# Distribution of Major Genotypes among Methicillin-Resistant Staphylococcus aureus Clones in Asian Countries

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To investigate the evolutionary pattern and genotypic characteristics of methicillin-resistant *Staphylococcus aureus* (MRSA) strains in the Asian region, 74 MRSA strains isolated from 12 Asian countries were analyzed by multilocus sequence typing (MLST) and SCC*mec* typing. Overall, a total of 16 genotypes based on sequence type and SCC*mec* types were identified among MRSA strains from Asian countries. Data revealed two major genotypes of MRSA strains in Asia, with unique geographic distributions. By MLST analysis, all strains from Korea and Japan except one belonged to clonal complex 5 (CC5) while most MRSA isolates from other Asian countries belonged to CC239. SCC*mec* typing showed that most isolates from Korea and Japan were SS*mec* type II whereas SCC*mec* type III (or IIIA) was the most common type in strains from other Asian countries. Our data documented a unique geographic distribution and evolutionary pattern of MRSA clones in Asia.

The widespread emergence of methicillin-resistant *Staphylococcus aureus* (MRSA), especially in various types of nosocomial infections, is a serious clinical problem worldwide. The incidence of methicillin resistance among nosocomial isolates of *S. aureus* is higher than 70% in some Asian countries such as Taiwan, China, and Korea (7, 16, 19). Recently, MRSA has also emerged in the community setting in some countries, including Asian countries (4, 21, 24, 32). One of the cardinal features of the rapid emergence of MRSA in many parts of the world is the dissemination of specific clones; this has contributed to the accelerated increases in the incidence of MRSA. Therefore, it is important to investigate the genotypic characteristics and evolutionary pathway of MRSA clones as well as the genetic relatedness of the strains isolated in different geographic regions.

SCCmec typing analyzes a mobile genetic element called the staphylococcal cassette chromosome mec (SCCmec), which contains the mecA gene encoding methicillin resistance (14). SCCmec is classified into four major types according to size and composition (17, 18, 20). Multilocus sequence typing (MLST) can also determine the genotypic characteristics of staphylococcal isolates by analyzing ~450-bp sequences of seven housekeeping genes (5, 10). Previous reports using MLST, pulsed-field gel electrophoresis, spa typing, or SCCmec typing have documented the clonality and evolutionary pathway of S. aureus (3, 11, 12, 29).

Population genetic studies based on MLST have shown that major MRSA clones have emerged from five clonal complexes (CCs), i.e., CC5, CC8, CC22, CC30, and CC45 (15–17), while pulsed-field gel electrophoresis has identified five major pan-

demic MRSA clones, i.e., Iberian, Brazilian, Hungarian, New York/Japan, and pediatric clones (27, 28). The Iberian, Brazilian, and Hungarian clones belong to CC8, and the New York/Japan and pediatric clones belong to CC5 (7, 28). Although some Asian countries showed the highest prevalence of MRSA in the world, very limited data are available with regard to the evolution and population genetics of MRSA isolates in this region (6, 7, 11, 25, 27).

In this study, we investigated the genotypic characteristics and evolutionary pattern of MRSA clones in Asian countries by using MLST and SCC*mec* typing. Based on these molecular typing methods, we characterized the genetic background of MRSA strains isolated in Asian countries.

## MATERIALS AND METHODS

Bacterial isolates. A total of 74 MRSA isolates from 12 Asian countries were analyzed in the study: 13 from Korea (Samsung Medical Center, Seoul), 7 from Indonesia (University of Indonesia, Jakarta), 6 from China (Beijing Children's Hospital, Beijing, Shanghai Children's Hospital, Shanghai), 6 from India (Christian Medical College), 6 from Japan (Nagoya University, Nagoya), 6 from the Philippines (Research Institute for Tropical Medicine, Manila), 5 from Saudi Arabia (King Saud University Hospital, Riyadh), 5 from Singapore (National University of Singapore, Singapore), 5 from Sri Lanka (University of Colombo, Colombo), 5 from Taiwan (Chang Gung Children's Hospital, Taipei), 5 from Thailand (Mahidol University, Bangkok), and 5 from Vietnam (Nhi Dong 2 Children's Hospital, Ho Chi-Minh). These isolates were randomly selected from the collection at the Asian Bacterial Bank, which had been isolated during the period from 1998 to 2003 by the Asian Network for Surveillance of Resistant Pathogens (ANSORP).

Antimicrobial susceptibility tests. In vitro susceptibility tests were performed by a broth microdilution test as described by the National Committee for Clinical Laboratory Standards (NCCLS) guidelines (22). Antimicrobial drugs tested included penicillin, amoxicillin-clavulanic acid, cefuroxime, ciprofloxacin, levo-floxacin, clarithromycin, trimethoprim-sulfamethoxazole, gentamicin, and vancomycin. The susceptibility interpretive criteria used were those by NCCLS M100-S13 (23).

MLST. Chromosomal DNA for MLST and multiplex PCR of SCC*mec* typing was extracted by a simple boiling-lysis method (2). MLST was performed as described previously (10). PCR fragments of the seven housekeeping genes,

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arcC, aroE, glpF, gmk, pta, tpi, and yqiL, were obtained from chromosomal DNA and were directly sequenced. PCRs were carried out with an initial denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 48°C for 30 s, and 72°C for 1 min. The reaction mixture was then maintained at 72°C for a further 5 min. The DNA fragments were purified using a PCR purification kit (CoreOne; Seoul, Korea) and then sequenced in an ABI3700 DNA sequencer.

**SCC***mec* **typing.** The SCC*mec* types were determined by a multiplex PCR strategy, as described previously; this strategy identified types I to IV and their variants, IA, IIIA, and IVA (26).

**Data analysis.** The allelic profiles of strains were defined by the alleles at the seven MLST loci in the order of *arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *yqiL*. Each unique allelic profile was designated a sequence type (ST), which was determined by comparing the database at the MLST website (http://saureus.mlst.net). Clustering of related STs, which were defined as clonal complexes (CCs), was determined by using the program eBURST (based upon related sequence types) (13). Single genotypes that do not correspond to any CC are defined as singletons. The MRSA genotypes were defined based on ST and SCC*mec* type as proposed by Robinson and Enright (29, 30).

#### RESULTS

Antimicrobial susceptibility. All MRSA strains were resistant to penicillin and gentamicin. The MIC of oxacillin ranged from 4 to 1,024 mg/liter. The majority of the isolates tested were resistant to amoxicillin-clavulanic acid (96%), cefuroxime (85%), clarithromycin (85%), and ciprofloxacin (84%), while about half of the strains were resistant to levofloxacin (49%) and trimethoprim-sulfamethoxazole (50%) (Table 1). All isolates were susceptible to vancomycin. Most MRSA strains were multiresistant to more than three classes of antibiotics, while a few strains from Korea were resistant to only β-lactam agents and gentamicin.

MLST analysis. MLST analysis showed 11 STs among 74 Asian MRSA isolates (Table 1). The most prevalent types were ST5 (17 strains), ST239 (28 strains), and ST241 (21 strains). ST241 is thought to be a single-locus variant (slv) of ST239 because alleles of ST239 and ST241 differ only at *yqiL* (allele 3 in ST239 and allele 30 in ST241). The other eight STs (ST8, ST59, ST88, ST254, ST5 slv, ST59 slv, ST239 slv, and ST240 slv) were found in only one isolate each. Four of these STs (ST5 slv, ST59 slv, ST239 slv, and ST240 slv) were newly identified in this study. Major CCs in Asian strains were CC239 (ST239, ST241, ST8, ST239 slv, ST254, and ST240 slv), CC5 (ST5 and ST5 slv), and CC59 (ST59 and ST59 slv).

SCCmec types. Four SCCmec types, II, III, IIIA, and IV, were found among 73 Asian MRSA isolates, and 1 isolate from India (I 93) showed a nontypeable SCCmec type (Table 1). The most common SCCmec type was type IIIA, lacking pT181, which was found in 42 strains. SCCmec type III was found in 7 strains (ST239 in 5 strains and ST241 in 2 strains). SCCmec type II was found in 18 strains: ST5 in 15 strains, ST5 slv in 1 strain, ST241 in 1 strain, and ST254 in 1 strain. Six strains showed SCCmec type IV, which had diverse STs: ST5 in 2 strains, ST8 in 1 strain, ST59 in 1 strain, ST59 slv in 1 strain, and ST88 in 1 strain.

**Distribution of MRSA genotypes.** Overall, a total of 16 MRSA genotypes (ST-MRSA-SCC*mec* type) based on STs and SCC*mec* types as proposed by Robinson and Enright (29, 30) were identified (Table 2). The distribution of MRSA genotypes showed a distinct demarcation according to the geographic origin of isolates, with Korea and Japan being distinct from other Asian countries (Table 2). By MLST analysis, 18 of 19 strains from Korea and Japan belonged to CC5 while no

strains from other Asian countries belonged to CC5. In contrast, most MRSA isolates from other Asian countries belonged to CC239 except three strains from the Philippines (Ph 31) and Taiwan (TW 7 and TW 23). Of 19 isolates from Korea and Japan, 17 showed SCC*mec* type II, while only 2 strains from Korea (Kor 12 and Kor 419) showed type IV. Of 52 strains from other Asian countries that belonged to CC239, 49 showed SCC*mec* type III or IIIA (Table 2). Two isolates from Philippines (Ph 9, ST241) and Taiwan (TW 28, ST8) were SCC*mec* types II and IV, respectively. Two strains belonging to a minor clonal complex (ST59-ST59 slv) were from Taiwan and showed SCC*mec* type IV.

## DISCUSSION

Data from this study revealed the genetic characteristics and evolution of MRSA isolates in Asia. Previous studies of the evolution and population genetics of MRSA isolates in the Asian region included isolates from Japan, Taiwan, and China (6, 7, 11, 25, 27). These studies showed that ST5 with SCC*mec* type II was prevalent in Japan (7, 11, 27) while ST239 and ST241 (a single-locus variant of ST239) with SCC*mec* type III or IIIA were prevalent in China and Taiwan (7). Since these studies analyzed MRSA isolates from a few Asian countries, however, data could not provide relevant information on the evolution or dissemination of MRSA strains at the level of the Asian continent.

Our data based on MLST and SCCmec typing identified two major genotypes of MRSA isolates in the Asian region. Interestingly, the distribution of genotypes was clearly demarcated by geographic region: Korea and Japan versus other Asian countries. By MLST analysis, most of the Korean and Japanese isolates belonged to CC5 while MRSA strains from other Asian countries such as China, India, Indonesia, the Philippines, Saudi Arabia, Singapore, Sri Lanka, Taiwan, Thailand, and Vietnam belonged to CC239, with few exceptions. The distribution of SCCmec types also followed this pattern of geographic difference. Most isolates from Korea and Japan showed SCCmec type II, while those from other Asian countries showed SCCmec type III or IIIA. Therefore, it is obvious that the MRSA clones in Korea and Japan are completely different from those in other parts of the Asian region. Although the exact reason is not clear, this unique geographic distribution of MRSA genotypes in the Asian region has not been reported to date. In this study, there were no isolates that belonged to other global clonal complexes such as CC22, CC30, or CC45.

CC5 in Korea and Japan has represented a highly diversified lineage of MRSA, as CC8 is (29). Genotype ST5-MRSA-II, which was found in most of the Korean and Japanese strains, had been named as the New York/Japan MRSA clone (9, 27, 28). This genotype has also been found in Finland and Ireland (27, 29). According to Robinson and Enright (29), ST5-MRSA-II might have occurred by acquisition of SCCmec type II of ST5-MRSA in Japan and has spread through Europe and the United States. However, data from our study with a limited number of strains suggest that this global MRSA clone has not been disseminated throughout Asia except in Korea and Japan. As a rare exception, two strains from Korea (Kor 12 and Kor 419) that belonged to CC5 (ST5 and ST5 slv) showed

TABLE 1. Genotypic and phenotypic characteristics of MRSA in Asian countries

Location and	Oxacillin MIC	Oxacillin MIC Antibiogram <sup>a</sup>									- CTT:		
strain	(mg/liter)	PEN	CIP	LEV	CLA	CFX SXT		GEN	AMXC	Allelic profile <sup>b</sup>	$ST^c$	CC	SCCmec
Korea Kor 12 Kor 16 Kor 100 Kor 115 Kor 130 Kor 136 Kor 162 Kor 186 Kor 257 Kor 312 Kor 319 Kor 419	16 152 256 1,024 512 512 512 512 512 4 512	R R R R R R R R R	S R R R R R R R R R S R	S R R R R R R R S R R	S R R R R R R R S R R	S R R R R R R R R R	S S S S S S S S S S S S S S S S S S S	R R R R R R R R R	R R R R R R R R R R	1-4-1-4-12-1-10 1-4-1-4-12-1-10 3-32-1-1-4-4-3 1-4-1-4-12-1-10 1-4-1-4-12-1-10 1-4-1-4-12-1-10 1-4-1-4-12-1-10 1-4-1-4-12-1-10 1-4-1-4-12-1-10 1-4-1-4-12-1-10 1-4-1-4-12-1-10 1-4-1-4-12-1-10 1-4-1-4-12-1-10 1-4-1-4-12-1-10	5 5 254 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	5 5 239 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	IV II I
Japan Jp 26 Jp 43 Jp 46 Jp 51 Jp 95 Jp 99	128 512 256 512 256 512	R R R R R	R R R R R	I I I I R	R R R R R	R R R R R	S S S S S	R R R R R	R R R R R	1-4-1-4-12-1-10 1-4-1-4-12-1-10 1-4-1-4-12-1-10 1-4-1-4-12-1-10 1-4-1-4-12-1-10 1-4-1-4-12-1-10	5 5 5 5 5 5	5 5 5 5 5 5	II II II II
China Ch B55 Ch B82 Ch B87 Ch S112 Ch S73 Ch S75	128 256 256 256 256 256 512	R R R R R	R R R R R	R R R R R	R R R R R	R R R R R	S S S S S	R R R R R	R R R R R	2-3-1-1-4-4-3 2-3-1-1-4-4-3 2-3-1-1-4-4-3 2-3-1-1-4-4-3 2-3-1-1-4-4-3	239 239 239 239 239 239	239 239 239 239 239 239	IIIA III IIIA IIIA IIIA
India I 22 I 93 I 103 I 116 I 123 I 127	64 64 64 512 8 128	R R R R R	R R R R R	I I I R I I	R R R R R	S S S R S R	R S R R R	R R R R R	R S R R R	2-3-1-1-4-4-30 2-3-1-1-4-4-30 2-3-1-1-21-64-3 2-3-1-1-4-4-3 2-3-1-1-4-4-3	241 241 240 239 239 241	239 239 239 239 239 239	IIIA NT <sup>d</sup> IIIA IIIA IIIA III
Indonesia IDN 46 IDN 126 IDN 137 IDN 148 IDN 165 IDN 189 IDN 208	128 128 128 64 512 64 256	R R R R R R	R R R R R R	I I I I R I	R R R I R	R R R R R R	R R R R R R	R R R R R R	R R R R S R	2-3-1-1-4-4-3 2-3-1-1-4-4-3 2-3-1-1-4-4-3 2-3-1-1-4-4-3 2-3-1-1-4-4-3 2-3-1-1-4-4-3	239 239 239 239 239 239 239 239	239 239 239 239 239 239 239 239	IIIA IIIA IIIA IIIA IIIA
Philippines Ph 7 Ph 9 Ph 17 Ph 22 Ph 31 Ph 34	512 256 128 512 256 512	R R R R R	R R R R S R	R I R R S R	I R I R S R	R R R R S R	R R R R S R	R R R R R	R R R R R	2-3-1-1-4-4-30 2-3-1-1-4-4-30 2-3-1-1-4-4-30 2-3-1-1-4-4-30 22-1-14-23-12-4-31 2-3-1-1-4-4-30	241 241 241 241 241 88 241	239 239 239 239 239	IIIA II IIIA IIIA IV IIIA
Saudi Arabia SA 6 SA 13 SA 21 SA 37 SA 41	256 256 256 512 256	R R R R	S S S R R	S S S I I	S S S R R	R R R R	S S R R R	R R R R	R R R R	2-3-1-1-4-4-30 2-3-1-1-4-4-30 2-3-1-1-4-4-30 2-3-1-1-4-4-30 2-3-1-1-4-4-30	241 241 241 241 241	239 239 239 239 239	IIIA IIIA IIIA IIIA
Singapore SI 6 SI 21 SI 45	256 256 256	R R R	R R R	I I R	R R R	R R R	R R R	R R R	R R R	2-3-1-1-4-4-3 2-3-1-1-4-4-3 2-3-1-1-4-4-3	239 239 239	239 239 239	IIIA IIIA IIIA

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TABLE 1—Continued

Location and strain	Oxacillin MIC	${\rm Antibiogram}^a$								Allelic profile <sup>b</sup>	$ST^c$	СС	SCCmec
	(mg/liter)	PEN	CIP	LEV	CLA	CFX	SXT	GEN	AMXC	Allene prome	21-	CC	SCCmec
SI 56	128	R	R	R	R	R	R	R	R	2-3-1-1-4-4-3	239	239	III
SI 100	128	R	R	I	R	R	R	R	R	2-3-1-1-4-4-3	239	239	IIIA
Sri Lanka													
SL 18	256	R	S	S	R	R	R	R	R	2-3-1-1-4-4-3	239	239	IIIA
SL 32	128	R	R	I	R	R	R	R	R	2-3-1-1-4-4-3	239	239	IIIA
SL 73	512	R	R	I	R	R	R	R	R	2-3-1-1-4-65-3	239	239	IIIA
SL 94	512	R	R	R	R	R	R	R	R	2-3-1-1-4-4-3	239	239	IIIA
SL 96	128	R	I	S	R	R	S	R	R	2-3-1-1-4-4-30	239	239	IIIA
Taiwan													
TW 7	32	R	S	S	R	R	R	R	R	19-23-15-2-19-20-15	59	59	IV
TW 23	128	R	S	S	R	R	S	R	R	19-23-15-6-19-20-15	59 slv	59	IV
TW 28	512	R	S	S	R	S	S	R	R	3-3-1-1-4-4-3	8	239	IV
TW 33	512	R	R	S	R	I	R	R	R	2-3-1-1-4-4-30	241	239	III
TW 60	512	R	R	I	R	R	R	R	R	2-3-1-1-4-4-30	241	239	IIIA
Thailand													
Th 1	128	R	R	R	R	R	R	R	R	2-3-1-1-4-4-30	241	239	IIIA
Th 2	256	R	R	R	R	R	R	R	R	2-3-1-1-4-4-30	241	239	IIIA
Th 6	256	R	R	R	R	R	R	R	R	2-3-1-1-4-4-30	241	239	IIIA
Th 86	64	R	R	R	R	R	R	R	R	2-3-1-1-4-4-30	241	239	IIIA
Th 106	512	R	R	R	R	S	S	R	R	2-3-1-1-4-4-3	239	239	III
Vietnam													
VN 9	256	R	R	R	R	R	R	R	R*	2-3-1-1-4-4-30	241	239	IIIA
VN 12	128	R	R	R	R	R	S	R	R	2-3-1-1-4-4-3	239	239	IIIA
VN 17	128	R	R	R	R	R	S	R	R	2-3-1-1-4-4-3	239	239	IIIA
VN 128	256	R	R	R	R	R	R	R	R	2-3-1-1-4-4-30	241	239	IIIA
VN 167	512	R	R	R	I	R	S	R	R	2-3-1-1-4-4-3	239	239	IIIA

<sup>&</sup>lt;sup>a</sup> Antibiotic abbreviations: PEN, penicillin; CIP, ciprofloxacin; LEV, levofloxacin; CLA, clarithromycin; CFX, cefuroxime; SXT, trimethoprim-sulfamethoxazole; GEN, gentamicin; AMXC, amoxicillin-clavulanic acid. R, resistant; S, susceptible; I, intermediate.

SCCmec type IV (ST5-MRSA-IV). These strains correspond to a pediatric clone which has been found in the United States, Poland, Portugal, and Colombia but not in Asian countries such as Japan, China, and Taiwan (6, 27-29). The strains were susceptible or intermediate in susceptibility to most antimicrobials tested except β-lactam agents and aminoglycoside. Other strains showing SCCmec type IV (Ph 31, TW 7, TW 23, and TW 28) with different STs had variable resistance to antimicrobial agents. Previous reports showed that SCCmec type IV was found in community-acquired MRSA isolates, which are usually more susceptible to most antimicrobial agents than are hospital-acquired MRSA isolates (1, 8, 14, 15, 25). However, a recent report from Taiwan indicated that only a few community-acquired MRSA isolates in Taiwan showed SCCmec type IV and that most were untypeable (32). Although we found six strains with SCCmec type IV from Asian countries in this study, we could not verify whether the infections were community acquired or hospital acquired due to lack of information.

CC239, which was found in most strains from other Asian countries, was regarded as a distinct lineage within CC8 (29). Robinson and Enright (29, 31) proposed an evolutionary pathway from ST8 to ST239 through the acquisition of SCC*mec* type III and recombination of a large portion of the sequence including the *arcC* locus. This type represents several epidemic

MRSA clones (EMRSA-1, EMRSA-4, EMRSA-7, EMRSA-9, and EMRSA-11) and the Hungarian, Portuguese, and Viennese clones (9, 27–29). Our data suggest that CC239 including ST239, ST241, and its variants, has disseminated in Asian countries, except for Korea and Japan, as in some European countries. For example, ST239 was reported to be highly prevalent in Nanjing, China (7) and was also found in strains from Beijing and Shanghai in this study.

SCCmec type IIIA, lacking pT181, was found in 42 strains with CC239. ST239-MRSA-IIIA, found in some Asian strains in this study, corresponds to the widespread Brazilian MRSA clone (26). The ST and antibiogram of three strains with SCCmec type III from China were identical to those of strains with SCCmec type IIIA. Therefore, they might have originated from the same ancestor, probably through the deletion of a portion including pT181. However, antibiograms of other isolates with SCCmec type III (I 127 from India, TW 33 from Taiwan, and Th 106 from Thailand) were different from those of strains isolated from the same country.

One Korean isolate (Kor 100) showed ST254 (3-32-1-1-4-4-3), which belonged to CC239 based on BURST analysis. This strain had a unique genotype, ST254-MRSA-II. Based on the MLST analysis, ST254 might be one of the first MRSA clones that emerged (29). However, MRSA strains isolated from the United Kingdom in 1962 and 1965 showed an ST254-MRSA-I

<sup>&</sup>lt;sup>b</sup> Allelic profile in order of arcC-aroE-glpF-gmk-pta-tpi-yqiL.

<sup>&</sup>lt;sup>c</sup> ST determined based on the MLST website (http://saureus.mlst.net/).

 $<sup>^{\</sup>it d}$  NT, nontypeable.

TABLE 2. Distribution of clonal complexes of MRSA in Asian countries

	No. of isolates with clonal complex and SCCmec type:										
Country (no. of isolates)	C	C5		С	C239	GGEO (III)	Other <sup>b</sup>				
,	II	IV	II	III	IIIA	IV	CC59 (IV)	(IV)			
Japan (6)	6										
Korea (13)	10	2	1								
China (6)				3	3						
India $(6)^a$				1	4						
Indonesia (7)					7						
Philippines (6)			1		4			1			
Saudi Arabia (5)					5						
Singapore (5)				1	4						
Sri Lanka (5)					5						
Taiwan (5)				1	1	1	2				
Thailand (5)				1	4	•	_				
Vietnam (5)				-	5						
Total (74) <sup>c</sup>	16	2	2	7	42	1	2	1			

<sup>&</sup>lt;sup>a</sup> One isolate (193), which was included in CC239, showed the nontypeable SCC*mec* type.

genotype which is different from that of this Korean strain. By MLST analysis, *tpi* of Kor CR11 represented a new allele, with more than a 20% difference with respect to other alleles; this might have resulted from gene recombination.

In summary, our study identified two distinct genotypes of MRSA strains in Asia. Based on MLST and SCCmec typing, CC5-MRSA-II is a prototype clone in Korea and Japan while CC239-MRSA-III (or IIIA) is a major clone in other Asian countries. These data demonstrated a unique geographic distribution of major genotypes of MRSA clones in the Asian region in relation to other global MRSA clones.

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## REFERENCES

- Baba, T., F. Takeuchi, M. Kuroda, K. Aoki, A. Oguchi, Y. Nagai, N. Iwama, K. Asano, T. Naimi, H. Kuroda, L. Cui, K. Yamamoto, and K. Hiramatsu. 2002. Genome and virulence determinants of high virulence communityacquired MRSA. Lancet 359:1819–1827.
- Baek, J. Y. K. S. Ko, W. S. Oh, S.-I. Jung, Y. S. Kim, H.-H. Chang, H. Lee, S. W. Kim, K. R. Peck, N. Y. Lee, and J.-H. Song. 2004. Unique variations of pbp2b sequences in penicillin-nonsusceptible Streptococcus pneumoniae isolated from Korea. J. Clin. Microbiol. 42:1746–1750.
- Branger, C., C. Cardye, J.-O. Galdbart, C. Deschamps, and N. Lambert. 2003. Genetic relationship between methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* strains from France and from international sources: delineation of genomic groups. J. Clin. Microbiol. 41:2946–2951.
- Chambers, H. F. 2001. The changing epidemiology of Staphylococcus aureus. Emerg. Infect. Dis. 7:3116–3120.
- Day, N. P. J., C. E. Moore, M. C. Enright, A. R. Berendt, J. Maynard Smith, M. F. Murphy, S.J. Peacock, B. G. Spratt, and E. J. Feil. 2001. A link between virulence and ecological abundance in natural population of *Staphylococcus aureus*. Science 292:114–116.
- de Sousa, M., and H. de Lencastre. 2003. Evolution of sporadic isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) in hospitals and their similarities to isolates of community-acquired MRSA. J. Clin. Microbiol. 41:3806–3815.
- de Sousa, M. A., M. I. Crisóstomo, I. S. Sanches, J. S. Wu, J. Fuzhong, A. Tomasz, and H. de Lencastre. 2003. Frequent recovery of a single clonal type

- of multidrug-resistant *Staphylococcus aureus* from patients in two hospitals in Taiwan and China. J. Clin. Microbiol. **41:**159–163.
- Eady, E. A., and J. H. Cove. 2003. Staphylococcal resistance revisited: community-acquired methicillin resistant *Staphylococcus aureus*—an emerging problem for the management of skin and soft tissue infections. Curr. Opin. Infect. Dis. 16:103–124.
- Enright, M. C. 2003. The evolution of a resistant pathogen—the case of MRSA. Curr. Opin. Pharmacol. 3:474–479.
- Enright, M. C., N. P. J. Day, C. E. Davies, S. J. Peacock, and B. G. Spratt. 2000. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J. Clin. Microbiol. 38:1008–1015.
- Enright, M. C., D. A. Robinson, G. Randle, E. J. Feil, H. Grundmann, and B. G. Spratt. 2002. The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). Proc. Natl. Acad. Sci. USA 99:7687–7692.
- Feil, E. J., J. E. Cooper, H. Grundmann, D. A. Robinson, M. C. Enright, T. Berendt, S. J. Peacock, J. Maynard Smith, M. Murphy, B. G. Spratt, C. E. Moore, and N. P. J. Day. 2003. How clonal is *Staphylococcus aureus*? J. Bacteriol. 185:3307–3316.
- Feil, E. J., B. C. Li, D. M. Aanensen, W. P. Hanage, and B. G. Spratt. 2004. eBURST: inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. J. Bacteriol. 186:1518–1530
- Hiramatsu, K., L. Cui, M. Kuroda, and T. Ito. 2001. The emergence and evolution of methicillin-resistant *Staphylococcus aureus*. Trends Microbiol. 9:486–493.
- Hiramatsu, K., K. Okuma, X. X. Ma, M. Yamamoto, S. Hori, and M. Kapi. 2002. New trends in *Staphylococcus aureus* infections: glycopeptide resistance in hospital and methicillin resistance in the community. Curr. Opin. Infect. Dis. 15:407–413.
- Huang, Y.-C., L.-H. Su, T.-L. Wu, C.-E. Liu, T.-G. Young, P.-Y. Chen, P.-R. Hseuh, and T.-Y. Lin. 2004. Molecular epidemiology of clinical isolates of methicillin-resistant *Staphylococcus aureus* in Taiwan. J. Clin. Microbiol. 42:307–310.
- Ito, T., Y. Katayama, K. Asada, N. Mori, K. Tsutsuminoto, C. Tiensasitorn, and K. Hiramatsu. 2001. Structural comparison of three types of staphylococcal cassette chromosome mec integrated in the chromosome in methicillin-resistant Staphylococcus aureus. Antimicrob. Agents Chemother. 45:1323–2336.
- Ito, T., K. Okuma, X. X. Ma, H. Yuzawa, and K. Hiramatsu. 2003. Insights on antibiotic resistance of *Staphylococcus aureus* from its whole genome: genomic island SCC. Drug Resist. Updates 6:41–52.
- Kim, H. B., W. B. Park, K. D. Lee, Y. J. Choi, S. W. Park, M. Oh, E.-C. Kim, and K. W. Choe. 2003. Nationwide surveillance for *Staphylococcus aureus* with reduced susceptibility to vancomycin in Korea. J. Clin. Microbiol. 41: 2279–2281
- Ma, X. X., T. Ito, C. Tiensasitorn, M. Jamklang, P. Chongtrakool, S. Boyle-Vavra, R. S. Daum, and K. Hiramatsu. 2002. Novel type of staphylococcal cassette chromosome mec identified in community-acquired methicillin-resistant Staphylococcus aureus strains. Antimicrob. Agents Chemother. 46: 1147–1152.
- Naimi, T. S., K. H. LeDell, D. J. Boxrud, A. V. Groom, C. D. Steward, S. K. Johnson, J. M. Besser, C. O'Boyle, R. N. Danila, J. E. Cheek, M. T. Osterholm, K. A. Moore, and K. E. Smith. 2001. Epidemiology and clonality of community-acquired methicillin-resistant *Staphylococcus aureus* in Minnesota, 1996–1998. Clin. Infect. Dis. 33:990–996.
- National Committee for Clinical Laboratory Standards. 2003. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A6, 6th ed. NCCLS, Wayne, Pa.
- National Committee for Clinical Laboratory Standards. 2003. Performance standards for antimicrobial susceptibility testing; 13th informational supplement, vol. 23, no. 1. M100-S13. NCCLS, Wayne, Pa.
- Nimmo, G. R., J. Schooneveldt, G. O'Kane, B. McCall, and A. Vickery. 2000. Community acquisition of gentimicin-sesitive methicillin-resistant *Staphylococcus aureus* in southeast Queensland, Australia. J. Clin. Microbiol. 38: 3926–3931.
- Okuma, K., K. Iwakawa, J. D. Turnidge, W. B. Grubb, J. M. Bell, F. G. O'Brien, G. W. Coombs, J. W. Pearman, F. C. Tenover, M. Kapi, C. Tiensasitorn, T. Ito, and K. Hiramatsu. 2002. Dissemination of new methicillin-resistant *Staphylococcus aureus* clones in the community. J. Clin. Microbiol. 40:4289–4294.
- Oliveira, D. C., and H. de Lencastre. 2002. Multiplex PCR strategy for rapid identification of structural types and variants of the *mec* element in methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. 36: 2155–2161.
- Oliveira, D. C., A. Tomasz, and H. de Lencastre. 2001. The evolution of pandemic clones of methicillin-resistant *Staphylococcus aureus*: identification of two ancestral genetic backgrounds and the associated *mec* elements. Microb. Drug Resist. 7:349–361.
- Oliveira, D. C., A. Tomasz, and H. de Lencastre. 2002. Secrets of success of a human pathogen: molecular evolution of pandemic clones of methicillinresistant Staphylococcus aureus. Lancet Infect. Dis. 2:180–189.

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 Robinson, D. A., and M. C. Enright. 2003. Evolutionary models of the emergence of methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. 47:3926–3934.

- Robinson, D. A., and M. C. Enright. 2004. Multilocus sequence typing and the evolution of methicillin-resistant *Staphylococcus aureus*. Clin. Microbiol. Infect. 10:92–97.
- Robinson, D. A., and M. C. Enright. 2004. Evolution of Staphylococcus aureus by large chromosomal replacements. J. Bacteriol. 186:1060– 1064.
- 32. Wang, C.-C., W.-W. Lo, M.-L. Chu, and L. K. Siu. 2004. Epidemiological typing of community-acquired methicillin-resistant. *Staphylococcus aureus* isolates from children in Taiwan. Clin. Infect. Dis. **39**:481–487.