

Dissemination of Multisusceptible Methicillin-Resistant *Staphylococcus aureus* in Singapore

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Analysis of hospital-acquired methicillin-resistant *Staphylococcus aureus* strains isolated from a tertiary public hospital in Singapore revealed that multisusceptible strains had gradually started to replace the endemic multiresistant strain (ST239-MRSA-III) since 2002. Molecular typing showed that this was a predominantly clonal outbreak of a UK-EMRSA-15 strain (ST22-MRSA-IV).

Methicillin-resistant *Staphylococcus aureus* (MRSA) was introduced into Singapore in the early 1980s (4), and a review of the records of the microbiology laboratories of Singapore General Hospital (SGH) and the National University Hospital of Singapore (NUH), two acute-care tertiary-level public hospitals in Singapore, showed that the proportion of methicillin-resistant strains isolated from all sites from inpatients with *Staphylococcus aureus* infections had been consistently above 40% since the mid-1990s.

Previous work done on representative strains isolated before 1998 showed that related multiresistant strains belonging to multilocus sequence type (ST) 239 and bearing the Type III staphylococcal chromosome cassette *mec* (SCC*mec*) had predominated in the 1990s (J. Sim and W. Grubb, personal communication). However, we observed a progressive increase in the number of isolates from all clinical specimens demonstrating susceptibility to tetracycline, trimethoprim-sulfamethoxazole, and gentamicin since 2002. A study was conducted to chart the resistance profiles of MRSA in SGH as well as to type the major circulating strains in both institutions.

We reviewed the records of the microbiology laboratory at SGH for MRSA strains isolated from clinical specimens from January 1997 to March 2004. Duplicate isolates from the same patient within a calendar year were eliminated on the basis of patients' names and identification numbers. The medical charts of patients infected by these MRSA were reviewed, and nine strains deemed to be possibly community acquired (isolation of MRSA within 48 h of hospitalization in patients who had not previously been in contact with any healthcare facility for at least 1 year) were excluded from the study. A total of 44 nonduplicate hospital-acquired MRSA strains isolated in March 2004 in NUH were contributed to the study.

Methicillin resistance was defined by resistance to oxacillin as determined by the Kirby-Bauer disk diffusion method and interpreted according to NCCLS guidelines (7). The MRSA strains were grouped according to their susceptibility to the following antimicrobials tested via the abovementioned method: trimethoprim-sulfamethoxazole, gentamicin, tetracycline,

rifampin, erythromycin, ciprofloxacin, clindamycin, fucidin, and vancomycin (7). Prior to 2004, it was the policy of the SGH laboratory that only isolates from blood, eye, urine, pleura, and peritoneal cultures underwent gentamicin susceptibility testing, whereas clindamycin susceptibility was not tested for urinary isolates.

Pulsed-field gel electrophoresis (PFGE) using restriction endonuclease SmaI (6) was used to type all contributed NUH strains and selected SGH strains isolated in 2003 and 2004. Gel findings were interpreted on the basis of standard criteria: strains were considered to be related if there was a difference of three bands or less on comparison with the predominant strain (9). Multilocus sequence typing (MLST) (3) was performed for selected strains belonging to each PFGE cluster. SCC*mec* of these isolates were typed following the multiplex-PCR method developed by Oliveira and de Lencastre (8).

Trends in antimicrobial resistance among SGH strains are shown in Fig. 1. All strains were susceptible to vancomycin and rifampin and resistant to erythromycin and ciprofloxacin.

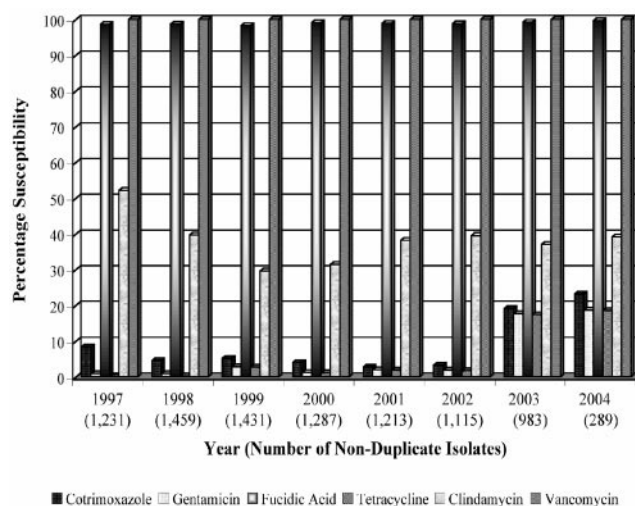


FIG. 1. Antimicrobial susceptibility of methicillin-resistant *Staphylococcus aureus* isolated at Singapore General Hospital, January 1997 to March 2004.

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TABLE 1. Antimicrobial susceptibility profiles and molecular typing of MRSA in SGH and NUH

Profile	Antimicrobial susceptibility ^a	No. of isolates/year (NUH)								No. of isolates tested ^c		PFGE cluster (n)	SCC _{mec}	MLST
		1997	1998	1999	2000	2001	2002	2003	2004 ^b	2003	2004			
I	C (variable), F (variable), V	290	321	360	378	445	353	261	222 (25)	0	68 (25)	A (93)	III	239
II	G (untested), C (variable), F (variable), V	835	1070	996	857	734	726	534	0	4	0	A (4)	III	239
III	G, C (variable), F (variable), V	3	2	1	0	1	0	0	0	0	0	Unknown	Unknown	Unknown
IV	S, C (untested), F (variable), V	27	14	53	23	11	7	13	13	3	6	A (9)	III	239
V	S, G (untested), F, V	17	10	2	4	3	9	14	0	5	0	A (5)	III	239
VI	S, G (untested), C, F, V	59	37	5	13	4	5	4	0	3	0	B (3)	II	5
VII	S, G (untested), T, C, F, V	0	4	3	7	5	14	93	0	12	0	C (12)	IV	22
VIII	S, G, T, C, F, V	0	1	9	5	8	1	63	53 (19)	20	53 (19)	C (92)	IV	22

^a C, clindamycin; F, fucidic acid; V, vancomycin; G, gentamicin; S, cotrimoxazole; T, tetracycline; and variable, either resistant or susceptible.
^b For the year 2004, only strains isolated from January to March are included. Number of NUH isolates is given in parenthesis.
^c Archived SGH isolates from 2003 and 2004. Number of NUH isolates is given in parenthesis.

Strains susceptible to gentamicin, trimethoprim-sulfamethoxazole, and tetracycline were first observed in 1998 but increased dramatically in 2003 and in the first quarter of 2004. In contrast, the proportion of strains susceptible to fucidic acid had remained constant over the years. The drop in absolute numbers of MRSA in 2003 resulted from better infection control measures and reduced hospital admission rates during the severe acute respiratory syndrome outbreak.

The different significant antimicrobial resistance profiles are shown in Table 1, and the 218 strains analyzed by PFGE could be grouped into three main clusters, a selected representation of which is shown in Fig. 2. As there are multiple possible overlapping antimicrobial resistance profiles in view of the testing strategies employed at SGH over the 7 years, we retested subject strains for which gentamicin or clindamycin susceptibility

testing had previously been omitted. The majority of strains initially deemed susceptible to clindamycin were found to have inducible resistance with a D-zone effect on testing (5).

All trimethoprim-sulfamethoxazole and gentamicin resistant-strains belonged to PFGE cluster A (ST239-MRSA-III) regardless of clindamycin and fucidic acid susceptibility. Trimethoprim-sulfamethoxazole-susceptible strains could be divided into three groups: those which were resistant to gentamicin and tetracycline belonged to PFGE cluster A (ST239-MRSA-III); a small number with tetracycline resistance but gentamicin and clindamycin susceptibility were clonal and belonged to PFGE cluster B (ST5-MRSA-II); and finally those susceptible to tetracycline, clindamycin, and gentamicin were clonal and belonged to PFGE cluster C (ST22-MRSA-IV). We were unable to type 1 profile because of the lack of archived

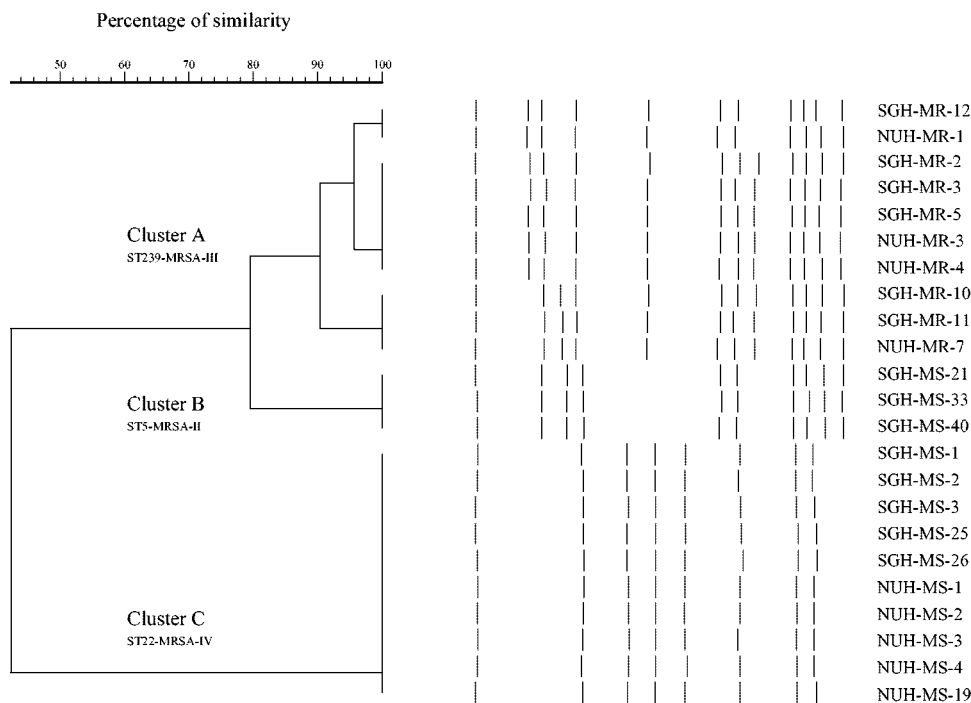


FIG. 2. Pulsed-field gel electrophoresis (PFGE) pattern of nosocomial MRSA from Singapore General Hospital and National University Hospital. SmaI restriction patterns were digitized and analyzed using Molecular Analyst version 1.6 software to calculate Dice coefficients of correlation and to generate a dendrogram by the unweighted pair group method using arithmetic averages (UPGMA) clustering.

strains that were trimethoprim-sulfamethoxazole and tetracycline resistant, gentamicin susceptible.

Our results confirm that the predominant local MRSA strain is still the multiresistant ST239-MRSA-III strain. Recent studies have indicated that endemic multiresistant MRSA from various European hospitals have gradually been replaced by more susceptible MRSA (1, 2, 10). A similar trend appears to be developing locally, although only time will tell if our situation will completely mirror that of these European hospitals.

There is just one predominant multisusceptible clonal type locally as opposed to the multiple diverse multisusceptible strains present in France and Belgium (1, 10). The MLST and SCC*mec* type indicate that this is likely to be UK-EMRSA-15, and representative strains were sent to the Staphylococcus Reference Laboratory, Centre for Infections, Health Protection Agency, United Kingdom, for confirmation. Phage typing done in the United Kingdom confirmed that these were EMRSA-15, and strains tested positive for enterotoxins C, G, and I. The PFGE profile matched that of EMRSA-15 variant B1.

Unfortunately, the lack of archived strains renders it impossible for us to determine the year of its importation, but it had probably been imported by either a patient or healthcare staff from overseas and had competed successfully with our endemic MRSA.

A small number of strains were related to the New York/Japan clone (ST5-MRSA-II). Although the true prevalence of this clone is unknown in view of noncomprehensive antimicrobial susceptibility testing, it is unlikely to be high.

While there are clearly short-term advantages in having multisusceptible MRSA replace the multiresistant strains, including having more antimicrobial options for treating MRSA infections, the lack of control over the spread of this clone implies a similar vulnerability should more virulent and resistant strains be imported.

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