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Abstract

The incidence of nosocomial infections with methicillin-resistant Staphylococcus aureus is of great concern in Japan and the developed world as a whole. Simple typing techniques like coagulase and phage typing are quick and useful for monitoring and evaluating these organisms. In view of this, the current status of antimicrobial susceptibility in Staphylococcus aureus (S. aureus) isolates in Okinawa typed by coagulase and phage typing was studied. Of 508 isolates, methicillin-resistant S. aureus (MRSA) comprised 54.3% (minimum inhibitory concentration (MIC) $>$ or = 16 micrograms/ml). Coagulase type II and phage group III were the most prevalent, comprising 65.2% and 38%, respectively. These were followed by phage non-typable group and coagulase type III with 36.6% and 12.7%, respectively. Compared to a previous study conducted in 1989, there has been an increase of about 17% in the MRSA isolation rate with a concomitant increase of about 11% in the coagulase type II MRSA isolation rate and a decrease of about 27% in the isolation rate of coagulase type III MRSA. Using a panel of 16 antibiotics, coagulase type II MRSA were resistant to all except Arbekacin and Vancomycin. Arbekacin and Vancomycin were the sole antibiotics to which resistance was not expressed by any of the isolates. With regard to the methicillin-sensitive S. aureus (MSSA), coagulase type III and phase group III were the most prevalent, comprising 25.9% and 32.3%, respectively.

KEYWORDS: coagulase type, phage type, antibiotic, MRSA, MSSA

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Current Status of Antimicrobial Susceptibility in MRSA Isolates Typed by Coagulase and Phage Typing in Okinawa

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The incidence of nosocomial infections with methicillin-resistant *Staphylococcus aureus* is of great concern in Japan and the developed world as a whole. Simple typing techniques like coagulase and phage typing are quick and useful for monitoring and evaluating these organisms. In view of this, the current status of antimicrobial susceptibility in *Staphylococcus aureus* (*S. aureus*) isolates in Okinawa typed by coagulase and phage typing was studied. Of 508 isolates, methicillin-resistant *S. aureus* (MRSA) comprised 54.3% (minimum inhibitory concentration (MIC) ≧ 16 μg/ml). Coagulase type II and phage group III were the most prevalent, comprising 65.2% and 38%, respectively. These were followed by phage non-typable group and coagulase type III with 36.6% and 12.7%, respectively. Compared to a previous study conducted in 1989, there has been an increase of about 17% in the MRSA isolation rate with a concomitant increase of about 11% in the coagulase type II MRSA isolation rate and a decrease of about 27% in the isolation rate of coagulase type III MRSA. Using a panel of 16 antibiotics, coagulase type II MRSA were resistant to all except Arbekacin and Vancomycin. Arbekacin and Vancomycin were the sole antibiotics to which resistance was not expressed by any of the isolates. With regard to the methicillin-sensitive *S. aureus* (MSSA), coagulase type III and phage group III were the most prevalent, comprising 25.9% and 32.3%, respectively.

Key words: coagulase type, phage type, antibiotic, MRSA, MSSA

The emergence and spread of multiple-antibiotic-resistant *Staphylococcus aureus* strains is of great concern worldwide (1-4). This characteristic is often associated with methicillin-resistant *S. aureus* (MRSA) which are noted for their nosocomial infections (5-7). Once these strains colonize a health institution, their eradication becomes an insurmountable problem.

Studies conducted in Okinawa and elsewhere in Japan indicate an increase in the isolation of coagulase type II MRSA in association with nosocomial infections (4, 8, 9). In Hiroshima University Hospital, for example, a study conducted in 1987 showed that over 97% of the isolates classified as highly resistant to methicillin (MIC > 100 μg/ml) were of coagulase type II (10). Many of the reports indicate that there have been changes in the most prevalent types of isolates (10-13).

The present study was conducted to evaluate any changes in Okinawa in the types of isolates and the current antimicrobial susceptibility status of *S. aureus* classified by coagulase and phage typing, with focus on the coagulase type II MRSA.

Materials and Methods

Bacterial isolates. A total of 508 *S. aureus* strains isolated in 1992 from patients in six major hospitals, geographically dispersed on the main island of Okinawa, were used for the study. These were selected on the basis of one isolate per patient.

Chemicals and reagents. Rabbit antisera against the eight coagulase types (I-VIII) and rabbit lyophilized plasma were purchased from Denka Seiken Co. (Tokyo, Japan). Bovine fibrinogen as well as tranexamic acid, acacia powder, glycerin and iron fillings (reduced) were obtained from Nacalai Tesque Inc. (Kyoto, Japan).

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Trisodium citrate was from Kishida Chemical Co., Ltd. (Osaka, Japan). Sodium azide was purchased from Katayama Chemical Co. (Osaka, Japan).

Antibiotics. Aminobenzyl penicillin (ABPC), methicillin (DMPPC), penicillin G (PCG), amikacin (AMK), and imipenem (IPM) were donated by Banyu Pharmaceutical Co., Ltd. (Tokyo, Japan). Cefazolin (CEZ), ceftizoxime (CZX), and cloxacillin (MCIPC) were donated by Fujisawa Pharmaceutical Co., Ltd. (Osaka, Japan). Gentamicin (GM), tobramycin (TOB), and vancomycin (VCM) were donated by Shionogi Pharmaceutical Co., Ltd. (Osaka, Japan). Minocycline (MINO) was donated by Japan Lederle Co., Ltd. (Tokyo, Japan). Ofloxacin (OFLX) was donated by Daiichi Pharmaceutical Co., Ltd. (Tokyo, Japan). Clindamycin (CLDM) was donated by Japan Upjohn Co., Ltd. (Tokyo, Japan). Erythromycin (EM) was donated by Dai Nippon Pharmaceutical Co., Ltd. (Osaka, Japan). Arbekacin (ABK) was donated by Meiji Seika Co., Ltd. (Tokyo, Japan).

Media. Mueller-Hinton agar (DIFCO Laboratories) was used for susceptibility testing. For methicillin susceptibility, 3% NaCl was incorporated into the medium.

Solutions. The diluent solution for coagulase typing consisted of 20 g/l polypeptone, 10 g/l trisodium citrate, 1 g/l tranexamic acid, 4 g/l gum arabic (acacia powder), and 1 g/l sodium azide. Bovine fibrinogen was dissolved in 50% (v/v) glycerin solution to a final concentration of 40 g/l. Equivalent volumes of fibrinogen solution and rabbit plasma (reconstituted with a half volume of water) were mixed together. This was then dispensed in small aliquots and kept at −20℃ until use, when it was diluted five times with the diluent solution.

Serotyping of coagulase. The method for serotyping coagulase described by Tajima *et al.* was used (14). Briefly, supernatant from an overnight culture of the organisms in brain heart infusion (BHI) broth was added to eight specific coagulase type antisera in a file of wells on a microtiter plate. After incubation for an hour, iron fillings and fibrinogen enriched rabbit plasma were added to each well and incubated for up to 12 h. The results were then read by positioning the plate on a magnetic stirer to observe the movement of the iron fillings in the absence of a clot.

Bacteriophage typing. The basic international set of 23 phages at routine test dilution (RTD) and 100 times RTD was employed according to standard procedures to type the isolates (15).

Antimicrobial susceptibility testing. The minimum inhibitory concentration (MIC) was determined by the agar dilution method (16). The antibiotic concentration range employed for the testing was 0.125-128 μg/ml. A multi-spot innoculator (Sakuma Manufacturing Co., Ltd., Tokyo, Japan) delivering approximately 5 μl of 10^6 CFU/ml was used to innoculate the antibiotic containing plates with an overnight culture of the organisms. These were incubated at 37℃ overnight. One set of DMPPC plates was incubated at 30℃ (17). Growth was considered to have occurred at a given dilution if more than five colonies were visible.

Statistical analysis. The MIC patterns of the various coagulase and phage groups were compared by the chi square method. A P value < 0.05 was considered significant.

Results

Fifty-four percent of the isolates were classified as resistant to methicillin (MIC $\geq 16\,\mu$g/ml) as shown in Table 1. According to coagulase type grouping (Table 1), coagulase type II MRSA was the most frequently isolated (65.2%). This was followed by coagulase type III with an isolation rate of 12.7%, and then coagulase type I with a rate of 10.9%. Coagulase type VI MRSA was not isolated. Coagulase type II MRSA was isolated mainly from inpatients. In the case of MSSA, coagulase type III was the most frequently isolated, with a rate of 25.9%. This was followed by coagulase type VII with 18.5%. Compared with coagulase type II which comprised 16.7% MSSA of 216 isolates, 63.2% of the 95 coagulase type III isolates were MSSA. Of the coagulase type VII isolates, 91.5% of 47 isolates were MSSA.

By phage typing, phage group III type MRSA was the most prevalent at 38% (Table 2). The second was the phage non-typable group which constituted 36.6%, followed by the mixed phage group (which was made up mostly of phages of groups I and III) at 17.4%. For MSSA, phage group III type was the most prevalent at 32.3%, followed by the phage non-typable group at 23.7%.

The isolation rates for the various coagulase types differentiated by phage groups are presented in Figs. 1, 2 for MRSA and MSSA, respectively. Considering the coagulase types and phage groupings together, coagulase type II and phage non-typable MRSA yielded 30.8%,

Table 1 Prevalence of *Staphylococcus aureus* by coagulase types

	Number of isolates										Total
	Coagulase types										
	I	II	III	IV	V	VI	VII	VIII	MX	NT	
MRSA[a]	30	180*	35	5	8	0	4	4	8	2	276
	(10.9)	(65.2)	(12.7)	(1.8)	(2.9)	(0.0)	(1.4)	(1.4)	(2.9)	(0.7)	(100.0)
MSSA	13	36	60	20	23	10	43	9	17	1	232
	(5.6)	(15.5)	(25.9)	(8.6)	(9.9)	(4.3)	(18.5)	(3.9)	(7.3)	(0.4)	(100.0)
Total	43	216	95	25	31	10	47	13	25	3	508
	(8.5)	(42.5)	(18.7)	(4.9)	(6.1)	(2.0)	(9.3)	(2.6)	(4.9)	(0.6)	(100.0)

*: $P < 0.05$, [a]: This comprised 54.3% of the *Staphylococcus aureus* isolates, Figures in parentheses indicate percent. MX: Mixed type; NT: Non-typable; MRSA: Methicillin-resistant *S. aureus*; MSSA: Methicillin-sensitive *S. aureus*.

Table 2 Prevalence of *Staphylococcus aureus* by phage types

	Number of isolates							Total
	Phage types							
	I	II	III	IV	V	MX	NT	
MRSA	10	11	105*	1	0	48	101	276
	(3.6)	(4.0)	(38.0)	(0.4)	(0.0)	(17.4)	(36.6)	(100.0)
MSSA	30	22	75	1	7	42	55	232
	(12.9)	(9.5)	(32.3)	(0.4)	(3.0)	(18.1)	(23.7)	(100.0)
Total	40	33	180	2	7	90	156	508
	(7.9)	(6.5)	(35.4)	(0.4)	(1.4)	(17.7)	(30.7)	(100.0)

*: $P < 0.05$, Figures in parentheses indicate percent. MRSA, MSSA, MX, and NT: See Table 1.

Table 3 Pattern of MRSA with antibiotic minimum inhibitory concentration (MIC) $\geq 16\mu g/ml$ classified by coagulase types

Type[b]	No.	Percent of isolates resistant to antibiotics[a] tested														
		ABPC	DMPPC	MCIPC	PCG	CEZ	CZX	GM	ABK	AMK	TOB	MINO	IPM	OFLX	EM	CLDM	VCM
I	30	70	100	13.3	83.3	60	96.7	93.3	0	26.7	100	6.7	3.3	20	80	16.7	0
II	180	93.3	100	77.8	96.7	92.2	95	69.4	0	24.4	89.4	51.1	52.8	26.1	75	65.6	0
III	35	91.4	100	62.9	94.3	91.2	100	80	0	8.6	71.4	14.3	14.3	54.3	25.7	20	0
IV	5	60	100	40	60	60	80	60	0	20	60	40	20	0	80	60	0
V	8	12.5	100	0	25	87.5	87.5	100	0	25	100	0	12.5	12.5	50	50	0
VI	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
VII	4	100	100	50	100	75	100	75	0	50	100	50	25	0	100	50	0
VIII	4	75	100	75	75	100	75	50	0	25	100	50	75	25	75	50	0
MX	8	50	100	50	50	87.5	87.5	75	0	25	100	12.5	12.5	37.5	62.5	50	0
NT	2	100	100	100	100	100	100	50	0	0	50	50	50	0	50	50	0
Total	276	85.9	100	64.6	90.3	87.7	94.9	74	0	22.7	88.4	39	39.4	27.8	68.6	53.1	0

No.: Number of isolates tested; [a]: Abbreviations for antibiotics are shown in Materials and Methods. [b]: Coagulase type; MRSA: See Table 1.

followed by coagulase type II and phage group III MRSA at 25.4%, and then coagulase type III and phage group III MRSA at 9.8% of 276 isolates. Eighty-four percent of the phage non-typable MRSA were coagulase type II isolates. The most prevalent MSSA was coagulase type III and phage group III type with 18.1% and then coagulase type VII and phage non-typable MSSA with 9.9% of 232 isolates.

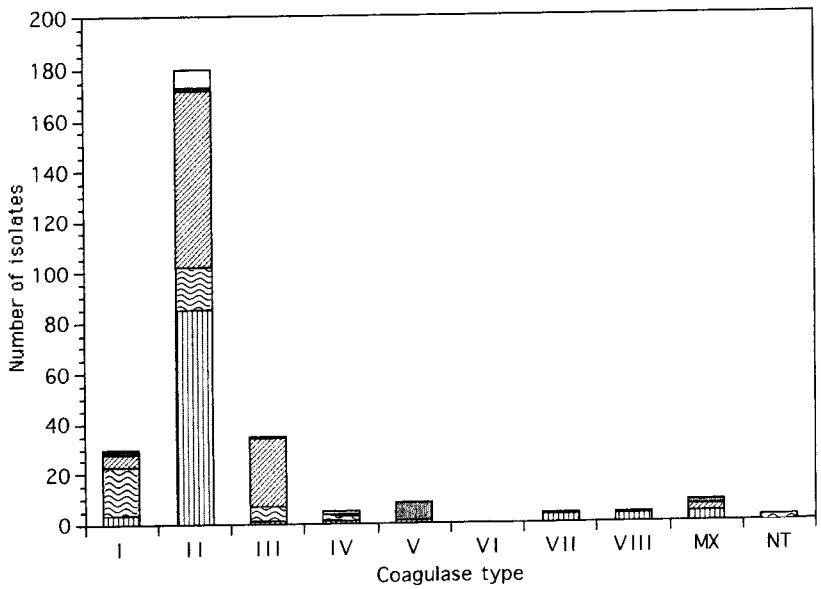

Fig. 1 Number of isolates of various MRSA phage groups categorized by coagulase type. The legend characters I, II, III, IV, V, MX and NT indicate the phage groups (□ I ■ II ▨ III ⊞ IV ▤ V ▧ MX ▥ NT). MX: Mixed type; NT: Non-typable; MRSA: Methicillin-resistant *S. aureus*.

Table 4 Pattern of MSSA isolates with antibiotic MIC ≥ 16 μg/ml classified by coagulase type

Type[b]	No.	Percent of isolates resistant to antibiotics[a] tested														
		ABPC	DMPPC	MCIPC	PCG	CEZ	CZX	GM	AMK	TOB	MINO	IPM	OFLX	EM	CLDM	VCM
I	13	0	0	0	23.1	0	76.9	69.2	7.7	69.2	0	0	0	38.5	0	0
II	36	19.4	0	2.8	66.7	2.8	44.4	19.4	8.3	25	5.6	5.6	0	16.7	5.6	0
III	60	16.7	0	0	35	3.3	56.7	15	8.3	26.7	11.7	0	10	8.3	5	0
IV	20	20	0	5	40	10	45	20	5	15	5	5	0	10	10	0
V	23	13	0	0	4.3	0	43.5	26.1	0	30.4	0	0	0	4.3	0	0
VI	10	10	0	0	30	0	40	10	0	10	0	0	10	10	0	0
VII	43	7	0	0	11.6	0	51.2	11.6	9.3	9.3	0	2.3	9.3	0	0	0
VIII	9	11.1	0	0	0	0	44.4	0	0	0	0	0	0	0	0	0
MX	17	5.9	0	0	23.5	0	35.3	17.6	23.5	23.5	5.9	0	0	5.9	0	0
NT	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	232	12.9	0	0.9	29.7	2.2	49.6	7.3	7.8	22.8	4.7	1.7	4.7	9.1	3	0

No.: Number of isolates tested; [a]: Abbreviations of antibiotics are shown in Meterials and Methods. [b]: Coagulase type; MSSA: See Table 1.

Using MIC ≥ 16 μg/ml as the cut-off, above which isolates are considered to be resistant to the antibiotics, the results obtained by coagulase type alone for resistant strains are shown in Tables 3 and 4 for MRSA and MSSA, respectively. Resistance was seen to all antibiotics tested except ABK and VCM. The frequency of MRSA-resistant strains was very high for some antibiotics such as CZX (94 %), PCG (90 %), ABPC (85 %), and GM (74 %).

The MIC profile for the three most prevalent MRSA, coagulase type II/phage non-typable group, coagulase type II/phage group III, and coagulase type III/phage group III, are presented in Tables 5, 6 and 7, respectively. Those for coagulase type III/phage group III, and

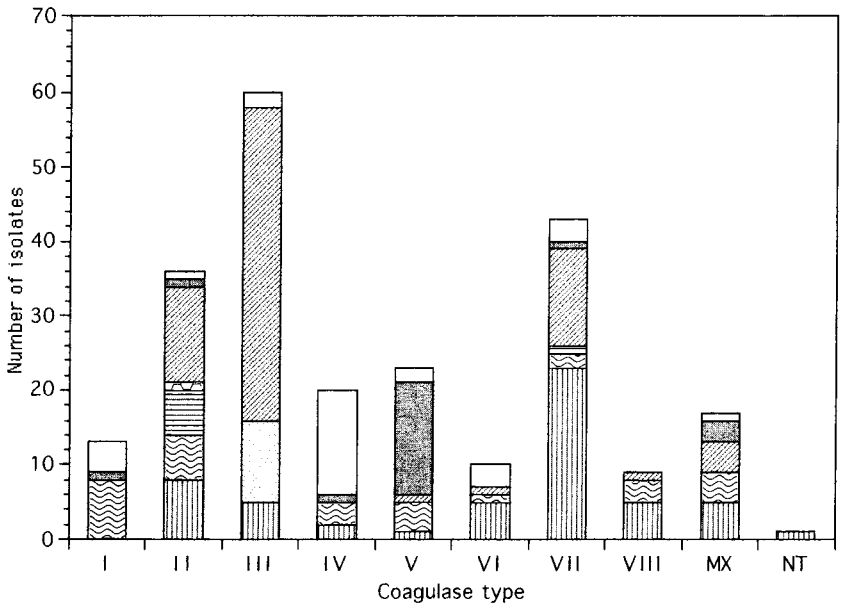

Fig. 2 Number of isolates of various methicillin-sensitive *S. aureus* (MSSA) phage groups categorized by coagulase type. The legend characters I, II, III, IV, V, MX and NT indicate the phage groups (□ I ▩ II ▨ III ⊞ IV ▤ V ▧ MX ▥ NT). MX, MT: See Fig. 1.

Table 5 MIC pattern of coagulase type II and phage non-typable group MRSA

Antibiotics	No. tested	MIC(μg/ml)			Rate of resistance (MIC ≧ 16μg/ml)
		MIC_{50}	MIC_{80}	MIC_{90}	
ABPC	85	32.0	64.0	64.0	98.8
DMPPC	85	>128.0	>128.0	>128.0	100.0
MCIPC	85	64.0	>128.0	>128.0	78.8
PCG	85	32.0	64.0	64.0	100.0
CEZ	85	>128.0	>128.0	>128.0	92.9
CZX	84	>128.0	>128.0	>128.0	98.8
GM	85	32.0	64.0	>128.0	65.9
AMK	85	8.0	16.0	16.0	25.9
TOB	85	128.0	>128.0	>128.0	90.6
MINO	85	16.0	32.0	32.0	52.9
IPM	84	16.0	32.0	64.0	57.1
OFLX	85	8.0	8.0	16.0	15.3
EM	85	>128.0	>128.0	>128.0	76.5
CLDM	85	>128.0	>128.0	>128.0	68.2
VCM	85	1	1	1	0.0

Abbreviations: See Tables 1 and 3.

Table 6 MIC pattern of coagulase type II and phage group III MRSA

Antibiotics	No. tested	MIC(μg/ml)			Rate of resistance (MIC ≧ 16μg/ml)
		MIC_{50}	MIC_{80}	MIC_{90}	
ABPC	70	32.0	64.0	64.0	98.6
DMPPC	70	>128.0	>128.0	>128.0	100.0
MCIPC	70	64.0	128.0	>128.0	78.6
PCG	70	64.0	64.0	64.0	100.0
CEZ	70	128.0	>128.0	>128.0	98.6
CZX	67	>128.0	>128.0	>128.0	100.0
GM	70	64.0	64.0	>128.0	78.6
AMK	70	8	16	16	25.7
TOB	70	128.0	>128.0	>128.0	94.3
MINO	70	16	32	32	60.0
IPM	69	16	32	32	56.5
OFLX	70	8	32	32	31.4
EM	70	>128.0	>128.0	>128.0	75.7
CLDM	70	>128.0	>128.0	>128.0	70.0
VCM	70	1	1	2	0.0

Abbreviations: See Tables 1 and 3.

coagulase type VII/phage non-typable MSSA are presented in Tables 8 and 9, respectively. The antibiotic MIC patterns (except OFLX) of the coagulase type II/phage non-typable group, and the coagulase type II/

Table 7 MIC pattern of coagulase type III and phage group III MRSA

Antibiotics	No. tested	MIC (μg/ml)			Rate of resistance (MIC $\geqq$ 16 μg/ml)
		MIC_{50}	MIC_{80}	MIC_{90}	
ABPC	27	32.0	64.0	64.0	88.8
DMPPC	27	64.0	64.0	128.0	100.0
MCIPC	27	32.0	32.0	128.0	70.4
PCG	27	32.0	64.0	64.0	96.3
CEZ	27	128.0	128.0	128.0	92.6
CZX	27	> 128.0	> 128.0	> 128.0	100.0
GM	27	64.0	64.0	128.0	77.8
AMK	27	4.0	8.0	8.0	7.4
TOB	27	32.0	64.0	128.0	74.1
MINO	27	0.25	0.5	32.0	11.1
IPM	27	2.0	4.0	16.0	14.8
OFLX	27	16.0	32.0	32.0	59.3
EM	27	0.5	128.0	> 128.0	22.2
CLDM	27	0.125	0.25	> 128.0	18.5
VCM	27	1.0	1.0	1.0	0.0

Abbreviations: See Tables I and 3.

Table 8 MIC pattern of coagulase type III and phage group III MRSA

Antibiotics	No. tested	MIC (μg/ml)			Rate of resistance (MIC $\geqq$ 16 μg/ml)
		MIC_{50}	MIC_{80}	MIC_{90}	
ABPC	42	4.0	8.0	16.0	16.7
DMPPC	42	2.0	4.0	4.0	0.0
MCIPC	42	0.5	1.0	1.0	0.0
PCG	42	8.0	16.0	16.0	31.0
CEZ	42	1.0	2.0	2.0	0.0
CZX	39	16.0	32.0	64.0	66.7
GM	42	0.25	8.0	32.0	16.7
AMK	42	2.0	4.0	8.0	9.5
TOB	42	0.5	16.0	16.0	33.3
MINO	42	0.25	0.25	16.0	14.3
IPM	42	0.125	0.125	0.125	0.0
OFLX	42	0.5	8.0	32.0	14.3
EM	42	0.5	1.0	128.0	11.9
CLDM	42	0.125	0.25	0.25	9.5
VCM	42	1.0	1.0	1.0	0.0

Abbreviations: See Tables I and 3.

phage group III types were similar ($P > 0.05$). Considering Tables 6 and 7, there were however obvious differences (especially in sensitivity to AMK, MINO, IPM, EM, and CLDM) between the coagulase type II/phage group III, and coagulase type III/phage group III types ($P < 0.05$). This was also reflected by the MIC modal frequency values and the percentages of isolates with MIC > 128 μg/ml (data not shown). Hence the antibiotic resistance pattern was more conveniently differentiated when categorized by coagulase type than by phage group.

Table 9 MIC pattern of coagulase type VII and phage non-typable group MSSA

Antibiotics	No. tested	MIC (μg/ml)			Rate of resistance (MIC $\geqq$ 16 μg/ml)
		MIC_{50}	MIC_{80}	MIC_{90}	
ABPC	23	1.0	2.0	4.0	0.0
DMPPC	23	2.0	4.0	4.0	0.0
MCIPC	23	0.25	0.5	1.0	0.0
PCG	23	1.0	2.0	4.0	0.0
CEZ	23	1.0	2.0	2.0	0.0
CZX	21	16.0	16.0	32.0	52.4
GM	23	0.5	0.5	0.5	8.7
AMK	23	2.0	2.0	4.0	8.7
TOB	23	0.25	0.5	0.5	8.7
MINO	23	0.25	0.25	0.5	0.0
IPM	23	0.125	0.125	0.125	0.0
OFLX	23	0.5	0.5	32.0	13.0
EM	23	0.25	0.5	0.5	0.0
CLDM	23	0.125	0.125	0.125	0.0
VCM	23	1.0	1.0	1.0	0.0

Abbreviations: See Tables I and 3.

Though coagulase type II MRSA comprised the majority in five of the hospitals studied, in the hospital designated as "A" (Table 10), coagulase type I constituted the majority. As shown in Table 10, both the phage group III and phage non-typable coagulase type II MRSA constituted only 13.7 % of the total MRSA in hospital A, while it constituted over 55 % in the other hospitals.

Discussion

The prevalence of coagulase type II MRSA in the study population is consistent with the earlier study conducted in 1989 and a study from the mainland of Japan (4, 8). Considering the two studies, there has been an appreciable increase of 17 % in the MRSA isolation rate, with a concomitant increase of about 11 % in the isolation rate of coagulase type II MRSA and a decrease of 27 % in the isolation rate of coagulase type III MRSA.

The differences in the coagulase and phage types as

Table 10 Prevalent *Staphylococcus aureus* strains by Institution

Institution	Coagulase type / Phage type						
	MRSA				MSSA		
	II / NT	II / III	III / III	Total	III / III	VII / NT	Total
A	3	1	0	29	3	2	18
	(10.3)	(3.4)	(0.0)	(100.0)	(16.7)	(11.1)	(100.0)
B	12	15	3	48	7	6	50
	(25.0)	(31.3)	(6.3)	(100.0)	(14.0)	(12.0)	(100.0)
C	9	8	4	29	18	10	63
	(31.0)	(27.6)	(13.8)	(100.0)	(28.6)	(15.9)	(100.0)
D	28	15	5	61	4	1	24
	(45.9)	(24.6)	(8.2)	(100.0)	(16.7)	(4.2)	(100.0)
E	20	20	11	66	4	3	27
	(30.3)	(30.3)	(16.7)	(100.0)	(14.8)	(11.1)	(100.0)
F	13	11	4	43	6	3	50
	(30.2)	(25.6)	(9.3)	(100.0)	(12.0)	(6.0)	(100.0)

Figures in parentheses indicate percent. Abbreviations: See Table 1.

well as the percentage contributions to the respective totals of the most prevalent MRSA and MSSA (Table 10) suggest that the MRSA are nosocomial strains, and are not due to drug selection of the indigenous bacteria haboured by the patients. It is, therefore, clear that coagulase type II MRSA is becoming established as a nosocomial strain not only in Okinawa, but also in other parts of Japan (4).

Based on MIC_{80} all the isolates were classified as susceptible to ABK and VCM. The coagulase type II MRSA isolates were classified as susceptible to only ABK and VCM. As shown in Table 3, these were the most multiple-antibiotic resistant strains. Coagulase type III MRSA were classified as susceptible to AMK, MINO, IPM, CLDM, ABK and VCM. The expression of multiple antibiotic resistance by coagulase type II MRSA may enhance their selection in the hospital environment. The selection and increase in coagulase type II MRSA isolation may contribute to the increase in the overall isolation rate of MRSA. Though moderately susceptible to AMK and OFLX (75.6 % and 73.9 %, respectively), the variability in their susceptibility to these antibiotics necessitates the determination of the antibiogram for each clinical isolate. This practice would also help to minimize selection of antibiotic resistant strains due to drug use. It is reported for example that the use of broad spectrum antibiotics is associated with the spread of MRSA (18, 19). It is also reported that improper use of third generation cephem derivative antibiotics has played a major role in producing multi-drug resistant bacteria (20). Though coagulase type II MRSA constituted the majority in five of the hospitals studied, coagulase type I MRSA (which was the least multiple antibiotic-resistant among the three most prevalent coagulase types) was the most prevalent in one hospital (A in Table 10) where penicillins and first generation cephems are given priority in chemotherapy. As shown in Table 10, both phage group III and phage non-typable coagulase type II MRSA comprised only 13.7 % of the total MRSA in the hospital A, while it constituted over 55 % in the other hospitals.

The results show that coagulase typing depicts a broader scope in categorizing the isolates as compared to phage grouping, and thus it complements phage typing. The differences observed in the antibiotic MIC pattern between coagulase types in the same phage group, rather than between phage groups of the same coagulase type, imply that the strains can be studied more closely using coagulase typing (Tables 5–7). The usefulness of coagulase typing is also shown by the results as for example 47 % of the coagulase type II MRSA were non-typable by phage typing (Fig 1). A similar trend has been reported by other investigators (21). Studies conducted in our laboratory over the years have shown that the phage non-typable MRSA have increased from a small percentage to the current 36.6 %. Almost half of the phage-typable isolates were lysed only at 100 times RTD. There have also been changes in the phage lytic patterns of the

predominant population of the isolates. Lytic patterns (54, 77) and 83A in phage group III tended to be isolated most frequently, compared with phage patterns 83A and (84, 85) in previous times. This variability may be due to multiple introductions into the study population or to the selection of preexisting strains due to drug pressure. For the latter case, the variability in the antibiotic susceptibility pattern of the isolates may indicate evolving drug resistance acquisition due to the different types of antibiotics they are exposed to, depending on the source of isolation. Our results showed two types of isolates with regard to GM susceptibility: the susceptible group had a mean MIC of $0.5\,\mu g/ml$, while the resistant group had a mean MIC of $64\,\mu g/ml$. A similar pattern was observed for MINO. For CLDM, there were two distinct classes: those with MIC $\leq 0.5\,\mu g/ml$, and the others with MIC $\geq 128\,\mu g/ml$. It is suggested, for example, that the treatment of patients habouring MRSA with GM may subsequently contribute to GM resistance in the MRSA (22, 23).

The search for new and potent antibiotics may not necessarily be the ultimate solution to the containment of these multiple antibiotic-resistant organisms, but a rigorous observance of monitoring and recommended regimen to hold the spread and establishment of these organisms in check may be in order (20, 24, 26). Though the isolation rate of coagulase type VII MRSA was comparatively low (1.4 %), the fact that it was susceptible to only OFLX, IPM, ABK, and VCM means that care has to be taken to prevent it from becoming established as a nosocomial strain. In Saga Medical School Hospital for example, it is reported that the prevalent strain changed from coagulase type II to type VII in 1990 (11).

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