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Epidemiological Study of *Staphylococcus aureus* Isolated from Six Hospitals in Okinawa

Dela Kwasi Lotsu* and Teisuke Imamura*,**

*Department of Microbiology and **Research Center of Comprehensive Medicine, School of Health Sciences, University of the Ryukyus, 207 Uehara, Nishihara, Okinawa 903-01

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ABSTRACT

The current status of coagulase types of *Staphylococcus aureus* in six major hospitals in Okinawa was investigated. Five hundred and thirteen strains of *S. aureus* isolated during the period from June to December 1992 were studied. The isolation rate for methicillin-resistant *S. aureus* (MRSA) ranged from 31%- 71.8% for the hospitals. Coagulase type II MRSA was the most prevalent isolate constituting 65.3% of the MRSA. This was followed by coagulase type III with 12.6%. Earlier studies in 1989 indicated 54% and 40% isolation rates for coagulase types II and III MRSA respectively. MRSA of all types, except VI, were isolated from all the hospitals, except hospital A which lacked type VIII. Coagulase type V MRSA were isolated only from hospitals A and E. Methicillin-sensitive *S. aureus* (MSSA) of all coagulase types were isolated in all the hospitals except A which lacked type I, VI, and VIII. The overall results show that coagulase type III MSSA is the most prevalent type of MSSA constituting over 25.4% of the MSSA isolates. The results indicate a shift toward a higher frequency of coagulase type II MRSA and MRSA as a whole in the hospitals studied. *Ryukyu Med. J., 14 (4) 225~230, 1994*

Key words : methicillin-resistant *S. aureus*, methicillin-sensitive *S. aureus*, coagulase type

INTRODUCTION

The incidence and prevalence of *Staphylococcus aureus* infaction have been an age-long problem, despite the regular discovery of several potent antibiotics. This persistence is due to the indiscriminate transfer of these organisms from one locality to another, coupled with their ability to modify their genetic expression with time and enviromental pressure[1-3]. These invariably have led to the selection of several multidrug resistant strains [4,5]. Noteworthy among these strains are the methicillin-resistant *S. aureus* (MRSA), which have become a problem worldwide, especially in nosocomial settings[6,7]. The threat these organisms pose has augmented efforts towards their successful typing and those of other *S. aureuns* strains in order to monitor their epidemiological spread[8-10]. Phage typing has been the most popular among the procedures employed over the years. Recent reports however indicate that many isolates of *S. aureus* are evading phage typing, sometimes constituting over 60% of the isolates of MRSA[7,11]. Coagulase typing, which is equally popular as phage typing in Japan is of tremendous help in this regard[11].

A study conducted at the Hiroshima University school of Medicine indicated that coagulase type IV MRSA were predominantly isolated at their hospital till 1984. After 1986, most of the isolates were coagulase type II[11,12]. At the Ogaki Municipal Hospital, coagulase type IV MRSA were isolated as at 1989. This changed to coagulase type II in 1990[5]. In a study of MRSA conducted at the Kumamoto University Hospital, the predominant MRSA isolated at the hospital in 1987 was coagulase type IV. This then shifted to coagualse type II in 1989[13]. This trend of change from coagulase type IV to type II MRSA was however different at the Saga Medical School Hospital. The most prevalent MRSA strain before 1989 was coagulase type II. This however changed to coagulase type VII after 1990[14]. A wide study conducted in 1990 revealed that coagulase type II MRSA is now the predominant strain in most areas of Japan. Coagulase type IV was isolated in high incidence on the Shikoku island[15].

In Okinawa, screening of *S. aureus* isolates at the Ryukyu University Hospital from 1986-1989 indicated

that coagulase type II MRSA was the predominant strain, constituting over 80% of the MRSA isolated. Coagulase type II methicillin-sensitive *S. aureus* (MSSA) also constituted not less than 25% of MSSA isolates [16]. In a study conducted in 1989 in six Okinawan hospitals (our study population), coagulase types II and III MRSA were predominantly isolated with frequencies of 54% and 40% respectively among the MRSA[17]. Considering the chemotherapeutic battle against the epidemiological spread of MRSA, and the changing trends in the isolates from the Japan mainland, strains isolated in 1992 from the six Okinawan hospitals were characterised to investigate any changes in the coagulase types.

MATERIALS AND METHODS

Bacterial isolates

A total of 513 *S. aureus* isolates, 47 from hospital A, 103 from hospital B, 92 from hospital C, 85 from hospital D, and 93 each from hospitals E and F isolated in 1992 were used for the study. The isolates were selected on the basis of one isolate per patient.

Chemicals and Reagents

Rabbit antisera against the eight coagulase types (I-VIII) was purchased from Denka Seiken Co. (Japan). Rabbit lyophilised plasma was purchased from Eiken Co. (Japan). Bovine fibrinogen as well as Tranexamic acid and acacia powder were obtained from Nacalai Tesque Inc., Japan. Glycerin and iron fillings (reduced) were purchased from Nakari Chemicals Co. Ltd (Japan). Trisodium citrate was from Kishida Chemical Co. Ltd (Japan). Sodium azide was purchased from Kitayama Chemical Co. (Japan).

Solutions

The diluent solution as desribed by Tajima *et al.*[18] consisted of 20g/l polypeptone, 10g/l trisodium citrate, 1g/l tranexamic acid, 4g/l gum arabic, and 1g/l sodium azide. Bovine fibrinogen was dissolved in 50% (v/v) glycerin solution to a final concentration of 40g/l. Equivalent volumes of fibrinogen solution and rabbit plasma (reconstituted with half volume of water) were mixed together. This was then dispensed into 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[18]. Briefly, *S. aureus* isolates were each cultured in 1 ml brain heart infusion (BHI) broth and incubated at 37℃ overnight. Culture supernatant was then obtained by centrifugation at 3000 rpm for 15 min. Ten microliter of the supernatant was pipetted into each of 9 wells in a file on a V-bottom shaped microtitre plate. Ten microliter of the eight type-specific antiserum were added to the first 8 wells with the culture supernatant in the file. Rabbit serum was added to the 9th well to act as a control against false positives. The plate was then incubated at 37℃ for 1 h to complete the antigen-antibody reaction. A small amount of reduced iron metal fillings was then added to each well. Twenty microliter of the diluted fibrinogen enriched rabbit plasma was thereafter added to each well and mixed. The plates were further incubated for up to 12 h. These were then read by placing them on a magnetic stirrer to check for movement of the iron fillings in the absence of a clot. A platform was raised on the magnetic stirrer to enhance the readability in the case of weak clots.

Methicillin sensitivity testing

The agar dilution method was used to determine the mininum inhibitory conentration (MIC) of all the isolates to methicillin[19]. The concentration range of methicillin used for the MIC was from 0.125 μg/ml-128 μg/ml. A multispot innoculator (Sakuma Manufacturing Co., Japan) delivering approximately 5 μl of 10^6 CFU/ml was used to innoculate the Mueller Hinton agar (DIFCO Laboratories) plates with an overnight culture of the isolates. One set of plates were incubated overnight at 37℃ while the other set was incubated at 30℃[20]. Growth was considered when five or more visible colonies could be counted at a dilution.

RESULTS AND DISCUSSION

The isolates were classified as MRSA when their minimum inhibitory concentration (MIC) for methicillin was $\geqq$ 16 μg/ml. In all the hospitals, MRSA were most frequently isolated from sputum and then from pus. MRSA constituted 54% of the total *S. aureus* isolates. The isolation rates were 61.7% for hospital A, 47.6% for hospital B, 31% for hospital C, 71.8% for hospital D, 71% for hospital E, and 46.2% for hospital F.

Sixty-five percent of the MRSA were constituted by coagulase type II. This was most frequently isolated from inpatients as shown in Table 2 (94.5%). It invariably means that this type has been established as a nosocomial strain. This observation is similar to reports from the mainland of Japan [12,13,15]. In the previous study conducted in 1989, coagulase type II MRSA constituted 54% of the total MRSA isolates [18]. In this study, it constituted 65.3% of the isolates indicating a rise of more than 11% in its isolation rate. Coagulase type III MRSA which constituted 40% of MRSA isolates in the previous study had a frequency of 12.6% in the present study. This was the second most prevalent after coagulase type II. This also was mostly isolated from inpatients. Coagulase type I MRSA which

Table 1 Number of isolates types of coagulase of MSSA from the various hopitals

Hospital	Total[1]		Coagulase type									
			I	II	III	IV	V	VI	VII	VIII	MX	NT
A	18	a	0(0)	2(11.1)	2(11.1)	1(5.5)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
		b	0(0)	0(0)	3(16.7)	5(27.8)	1(5.5)	0(0)	1(5.5)	0(0)	3(16.7)	0(0)
B	54	a	2(3.7)	8(14.8)	5(9.3)	5(9.3)	2(3.7)	2(3.7)	5(9.3)	0(0)	8(14.8)	0(0)
		b	0(0)	4(7.4)	4(7.4)	2(3.7)	0(0)	0(0)	6(11.1)	1(1.9)	0(0)	0(0)
C	63	a	0(0)	1(1.6)	19(30.2)	1(1.6)	1(1.6)	1(1.6)	6(9.5)	0(0)	2(3.2)	0(0)
		b	1(1.6)	5(7.9)	6(9.5)	1(1.6)	3(4.8)	3(4.8)	9(14.3)	1(1.6)	3(4.8)	0(0)
D	24	a	1(4.2)	5(20.8)	5(20.8)	0(0)	2(8.3)	0(0)	0(0)	1(4.2)	1(4.2)	0(0)
		b	2(8.3)	2(8.3)	0(0)	1(4.2)	0(0)	1(4.2)	3(12.5)	0(0)	0(0)	0(0)
E	27	a	2(7.4)	2(7.4)	6(22.2)	0(0)	2(7.4)	1(3.7)	3(11.1)	1(3.7)	0(0)	0(0)
		b	0(0)	0(0)	1(3.7)	0(0)	5(18.5)	0(0)	2(7.4)	1(3.7)	1(3.7)	0(0)
F	50	a	0(0)	2(4)	2(4)	0(0)	1(2)	0(0)	0(0)	2(4)	0(0)	0(0)
		b	5(10)	6(12)	7(14)	5(10)	6(12)	2(4)	8(16)	2(4)	1(2)	1(2)
Total[2]	236		13(5.5)	37(15.7)	60(25.4)	21(8.9)	23(9.7)	10(4.2)	43(18.2)	9(3.8)	19(8.1)	1(0.4)

Numbers in parenthesis indicate percent of isolates.
a: inpatients.
b: outpatients.
[1]Total number of MSSA isolates from the hospital.
[2]Total number of that coagulase type.

was previously not isolated is present in all the hospitals[17]. This strain tended to be isolated mostly from outpatients. Unlike the other hospitals where coagulase type II MRSA was the most prevalent, coagulase type I MRSA was the predominant strain in hospital A (Fig. 1). This was followed by coagulase type V and then type II. In hospital C, the most prevalent strain was coagulase type II followed by type I and then type III (Fig. 3). Apart from the two hospitals mentioned above, the general trend in the other four hospitals was that coagulase type II was the most prevalent, followed by coagulase type III and then coagulase type I as shown in Figs. 2, 4, 5, and 6. The overall picture of the strain isolation showed same pattern. Coagulase type V MRSA was not isolated in the previous study. In this study it was isolated from only A and E hospitals. All the isolates from hospital A were from outpatients. Coagulase type V MRSA were also not isolated in the previous study[17]. The isolation rates for the other coagulase types of MRSA were comparatively low. Coagulase type VI MRSA was not isolated from any of the hospitals.

In the case of MSSA, all coagulase types were isolated from all the hospitals with the exception of hospital A which lacked coagulase types I and VIII. Coagulase type III MSSA was the most frequently isolated, constituting approximately 25.4% of the total MSSA isolates. This was followed by coagulase type VII with 18.2% and then coagulase type II 15.7%. Coagulase type VIII had the least isolation rate of 3.8%. The results of this study indicate that there has been a shift in the pattern of MRSA isolation in the

Table 2 Number of isolates of coagulase types of MRSA from the various hopitals

Hospital	Total[1]		Coagulase type									
			I	II	III	IV	V	VI	VII	VIII	MX	NT
A	29	a	4(13.8)	5(17.2)	1(3.4)	0(0)	0(0)	0(0)	2(6.9)	0(0)	0(0)	0(0)
		b	6(20.7)	1(3.4)	1(3.4)	0(0)	7(24.1)	0(0)	0(0)	0(0)	2(6.9)	0(0)
B	49	a	2(4.1)	34(69.4)	3(6.1)	2(4.1)	0(0)	0(0)	0(0)	0(0)	1(2)	0(0)
		b	1(2)	3(6.1)	1(2)	1(2)	0(0)	0(0)	0(0)	1(2)	0(0)	0(0)
C	29	a	1(3.4)	14(48.3)	2(6.9)	0(0)	0(0)	0(0)	0(0)	0(0)	1(3.4)	0(0)
		b	5(17.2)	3(10.3)	2(6.9)	1(3.4)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
D	61	a	2(3.3)	45(73.8)	5(8.2)	0(0)	0(0)	0(0)	1(1.6)	1(1.6)	1(1.6)	0(0)
		b	3(4.9)	0(0)	2(3.3)	1(1.6)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
E	66	a	3(4.5)	46(69.7)	11(16.7)	0(0)	1(1.5)	0(0)	1(1.5)	0(0)	2(3)	1(1.5)
		b	0(0)	0(0)	1(1.5)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
F	43	a	2(4.7)	27(62.8)	3(7)	0(0)	0(0)	0(0)	0(0)	2(4.7)	1(2.3)	0(0)
		b	1(2.3)	3(7)	3(7)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(2.3)
Total[2]	277		30(10.8)	181(65.3)	35(12.6)	5(1.8)	8(2.9)	0(0)	4(1.4)	4(1.4)	8(2.9)	2(0.7)

Numbers in parenthesis indicate percent of isolates.
a: inpatients.
b: outpatients.
[1]Total number of MSSA isolates.
[2]Total number of Coagulase type.

six hospitals. There has been a decrease in the isolation rate of coagulase type III MRSA coupled with an increase in the rate of coagulase type II MRSA isolation.

Coagulase types I and V are new isolates probably more prevalent in outpatients due to disparites in antibiotic use. Should care not be taken these could become established as community strains. The rate of MRSA isolation has also increased from an isolation rate range of 13%-52% in 1989 to 31%-71.8% for the hospitals as at 1992. Coagulase type II MRSA seems to be preferably established in health institutions when the results of this study are compared with those elsewhere [12,13,15]. It is therefore necessary to investigate the mechanism (s) that favours its colonisation of the health institutions.

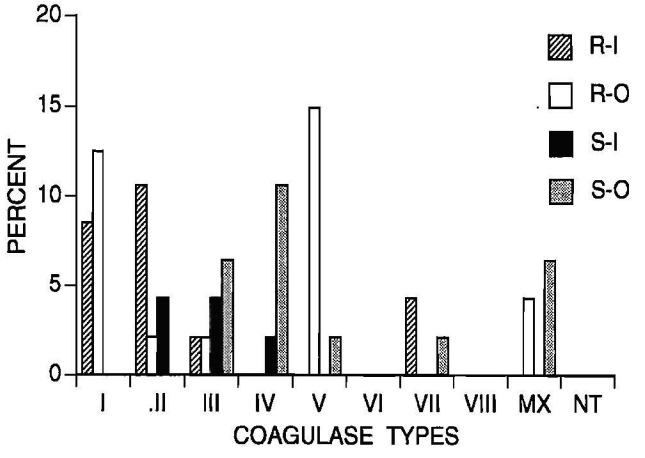

Fig. 1 Profile of *S. aureus* strains at Hospital A. (R-I: MRSA from inpatients; R-O: MRSA from outpatients; S-I: MSSA from inpatients; S-O: MSSA from outpatients)

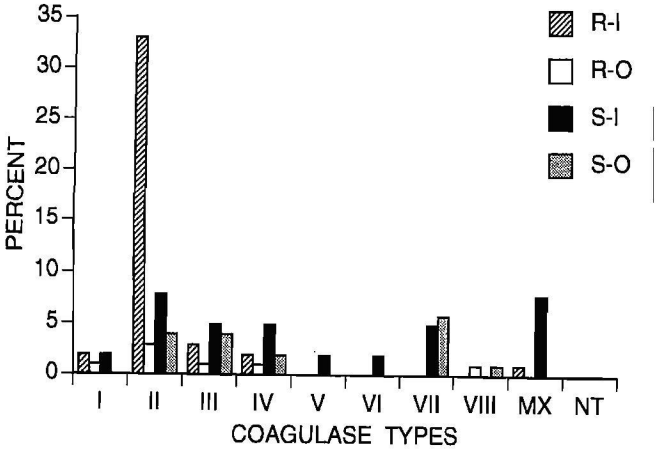

Fig. 2 Profile of *S. aureus* strains at Hospital B. (R-I: MRSA from inpatients; R-O: MRSA from outpatients; S-I: MSSA from inpatients; S-O: MSSA from outpatients)

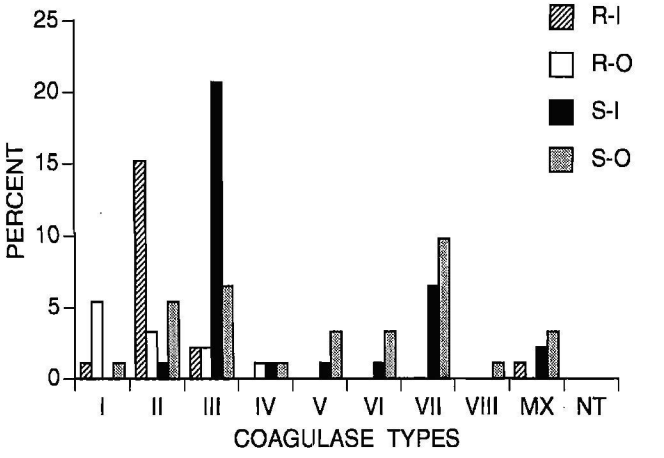

Fig. 3 Profile of *S. aureus* strains at Hospital C. (R-I: MRSA from inpatients; R-O: MRSA from outpatients; S-I: MSSA from inpatients; S-O: MSSA from outpatients)

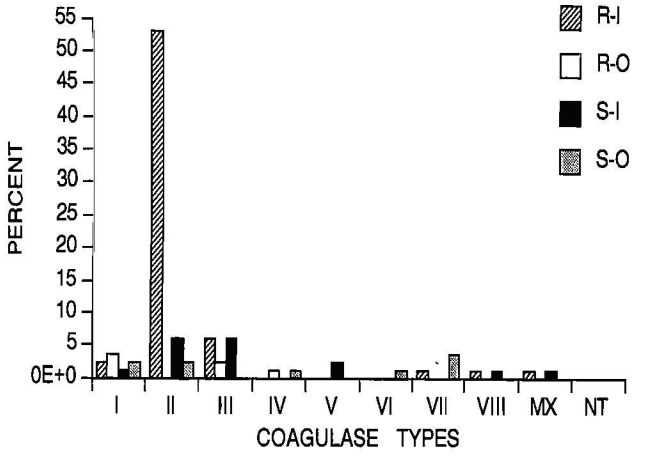

Fig. 4 Profile of *S. aureus* strains at Hospital D. (R-I: MRSA from inpatients; R-O: MRSA from outpatients; S-I: MSSA from inpatients; S-O: MSSA from outpatients)

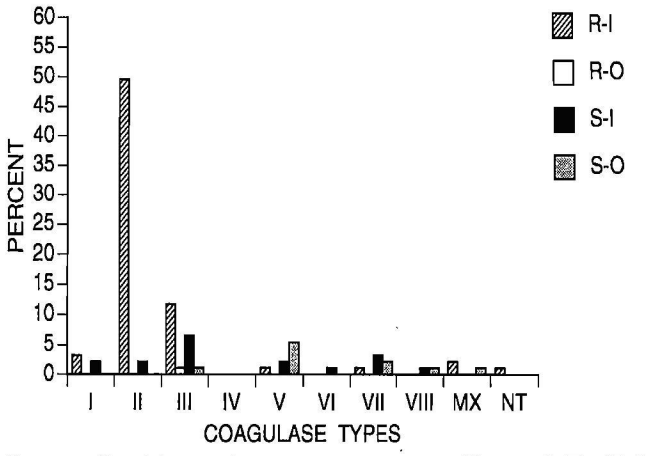

Fig. 5 Profile of *S. aureus* strains at Hospital E. (R-I: MRSA from inpatients; R-O: MRSA from outpatients; S-I: MSSA from inpatients; S-O: MSSA from outpatients)

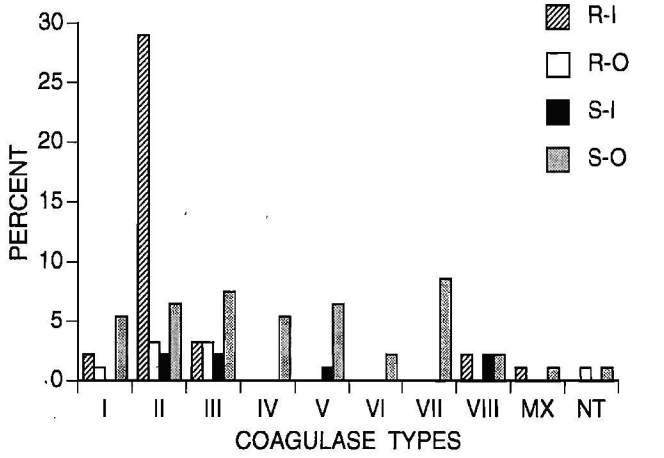

Fig. 6 Profile of *S. aureus* strains at Hospital F. (R-I: MRSA from inpatients; R-O: MRSA from outpatients; S-I: MSSA from inpatients; S-O: MSSA from outpatients)

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