DISCRIMINATORY POWERS OF MOLECULAR TYPING TECHNIQUES FOR METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* IN A UNIVERSITY HOSPITAL, THAILAND

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Abstract. Discriminatory powers of various molecular techniques were evaluated for typing of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated in Siriraj Hospital, Bangkok, Thailand. Thirty MRSA isolates were randomly selected in this study. They were characterized by pulsed-field gel electrophoresis, *Cla*I-*mecA* and *Cla*I-Tn*554* polymorphisms, ribotyping, and PCR-based methods including SCC*mec* typing, *spa* and *coa* gene polymorphism, and repeat units in hypervariable region downstream of *mecA*. Individual molecular typing technique distinguished those MRSA isolates into 2 to 5 types. Eleven genetic backgrounds of MRSA isolates were elucidated by combination of typing methods with trimethoprim/sulfamethoxazole (TMP/SXT) susceptibility. Combination of all typing methods including TMP/SXT susceptibility yielded a discriminatory index of 0.94. Combination of PCR-based methods and TMP/SXT susceptibility, with the discriminatory index of 0.89, is a practical typing approach suitable for rapid epidemiological investigation of MRSA isolates in a hospital setting.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most important nosocomial pathogens worldwide. It has firstly been recognized from outbreaks in the United Kingdom and other European countries more than 40 years ago (Jevon, 1961). Nowadays, it accounts for more than 40% of *Staphylococcus aureus* isolates especially in many hospitals in Asia (Bell *et al*, 2002). MRSA differs from susceptible strain in harboring a special mobile genetic element, named Staphylococcal cassette chromosome *mec* (SCC*mec*), of which *mecA* and its regulatory genes are the main components (Ito *et al*, 2001).

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Effective control measures need knowledge of the bacterium's epidemiology, which requires typing techniques that can be ideally applied to all situations. For the last decade, typing methods have been developed for differentiation of MRSA strains, such as antibiogram, phage typing, serotyping, protein electrophoresis, plasmid analysis, Southern blot hybridization, pulsed-field gel electrophoresis (PFGE) and PCR-based typings (Weller, 2000). PFGE has been suggested to be a gold standard for typing of MRSA (Murchan *et al*, 2003); however, it needs expertise and special instrument, and takes time to conduct (van Belkum *et al*, 1998; Deplano *et al*, 2000). PCR-based typing methods provide a feasible alternative tool, which is more rapid and cost-effective than other molecular typing systems (Deplano *et al*, 2000; Stranden *et al*, 2003). Recently, SCC*mec* typing has been introduced as an additional PCR typing technique for epidemiological investigation (Okuma *et al*, 2002; Oliviera *et al*, 2002). In this study, we evaluated

the discriminatory powers of PFGE, Southern blot hybridization and PCR-based methods for differentiation of MRSA in a 2,400-bed university hospital in Bangkok, Thailand based on calculation of Simpson's discriminatory index (Hunter and Gaston, 1998).

MATERIALS AND METHODS

Bacterial isolates

A total of 30 methicillin-resistant *Staphylococcus aureus* isolates were investigated in this study. These bacterial isolates were randomly selected from individual patients admitted to Siriraj Hospital in 1999 (4 isolates), 2002 (19 isolates), and 2003 (7 isolates). Nine isolates of year 2002 were obtained during an outbreak in an intensive care unit. These MRSA isolates were derived from blood, cerebrospinal fluid, nasal swab, pus, sputum, and soft tissues. Identification of bacterial species had been performed by routine laboratory procedures (Bannerman, 2003). All isolates were confirmed to harbor *mecA* gene by PCR using the primer set described by Ishino *et al* (2002). Control strains in this study were methicillin-resistant *S. aureus* strain NCTC 10442 (with type-I SCC*mec*), strains N315, Mu50, Mu3 (with type-II SCC*mec*), strain 85/2082 (with type-III SCC*mec*), methicillin-susceptible *S. aureus* ATCC 25923.

Antimicrobial susceptibility testing

Antimicrobial susceptibilities of MRSA isolates were performed by disk diffusion technique under standard recommendation by CLSI (formerly NCCLS) (NCCLS, 2000). All MRSA isolates were tested for resistance to ampicillin, oxacillin, cefazolin, cefuroxime, cefotaxime, ceftriaxone, ceftazidime, cefpirome, cefepime, amoxicillin/clavulanic acid, sulbactam/cefoperazone, tazobactam/piperacillin, imipenem, meropenem, erythromycin, clindamycin, amikacin, gentamicin, netilmicin, ciprofloxacin, fosfomycin, fusidic acid, vancomycin, teicoplanin and linezolid.

Chromosomal DNA preparation

Chromosomal DNA of clinical isolates and control strains of MRSA was purified by using phenol-chloroform extraction (Sambrook and Russel, 2001) and 20 μl of 0.2 mg/ml of lysostaphin (Sigma diagnostic, ST Louis, USA) was added as lysis agent in the extraction process. Purified chromosomal DNA was stored at -20ºC until used.

Pulsed-field gel electrophoresis (PFGE)

*Sma*I-pulsed-field gel electrophoresis (*Sma*I-PFGE) was performed as described previously by Prevost *et al* (1992) with some modifications. PFGE was carried out in a contour-clamped homogeneous electric field apparatus (CHEF-DRII or GenPath, BioRad) with an initial time of 5 seconds, 6.0 volts/cm for 20 hours and a final time of 40 seconds. Lambda marker was used as molecular size marker. After the electrophoresis was completed, the gel was stained by 0.5 μg/ml of ethidium bromide and destained with distilled water. Gels were photographed and analyzed as described by Tenover *et al* (1995).

Ribotyping

Ribotyping was performed as described by Blumberg *et al* (1992). In brief, 3 μl of chromosomal DNA of tested isolates were digested by 2 μl of *Eco*RI (30 U) (Amersham Biosciences) in 30 μl of reaction solution and left at 37ºC. After overnight incubation, 5 μl of loading dye were added to stop the reaction. Southern blot hybridization was performed following a protocol described elsewhere (Sambrook and Russel, 2001) using the Gene Images™ random prime labeling module and Gene Images CDP-Star detection module (Amersham Pharmacia Biotech) with 16S rDNA fragment prepared by polymerase chain reaction, using 16S rDNA-1 forward primer, 5´-AGC GGCGGACGGGTGAGTAA-3´ and 16S rDNA-2 reverse primer, 5´-AAGGGTGCGCTCGTTGCGG-3´ (GenBank accession number BA000018; position 506260-507287, MRSA strain N315).

Determination of *mecA* and Tn*554* polymorphisms

mecA and Tn*554* polymorphisms were studied based on Southern blot hybridization using *Cla*I restriction enzyme and probed with DNA fragment of *mecA* gene and transposase B of Tn*554* following the technique described previously (Krieswirth *et al*, 1993).

SCC*mec* typing

SCC*mec* typing was performed by PCR as

described previously by Okuma *et al* (2002) and Oliviera *et al* (2002).

Determination of hypervariable region downstream of *mecA* gene

PCR amplification of hypervariable region downstream of *mecA* was performed as described by Senna *et al* (2002) using primer HVR1, 5´-ACTATTCCCTCAGGCGTCC-3´ (position 338-356 of GenBank accession number X52594) and HVR2, 5´-GGAGTTAATCTACGTCTCATC-3´ (position 912-892 of GenBank accession number X52594). The amplicons were separated in 4% agarose gel and stained by 0.5 µg/ml of ethidium bromide. The images were recorded by a CCD camera via UV-transluminator.

PCR-restriction enzyme analysis for *spa* and *coa* repeat units

The polymorphic region of *spa* encoding surface protein A was performed by protocol described by Frenay *et al* (1994) by PCR using forward primer, 5´-TGTAAAACGACGGCCAG TGCTAAAAAGCTAAACGATGC-3´ and reverse primer, 5´-CAGGAAACAGCTATGACCCCACC AAATACAGTTGTACC-3´. After PCR, 10 µl of amplified products were digested with 8 U of *Rsa*I (Amersham Biotech), according to the manufacturer's recommendation, and incubated at 37ºC for an hour. The reaction was terminated by adding 5 µl of loading dye into each tube. The digested products were separated in 4% agarose gel.

Similar to the protocol for *spa* typing, *coa* gene polymorphisms of MRSA isolates were also performed by PCR using coa2, 5´-CGAG ACCAAGATTCAACAA G-3´, and coa3, 5´-AAAG AAAACCACTCACATCA-3´, primers as described by Goh *et al* (1992). Ten µl of the amplicons were digested with 10 U of *Alu*I (Amersham Biotech), following the manufacturer's instruction, and incubated at 37ºC for one hour. After stopping the reaction, the digested products were separated in 4% agarose gel.

Calculation of discriminatory index

Discriminatory indices of typing systems and their combinations were calculated based on the formula described by Hunter and Gaston (1988).

RESULTS

Thirty isolates of methicillin-resistant *Staphylococcus aureus* showed multi-drug resistance to many varieties of antibiotics. They were susceptible to vancomycin, fosfomycin, fusidic acid, teicoplanin, and linezolid and some isolates were additionally susceptible to trimethoprim-sulfamethoxazole (TMP/SXT), and we used this phenotype as one of the criteria for differentiation of MRSA isolates in our hospital. MRSA isolates in this study were typeable by individual molecular methods with different degrees of discriminatory powers. Based on restriction fragment analysis, such as ribotyping, *Cla*I-*mecA* and *Cla*I-Tn*554* polymorhisms, and *Sma*I-PFGE, we could distinguish MRSA into 2, 4, 4 and 5 groups, respectively. Whereas PCR-based methods, such as PCR-REA of *coa* gene, *spa* gene, SCC*mec* typing and HVR-PCR differentiated the isolates into 3, 4, 4 and 5 groups, respectively. Table 1 shows 11 groups of MRSA categorized primarily by ribotyping, TMP/SXT susceptibility, SCC*mec* typing, *spa* and *coa* polymorphisms and a number of repeat units in hypervariable region downstream of *mecA* gene, following *Sma*I-PFGE and *Cla*I-*mecA*/*Cla*I-Tn*554* polymorphisms as supplementary methods. It was noticeable that *Sma*I-PFGE distinguished MRSA into 5 closely related groups designated as A and A1 to A4, the majority of which were classified into pulsotype A.

Table 2 shows the discriminatory indices of individual typing methods and their combinations. Individual tests provided quite low discriminatory indices of 0.07, 0.40, 0.48, 0.51, 0.58, 0.60, 0.61 and 0.75 corresponding to ribotyping, *Cla*I-Tn*554* polymorphisms, *coa* typing, *spa* typing, SCC*mec* typing, *Cla*I-*mecA* polymorphisms, *Sma*I-PFGE and HVR typing, respectively. Nevertheless, discriminatory indices of their combinations with TMP/SXT susceptibility were further calculated in order to elucidate which combinations should be appropriate for MRSA typing. Combination of all tests, excluding ribotyping, provided highest discriminatory index of 0.94. They could differentiate MRSA isolates in this study into 18 groups. The typing techniques associated with restriction fragment analysis

Table 1
Phenotypic and genotypic characteristics of MRSA isolated at Siriraj Hospital, Bangkok, in 1999, 2001 and 2002.

Group	Isolate	Ribotyping	TMP/SXT susceptibility	SCC*mec*	*spa* type	*coa* type	HVR (DRUs)	Pulsotype	*Cla*I-*mecA*/ *Cla*I-Tn*554*
1	466/99	A	R	III	A	B	7	A	A/A
	777/99	A	R	III	A	B	7	A	A/A
2	94/02	A	R	III	A	B	9	A	A/B
3	19/02	A	R	III	A	B	13	A	A/A
	32/02	A	R	III	A	B	13	A	A/B
	216/02-ICU	A	R	III	A	B	13	A	A/B
	92/03	A	R	III	A	B	13	A	A/A
4	20/02	A	R	III	A	B	15	A	A/A
	269/02-ICU	A	R	III	A	B	15	A4	A/A
	26/03	A	R	III	A	B	15	A	A/A
5	3/02-ICU	B	S	IIIA	A	A	2	A1	B/B
6	1/02	A	S	IIIA	A	A	9	A1	B/A
7	786/99	A	R	IIIA	A	B	7	A	B/A
	102/02	A	R	IIIA	A	B	7	A	A/A
	9/02-ICU	A	R	IIIA	A	B	7	A	B/A
	38/02-ICU	A	R	IIIA	A	B	7	A	B/A
	152/02-ICU	A	R	IIIA	A	B	7	A	B/A
	234/02-ICU	A	R	IIIA	A	B	7	A	B/A
	17/03	A	R	IIIA	A	B	7	A	B/A
	82/03	A	R	IIIA	A	B	7	A	B/A
8	34/02-ICU	A	S	IIIA	B	A	9	A4	B/A
	209/02-ICU	A	S	IIIA	B	A	9	A4	B/A
9	7/02	A	S	IIIA	C	A	9	A4	B/A
	31/02	A	S	IIIA	C	A	9	A4	B/A
	240/02-ICU	A	S	IIIA	C	A	9	A1	B/A
	23/03	A	S	IIIA	C	A	9	A1	B/A
	38/03	A	S	IIIA	C	A	9	A4	B/A
10	107/02	A	R	IIIB	C	A	13	A2	C/C
	78/03	A	R	IIIB	C	A	13	A3	C/C
11	643/99	A	S	II	D	C	9	A	D/D
Control	NCTC10442	-	S	I	ND	ND	ND	-	II*
	N315	A	S	II	D	C	9	-	D/D
	85/2085	B	R	III	A	B	10	-	IV*

TMP/SXT: trimethroprim/sulfamethoxazole; SCC*mec*: staphylococcal cassette chromosome *mec*; *spa*: surface protein A gene; *coa*: coagulase gene; HVR: hypervariable region downstream of *mecA*; DRU: direct repeat units; ND: not done; *: *Cla*I-*mecA*

(*Sma*I-PFGE, *Cla*I-*mecA* and *Cla*I-Tn*554* polymorphisms) could distinguish MRSA isolates into 11 groups with discriminatory index of 0.88, while combination of PCR-based methods with TMP/SXT susceptibility gave a discriminatory index of 0.89.

DISCUSSION

Molecular typing methods provide valuable information of the genetic background of methicillin-resistant *S. aureus* isolates. These techniques have been useful for differentiation among

Table 2
Discriminatory indices of individual typing techniques and their combination for typing of methicillin-resistant *S. aureus* at Siriraj Hospital, Bangkok.

Typing technique(s)	No of types	Discriminatory index
Pulsed-field gel electrophoresis (PFGE)	5	0.61
Ribotyping	2	0.07
*Cla*I-*mecA*	4	0.60
*Cla*I-Tn*554*	4	0.40
SCC*mec* typing	4	0.58
spa typing (*spa*)	4	0.51
coa typing (*coa*)	3	0.48
Hypervariable region typing (HVR)	5	0.75
*Cla*I-*mecA*, *Cla*I-Tn*554*	6	0.69
PFGE, *Cla*I-*mecA*, *Cla*I-Tn*554*	11	0.88
SCC*mec*, *spa*	6	0.77
SXT, SCC*mec*, *spa*	7	0.80
SXT, SCC*mec*, *spa*, *coa*	7	0.80
SXT, SCC*mec*, *spa*, HVR	11	0.89
SXT, SCC*mec*, *spa*, *coa*, HVR	11	0.89
PFGE, HVR	12	0.89
PFGE, TMP/SXT, SCC*mec*, *spa*, *coa*, HVR	16	0.91
PFGE, TMP/SXT, SCC*mec*, *spa*, HVR	16	0.91
SXT, SCC*mec*, *spa*, HVR, *Cla*I-*mecA*	12	0.90
SXT, SCC*mec*, *spa*, *coa*, HVR, *Cla*I-*mecA*, *Cla*I-Tn*554*	13	0.91
PFGE, TMP/SXT, SCC*mec*, *spa*, *coa*, HVR, *Cla*I-*mecA*	17	0.93
PFGE, TMP/SXT, SCC*mec*, *spa*, *coa*, HVR, *Cla*I-*mecA*,*Cla*I-Tn*554*	18	0.94

MRSA strains and further utilized as tools for epidemiological purposes (van Belkum *et al*, 2001). The goal of this study was to evaluate the discriminatory indices of various techniques, which may be used as typing tools for MRSA in a hospital setting.

Individual typing methods showed low discriminatory power, ranging from 0.07 to 0.75. PFGE is the method of choice for genotyping of MRSA; however, it is time-consuming and expensive (Tenover *et al*, 1994; Bannerman *et al*, 1995). Moreover, it may also provide low discriminatory power when the outbreak is caused by clonal pathogens (Tenover *et al*, 1994; Dos Santos Soares *et al*, 2001). In this study, PFGE distinguished 30 MRSA isolates into five related pulsotypes, which were classified into A to A4 corresponding to the criteria described by Tenover *et al* (1995), with a discriminatory index of 0.61. This finding implies that MRSA strains spread in our hospital represent closely related clones, which was also demonstrated by *Cla*I-*mecA* polymorphisms and SCC*mec* typing. The polymorphisms of *Cla*I-*mecA* and *Cla*I-Tn*554* restriction fragment analysis did not differ as much as the study of Krieswirth *et al* (1993) and Dominguez *et al* (1994). This typing requires well-defined strains for comparing patterns among tested isolates.

Based on SCC*mec* typing, 29 of the tested isolates were classified as Brazilian/Hungarian clone (type-III SCC*mec* harboring MRSA), while only one isolate was identified as New York/Japan clone (Oliviera *et al*, 2001, 2002; Okuma *et al*, 2002). Type-III SCC*mec* and its variants (type-IIIA and IIIB) have been primarily reported from Brazil, Hungarian, Spain and Portugal including Australia (Oliviera *et al*, 2001). Recently, it was noticed that type-III SCC*mec* element harboring MRSA strains are mainly distributed in Asian

countries, such as Saudi Arabia, India, Sri Lanka, China, Taiwan, Hong Kong, Indonesia, Philippines, Singapore, and Thailand, while type-II SCC*mec* MRSA strains have been found mainly in Japan and Korea (Aires de Sousa *et al*, 2003; Ip *et al*, 2003; Ko *et al*, 2005). Not only could SCC*mec* typing distinguish MRSA isolates, but it also provided the principal epidemiological data of the spread of MRSA in our hospital.

The other epidemiological marker for MRSA typing was detection of variable units in hypervariable regions downstream of *mecA* gene. Nichi *et al* (1995) designed a primer set for amplification of this region and performed PCR among MRSA isolates in Japan. They demonstrated five different PCR products among 61 MRSA isolates. Further study by Nahvi *et al* (2001) showed that the number of repeat units in HVR is not associated with allotype of SCC*mec* element, which was also observed in this study.

In case of *coa* polymorphism and ribotyping, these two typing techniques had been claimed to have low power for differentiation of MRSA isolates (Tenover *et al*, 1994; Schwarzkopf and Karch, 1994; Hoefnagels-Schuermans *et al*, 1997). In this study, typing by *coa* polymorphism distinguished thirty isolates of MRSA into three groups with discriminatory power of 0.48, whereas ribotyping gave only 0.07. The discriminatory indices made clear that these two techniques are not appropriate for MRSA typing.

Combinations of typing methods are considerably useful for typing of MRSA (Tenover *et al*, 1994; Schmitz *et al*, 1998; Dos Santos Soares *et al*, 2001). In this study, combination of all techniques including trimethoprim/sulfamethoxazole (TMP/SXT) susceptibility provided the best resolution; it was able to identify 18 genotypes among 30 MRSA isolates with discriminatory index of 0.94. However, SCC*mec*, *spa* polymorphism, and HVR-PCR combined with TMP/SXT susceptibility could distinguish MRSA isolates into 11 types with discriminatory power of 0.89. PCR-based methods have the advantage of rapid performance and easy handling; however, PFGE has been demonstrated superior in some studies (Schmitz *et al*, 1998; Senna *et al*, 2002; Stranden *et al*, 2003).

In summary, combination of molecular typing techniques is essential for MRSA typing. PCR-based typing methods including SCC*mec* typing, PCR-REA of *spa* gene and PCR for detection of variable repeat units located downstream of *mecA* gene combined with TMP/SXT susceptibility may be usable tools for MRSA typing in a hospital setting.

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REFERENCES

Aires de Sousa M, Crisostomo MI, Sanches IS, *et al*. Frequent recovery of a single clonal type of multidrug-resistant *Staphylococcus aureus* from patients in two hospitals in Taiwan and China. *J Clin Microbiol* 2003; 41: 159-63.

Bannerman T, Hancock G, Tenover FC, Miller J. Pulsed-field gel electrophoresis as a replacement for bacteriophage typing of *Staphylococcus aureus*. *J Clin Microbiol* 1995; 33: 551-5.

Bannerman T. *Staphylococcus*, *Micrococcus*, and other catalase-positive cocci that grow aerobically. In: Murray P, Baron E, Jorgensen J, Pfaller M, Yolken R, eds. Manual of clinical microbiology. 8th ed. Washington DC: American Society for Microbiology, 2003: 384-404.

Bell JM, Turnidge JD. SENTRY APAC participants. High prevalence of oxacillin-resistant *Staphylococcus aureus* isolates from hospitalized patients in Asia-Pacific and South Africa: results from SENTRY antimicrobial surveillance program, 1998-1999. *Antimicrob Agents Chemother* 2002; 46: 879-81.

Blumberg H, Rimland D, Kiehlbauch J, Terry P, Wachsmuth I. Epidemiologic typing of *Staphylococcus aureus* by DNA restriction fragment length polymorphisms of rRNA genes: elucidation of the clonal nature of a group of bacteriophage-nontypeable, ciprofloxacin- resistant, methicillin-susceptible *S. aureus* isolates. *J Clin Microbiol* 1992; 30: 362-9.

Deplano A, Schuermans A, Van Eldere J, *et al*. Multicenter evaluation of epidemiological typing of methicillin-resistant *Staphylococcus aureus* strains by repetitive-element PCR analysis. *J Clin Microbiol* 2000; 38: 3527-33.

Dominguez MA, de Lencastre H, Linares J, Tomasz A. Spread and maintenance of a dominant methicillin-resistant *Staphylococcus aureus* (MRSA) clone during an outbreak of MRSA disease in a Spanish hospital. *J Clin Microbiol* 1994; 32: 2081-7.

Dos Santos Soares M, Teixeira L, Do Rosario Nunes M, *et al*. Analysis of different molecular methods for typing methicillin-resistant *Staphylococcus aureus* isolates belonging to the Brazilian epidemic clone. *J Med Microbiol* 2001; 50: 732-42.

Frenay HM, Theelen JPG, Schouls LM, *et al*. Discrimination of epidemic and nonepidemic methicillin-resistant *Staphylococcus aureus* strains on the basis of protein A gene polymorphism. *J Clin Microbiol* 1994; 32: 846-7.

Goh SW, Byrne SK, Zhang JL, Chow AW. Molecular typing of *Staphylococcus aureus* on the basis of coagulase gene polymophisms. *J Clin Microbiol* 1992; 30: 1642-5.

Hoefnagels-Schuermans A, Peetermans W, Struelens M, Van Lierde S, Van Eldere J. Clonal analysis and identification of epidemic strains of methicillin - resistant *Staphylococcus aureus* by antibiotyping and determination of protein A gene and coagulase gene polymorphisms. *J Clin Microbiol* 1997; 35: 2514-20.

Hunter P, Gaston M. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. *J Clin Microbiol* 1988; 26: 2465-6.

Ishino K, Tsuchizaki N, Bok S, Kikuchi K, Totsuka K, Hotta K. Relationship between aminoglycoside (AG) resistance and AG modifying enzyme gene profiles in MRSA. Tsukuba, Japan: 10th International Symposium on Staphylococci and Staphylococcal Infections, Oct 16-19; 2002: 149.

Ip M, Lyon D, Chio F, Enright M, Cheng A. Characterization of isolates of methicillin-resistant *Staphylococcus aureus* from Hong Kong by phage typing, pulsed-field gel electrophoresis, and fluorescent amplified-fragment length polymorphism analysis. *J Clin Microbiol* 2003; 41: 4980-5.

Ito T, Katayama Y, Asada K, *et al*. Structural comparison of three types of staphylococcal cassette chromosome *mec* intergrated in the chromosome in methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2001; 45: 1323-36.

Jevons MP. "Celbenin"-resistant staphylococci. *BMJ* 1961; 124: 124-5.

Ko K, Lee J-Y, Suh J, *et al*. Distribution of major genotypes among methicillin-resistant *Staphylococcus aureus* clones in Asian countries. *J Clin Microbiol* 2005; 43: 421-6.

Kreiswirth B, Kornblum J, Arbeit RD, *et al*. Evidence for a clonal origin of methicillin resistance in *Staphylococcus aureus*. *Science* 1993; 259: 227-30.

Murchan S, Kaufmann ME, Deplano A, *et al*. Harmonization of pulsed-field gel electrophoresis protocols for epidemiological typing of strains of methicillin-resistant *Staphylococcus aureus*: a single approach developed by consensus in 10 European laboratories and its application for tracing the spread of related strains. *J Clin Microbiol* 2003; 41: 1574-85.

Nahvi M, Fitzgibbon J, John J, *et al*. Sequence analysis of *dru* regions from methicillin-resistant *Staphylococcus aureus* and coagulase-negative staphylococcal isolates. *Microb Drug Resist* 2001; 7: 1-12.

National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disk susceptibility tests; approved standard. 7th ed. Pensylvania: National Committee for Clinical Laboratory Standards, 2000.

Nichi J, Miyanohara H, Nakajima T, *et al*. Molecular typing of the methicillin resistance determinant (*mec*) of clinical stains of *Staphylococcus* based on *mec* hypervariable region length polymorphisms. *J Lab Clin Med* 1995: 29-35.

Okuma K, Iwakawa K, Turnidge JD, *et al*. Dissemination of new methicillin-resistant *Staphylococcus aureus* clones in the community. *J Clin Microbiol* 2002; 40: 4289-94.

Oliveira DC, Tomasz A, de Lencastre H. The evolution of pandemic clones of methicillin-resistant *Staphylococcus aureus*: identification of two ancestral genetic backgrounds and the associated mec element. *Microb Drug Resist* 2001; 7: 349-61.

Oliveira DC, de Lencastre H. Multiplex PCR strategy for rapid identification of structural types and variants of the *mec* element in methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother*. 2002; 46: 2155-61.

Prevost G, Jaulhac B, Piemont Y. DNA fingerprinting by pulsed-field gel electrophoresis is more effective than ribotyping in distinguishing among methicil-

lin-resistant *Staphylococcus aureus* isolates. *J Clin Microbiol* 1992; 17: 255-69.

Sambrook J, Russell D. Molecular cloning, a laboratory manual. 3rd ed. New York: Cold Spring Harrbor, 2001.

Schmitz F-J, Steiert M, Tichy H-V, *et al*. Typing of methicillin-resistant *Staphylococcus aureus* isolates from Dusseldorf by six genotypic methods. *J Med Microbiol* 1998; 47: 341-51.

Schwarzkopf A, Karch H. Genetic variation in *Staphylococcus aureus* coagulase genes: potential and limits for use as epidemiological marker. *J Clin Microbiol* 1994; 32: 2407-12.

Senna JPM, Pinto CA, Carvalho LPS, *et al*. Comparison of pulsed-field gel electrophoresis and PCR analysis of polymorphisms on the *mec* hypervariable region for typing methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol* 2002; 40: 2254-6.

Stranden A, Frei R, Widmer AF. Molecular typing of methicillin-resistant *Staphylococcus aureus*: can PCR replace pulsed-field gel electrophoresis? *J Clin Microbiol* 2003; 41: 3181-6.

Tenover FC, Arbeit R, Archer G, e*t al*. Comparison of traditional and molecular methods of typing isolates of *Staphylococcus aureus*. *J Clin Microbiol* 1994; 32: 407-15.

Tenover FC, Arbeit R, Goering R, *et al*. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 1995; 33: 2233-9.

van Belkum A, van Leeuwen W, Kaufmann M, *et al*. Assessment of resolution and intercenter reproducibility of results of genotyping *Staphylococcus aureus* by pulsed-field gel electrophoresis of *Sma*I macrorestriction fragments: a multicenter study. *J Clin Microbiol* 1998; 36: 1653-59.

van Belkum A, Struelens M, de Visser A, *et al*. Role of genomic typing in taxonomy, evolutionary genetics, and microbial epidemiology. *Clin Microbiol Rev* 2001; 14: 547-60.

Weller T. Methicillin-resistant *Staphylococcus aureus* typing methods: which should be the international standard? *J Hosp Infect* 2000; 44: 160-72.