especially diffuse panbronchiolitis, is remarkably improved by long-term administration of low-dose macrolides. However, in some cases, patients are refractory to macrolide treatment and show a low or no response; therefore, new treatment strategies are required. Here we present a patient refractory to either single low-dose clarithromycin or azithromycin but responded remarkably to the combination usage of both macrolides.

Key words: chronic respiratory tract infection, *Pseudomonas aeruginosa*, clarithromycin, azithromycin

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Introduction

The prognosis of patients with refractory chronic respiratory tract infections such as diffuse panbronchiolitis, has been dramatically improved owing to the long-term administration of low-dose macrolides. There are some cases, however, that are refractory to such treatment, and newer or improved strategy of treatment is urgently required in clinical settings. We here report a case of refractory chronic respiratory infection caused by *Pseudomonas aeruginosa* that was not well controlled by administration of either single low-dose clarithromycin (CAM) or azithromycin (AZM) but good control was achieved by combined therapy with both macrolides.

Case Report

A 60-year-old woman, previously diagnosed with systemic lupus erythematosus, interstitial pneumonia due to collagen diseases, Sjögren syndrome, and antiphospholipid syndrome, in 1992, 1994, 2003, and 2008, respectively, was followed up clinically at the Department of Dermatology and Respiratory Medicine of Nagasaki University Hospital (NUH), Nagasaki, Japan. She had been taking oral prednisolone (5 mg/day) continuously for 16 years. Interstitial pneumonia gradually progressed, and the lungs showed a honeycomb appearance. The severity of clinical symptoms such as cough and sputum production had gradually increased since 2008. Moreover, the frequency of recurrences of chronic respiratory tract infection due to *P. aeruginosa* had increased since May 2008. Administration of oral CAM (200 mg/day) was initiated in August 2008. The patient was admitted to NUH on January 28, 2009, because the cough and sputum production worsened and she had a persistent high fever.

On admission, she was alert and vital signs were as follows: body temperature, 39.2℃; heart rate, 136 beats/min with a regular rhythm; SpO_2, 89% (on room air); respiratory rate, 24 breaths/min with regular rhythm; and blood pressure, 106/71 mmHg. Physical examination revealed emaciation (height =156.0 cm and body weight =42.5 kg) and diminished respiratory sounds with moist rales in both the

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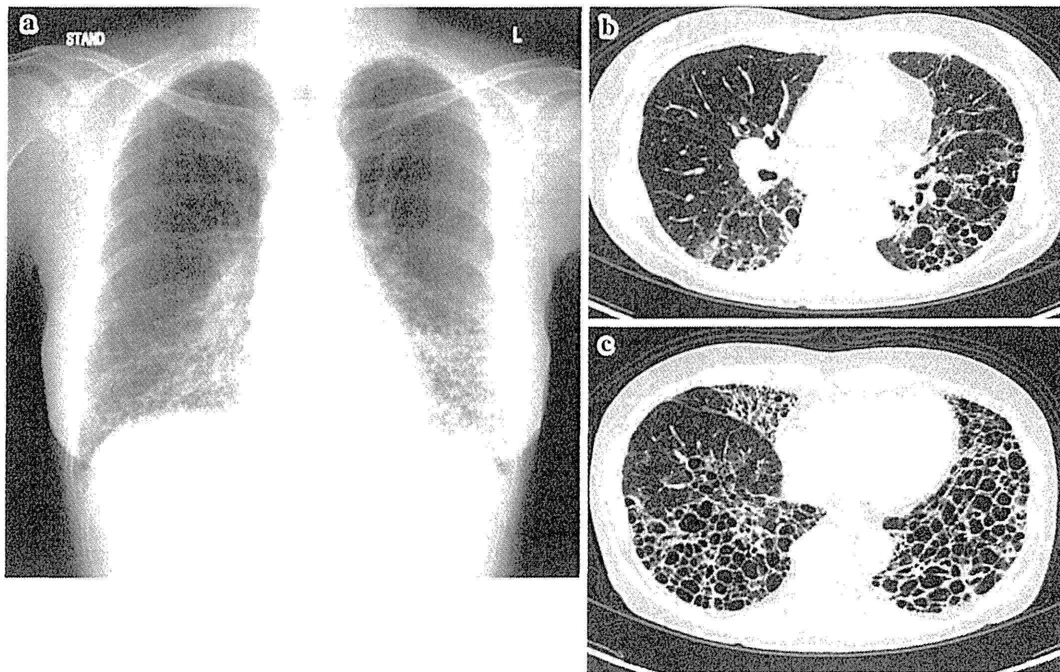

Figure 1. **Radiological findings on admission. a) Chest radiograph showing severe cystic and reticular shadows in both the right and left lower lung fields. b) and c) Chest computed tomography scan images showing the honeycomb appearance of the lungs.**

right and left lower lung fields. No signs of systemic lymphadenopathy, hepatosplenomegaly, or pre-tibial edema were observed. On admission, her white blood cell count was $11.1\times10^3/\mu L$ with a shift to the left (neutrophils, 83%) and C-reactive protein level was 10.1 mg/dL. The findings of the blood gas analysis were as follows: pH, 7.488; PCO_2, 34.9 torr; PO_2, 91.5 torr; and HCO_3^-, 25.9 mmol/L (O_2 nasal, 1.5 L/min). A microbiological test of the sputum revealed the presence of *P. aeruginosa* at 1×10^5 CFU/mL, and a drug susceptibility test indicated that the minimum inhibitory concentrations (MICs) of gentamycin, ciprofloxacin, and meropenem were 2.0, <0.25, and <0.25 μg/mL, respectively. Chest radiographs showed severe cystic shadows in both the right and left lower lung fields. Computed tomography scans showed a honeycomb appearance of the lungs (Fig. 1). Fig. 2 illustrates the clinical course of this case. Recurrence of chronic respiratory infection was diagnosed on admission, and the administration of tazobactam/piperacillin (4.5gx4/day) was started. Her clinical symptoms and fever were rapidly recovered and tazobactam/piperacillin was continued for 14 days then the patient was discharged. Three days after the discharge, however, the patient was re-admitted to NUH because of high fever. Refractory chronic pulmonary infection due to *P. aeruginosa* was diagnosed again. Although inhaled tobramycin with intravenous ciprofloxacin, followed by tazobactam/piperacillin with intravenous amikacin, and colistin were administered, she did not recover completely. As for long-term macrolide treatment, the previously administered CAM at 200 mg/day was switched to AZM at 250 mg/day every other day; however, this treatment was not effective at all. A single treatment with AZM was not effective; therefore, in August 2009, we initiated combined therapy with CAM at 400 mg/day and AZM at 250 mg/day once daily. This combined treatment reduced the event of high fever but low grade fever continued occasionally after December, 2010. The patient has been receiving both CAM and AZM for 2 years, and only 1 apparent episode of recurrence of chronic respiratory infection which required hospitalization and intravenous antibiotics administration (meropenem and ciprofloxacin for a week in January 2010) has been observed. Combined treatment with CAM and AZM is currently administered at the outpatient clinic. Additionally, an increase in the sensitivity of *P. aeruginosa* to almost all anti-*Pseudomonas* antimicrobial agents was observed after combined administration of anti-pseudomonas antimicrobial agents (Table 1).

Discussion

Long-term administration of low-dose erythromycin treatment, established by Kudoh et al., has remarkably improved the prognosis of patients with diffuse panbronchiolitis (1, 2). Apart from their anti-microbial activity, macrolides were found to have immunomodulatory effects, and these effects were extensively studied in Japan. These macrolides have been found to be highly effective in (a) reducing the amount of sputum produced via suppression of mucin secretion by blocking chloride channels of bronchial epithelial cells (3, 4); (b) blocking and inhibiting the accumulation of neutrophils and lymphocytes, neutrophil elastase activity, cytokine production, and adherence to cells (5-7); (c) decreasing and disrupting biofilm formation by *P. aeruginosa* (8); and (d) suppressing *P. aeruginosa* quorum-sensing systems as cell-to-cell communication (9). In fact, these effects work

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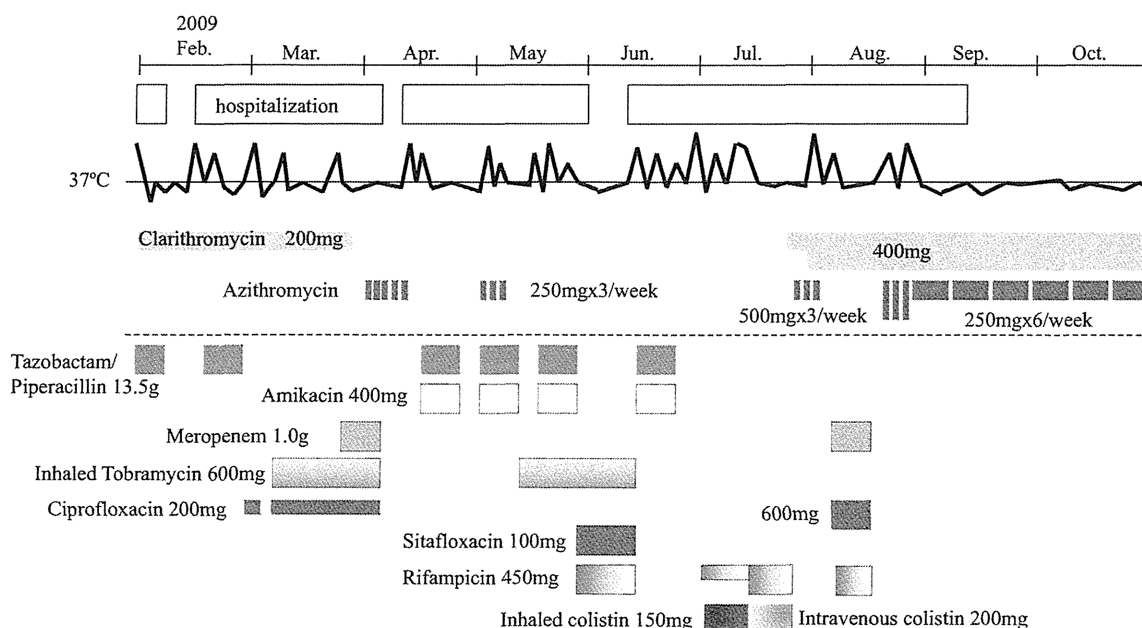

Figure 2. **The clinical course of the patient. Fever was not reduced by either single low-dose clarithromycin or azithromycin, but the patient did markedly respond to the combination of both macrolides.**

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).

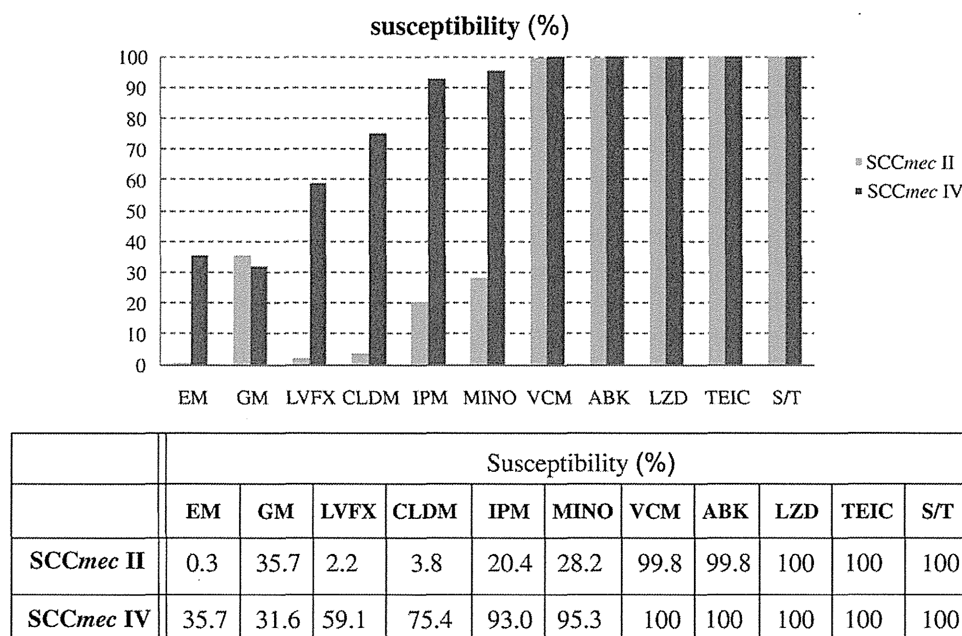
3.2. Distribution of SCCmec types and pathogenic genes

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, ≥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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