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Epidemiologic Study of methicillin-resistant *Staphylococcus aureus* by Coagulase Gene Polymorphism

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ABSTRACT: An epidemiologic study of methicillin-resistant *Staphylococcus aureus* (MRSA) was conducted by antibiotype, coagulase gene typing and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. A total of 129 MRSA strains were isolated from 17 hospitals in the regions of the central, northern, northeastern and eastern Thailand during November 2003 – March 2004. Antimicrobial susceptibility testing with a panel of 10 antimicrobial agents showed 9 different antibiotypes. The antibiotypes 1 and 2 were the most common phenotypes with 44.2% and 35.6% of the isolates, respectively. Coagulase gene typing of MRSA strains generated 4 different genotypes: I, II, III, IV, the PCR products of which were 492±20, 654±20, 735±20 and 816±20 bp with the percentages of 1.5 (2/129), 2.3 (3/129), 82.2 (106/129) and 14 (18/129), respectively. Coagulase gene PCR-RFLPs exhibited 4 patterns: A, B, C and D, with *Alu*I digested PCR product fragments at 220±20 and 220±20 bp (pattern A); 400±20 and 220±20 bp (pattern B); 420±20 and 220±20 bp (pattern C); and 510±20 and 220±20 bp (pattern D). The percentage values for each pattern were compatible with those from the coagulase gene typing method. The results indicated that antibiotypes 1, 2, coagulase gene type III and PCR-RFLP pattern C were the epidemic strains while the rest were sporadic strains.

KEYWORDS: epidemiologic study, methicillin-resistant *Staphylococcus aureus*, antibiotype, coagulase gene typing, polymerase chain reaction-restriction fragment length polymorphism.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) which causes nosocomial infections, is among the most important multi-resistant pathogens worldwide[1-2]. A variety of typing techniques have been developed to discriminate the related strains from unrelated strains. Accurate and rapid typing are crucial for the control of MRSA outbreaks[3]. Both phenotypic and genotypic characterizations can be used to identify epidemic MRSA. A bacteriophage typing has been internationally accepted since 1951[4-5]. However, some MRSA strains may not be typable with phages[6]. The antibiotype has been the main typing tool in many hospital outbreaks because the technique is widely available and standardized. A large number of molecular methods have been developed for epidemiologic typing of MRSA strains, including pulsed-field gel electrophoresis (PFGE), polymerase chain reaction (PCR) of the coagulase (*coa*) gene, the protein A (*spa*) gene and hypervariable (HVR) regions adjacent to the *mec*A gene, DNA sequencing and polymerase chain reaction-restriction fragment length polymorphism of *the coa* and *spa* genes[7,8,9,10,11,12]. PCR products of the *coa* and *spa* genes can be further discriminated by *Alu*I/*Cfo*I/*Hae*III and *Rsa*I digestion, respectively[10,11,12]. Macrorestriction followed by PFGE is considered to be one of the most reliable, discriminatory and reproducible typing procedures, which allows the detection of a high degree DNA polymorphism. However, PFGE is costly, technically complex, time-consuming and lacks an agreed criterion for the interpretation of banding patterns[8,9,12,13,14,15]. Variations in the sequence of the *coa* and *spa* genes and the HVR adjacent to the *mec*A gene, have been the basis for the most widely used forms of PCR typing for MRSA[13], which show a good correlation with PFGE typing[12,13,16,17]. The heterogeneous *coa* gene region contains 81-base pairs tandem repeats at the 3′ ends. The X region in the *spa* gene includes a variable number tandem repeat (VNTR) of 24-base pairs. PCR amplifications of these particular regions produce DNA fragments of different sizes and are highly polymorphic with regard to the number and sequence of the repeats[8,12,17]. Therefore, the aim of this study was to investigate epidemiologic typing of MRSA

isolates in some regions of Thailand by using antibiotype, coagulase gene typing and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of the coagulase gene.

Material and Methods

Bacterial Isolates

A total of 129 MRSA isolates were obtained from clinical specimens, such as pus, sputum, urine and haemoculture, from 17 hospitals in four regions of Thailand (Central, North, Northeast and East). All isolates were collected between November, 2003 and March, 2004. Two coagulase-negative staphylococci (*Staphylococcus epidermidis* and *S. saprophyticus*), *Escherichia coli* and *Pseudomonas aeruginosa* were included as negative controls for PCR testing of the *coa* gene polymorphism. Stock cultures were maintained in a brain-heart infusion broth-glycerol mixture (10% V/V) at –20° C until further analysis.

Identification of MRSA

MRSA isolates were identified by standard microbiological methods including Gram stain, catalase, coagulase, DNase and growth on mannitol salt agar. Resistance to oxacillin was determined by the disc diffusion method according to the National Committee for Clinical Laboratory Standard (NCCLS) guidelines[18].

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed by the disc diffusion method for erythromycin (15 μg), gentamicin (10 μg), tetracycline (30 μg), trimethoprim-sulfamethoxazone (1.25/23.75 μg), amikacin (30 μg), chloramphenicol (30 μg), rifampicin (5 μg), ciprofloxacin (5 μg), vancomycin (30 μg) and teicoplanin (30 μg) according to NCCLS guidelines[18]. Bacterial isolates were considered to belong to different antibiotypes if at least one difference was observed.

DNA extraction

Genomic DNA was isolated by using DNAzol reagent (Invitrogen). One loopful of MRSA cells was harvested from a blood agar plate, suspended in 100 μl UltraPure water (Gibco BRL) and gently mixed with 900 μl of DNAzol reagent. The sample was centrifuged at 10,000 rpm for 10 min at 4°C. The DNA was precipitated from the supernatant by adding 500 μl of absolute ethanol and mixing by inversion at room temperature for 1-3 min. The DNA pellet was washed with 1 ml of 75% ethanol, air-dried and dissolved in 100 μl 8 mM NaOH. The DNA was kept at -20° C until use.

Coagulase Gene Typing by Polymerase Chain Reaction

The entire 3′ tandem repeat end region of the *coa* gene was amplified with two primers, as described by Hooky J *et al*[10,19]. The forward primer 5′ ATA GAG ATG CTG GTA CAG G 3′ (1513 to 1531; nucleotide numbering according to the work of Kaida *et al*[20] MRSA 213, accession no. X16457) and the reverse primer 5′ GCT TCC GAT TGT TCG ATG C 3′ (2188 to 2168) were chosen. Five microliters of DNA solution was added to a 45 μl PCR mixture containing 35 μl of UltraPure water, 5 μl of 10X PCR buffer, 1.5 μl of 50 mM $MgCl_2$, 1 μl of 10 mM deoxyribonucleotide triphosphate (Invitrogen), 1 μl of 10 mM solutions of each primer and 0.5 μl of 5 unit/μl *Taq* DNA polymerase (Invitrogen). DNA amplifications were performed in a P.E 2400 Thermal cycler (Perkin-Elmer 2400) as the following cycling parameters: an initial step at 94°C for 3 min; 30 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1 min; and a final step at 72°C for 5 min. PCR products (10 μl) were subjected to electrophoresis on 2% (W/V) agarose gel (Seakem) and the sizes of the PCR products were determined by comparison to the 100-1,500 base pairs ladder DNA marker (Roche Applied Science). The gel was stained with ethidium bromide and

Table 1. Antimicrobial susceptibilities of MRSA isolates in this study.

Antibiotype	Susceptibility to Antibiotics[a]										No. of isolates (%)
	ERY	GEN	TET	SXT	AMK	CHL	RIF	CIP	VAN	TEC	
Ant 1	R	R	R	R	R	S	R	R	S	S	57 (44.2)
Ant 2	R	R	R	R	R	S	S	R	S	S	46 (35.6)
Ant 3	R	R	R	R	R	R	S	R	S	S	12 (9.3)
Ant 4	R	R	R	R	R	R	R	R	S	S	5 (3.8)
Ant 5	R	R	R	S	R	R	S	R	S	S	2 (1.6)
Ant 6	R	R	S	S	R	S	S	R	S	S	2 (1.6)
Ant 7	R	R	S	S	S	S	S	R	S	S	1 (0.8)
Ant 8	R	R	S	R	S	S	S	R	S	S	1 (0.8)
Ant 9	R	R	S	R	R	S	R	R	S	S	3 (2.3)

[a]Abbreviations for susceptibility: R, resistant; S, susceptible.
Abbreviations for antimicrobial agents: ERY: Erythromycin, GEN: Gentamicin, TET: Tetracycline, SXT: Trimethoprim-sulfamethoxazone, AMK: Amikacin, CHL: Chloramphenicol, RIF: Rifampicin, CIP: Ciprofloxacin, VAN: Vancomycin, TEC: Teicoplanin.

photographed on a UV illuminator.

Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) of the Coagulase Gene

Ten microliters of PCR product was digested with 2 units of restriction endonuclease *Alu*I (Invitrogen) at 37°C for 1 hr 30 min. The restriction digested fragments were electrophoresis on 2% agarose gels, which were then stained with ethidium bromide and visualized under UV light.

Results

Antibiotypes

Nine different antibiotypes (Ant 1 - 9) were identified using a panel of 10 antimicrobial agents (Table 1). The most common antibiotypes, namely antibiotypes 1 and 2, were found in 44.2% (57/129) and 35.6% (46/129) of the isolates, respectively. Antibiotype 1 was sensitive to chloramphenicol, vancomycin and teicoplanin, and resistant to erythromycin, gentamicin, tetracycline, rifampicin, trimethoprim-sulfamethoxazone and amikacin. Antibiotype 2 was similar to antibiotype 1 excepted that it was sensitive to rifampicin. Antibiotypes 1 and 2 were involved in epidemic outbreak, while the others (antibiotypes 3 - 9) were found in sporadic cases.

Coagulase Gene Typing and Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) of Coagulase Gene

PCR amplification of the coagulase gene from 129 isolates generated 4 different types of products: I, II, III and IV, which had sizes of 492±20, 654±20, 735±20 and 816±20 base pairs (Figure 1A) and were found at 1.5% (2/129), 2.3% (3/129), 82.2% (106/129) and 14% (18/129), respectively (Table 2). Two coagulase-negative staphylococci, *E. coli*, *Ps. aeruginosa* produced no DNA products upon PCR amplification. PCR-RFLP analysis of the coagulase gene yielded 4 patterns of *Alu*I digested PCR products: A, B, C and D, which had DNA products at 220±20 and 220±20 base pairs (pattern A); 400±20 and 220±20 base pairs (pattern B); 420±20 and 220±20 base pairs (pattern C); and 510±20 and 220±20 base pairs (pattern D), respectively (Figure 1B). The percentages of these patterns were compatible with those of the coagulase gene typing method. The band of 220±20 base pairs was a common one found in all patterns, while the others varied in size. Coagulase gene type III / PCR-RFLP pattern C was the most common (n = 106; 82.2%) and accounted for all epidemic strains, while the rest of coagulase gene types / PCR-RFLP patterns were found in sporadic strains. Figures 2 and 3 show the coagulase gene type / PCR-RFLP pattern for

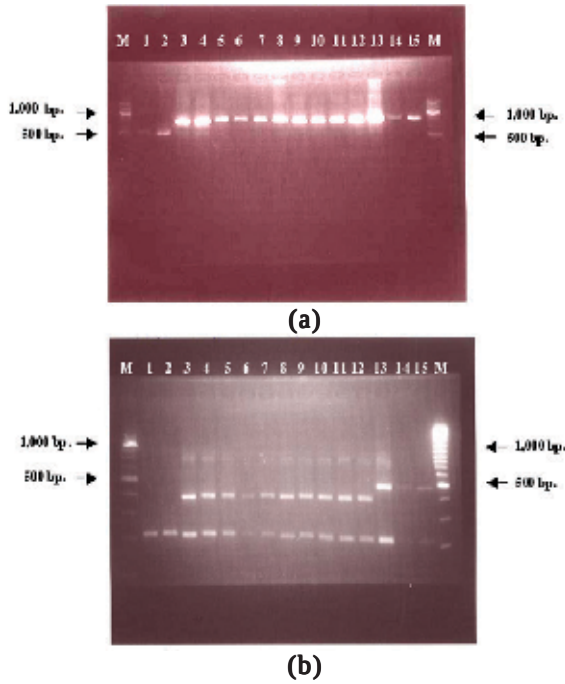

Fig 1. Agarose gel electrophoresis of coagulase gene products obtained from representative isolates of MRSA. A) Undigested coagulase gene PCR products. Lanes 1-2: coagulase gene type I (492 ±20 bp); lane 3: coagulase gene type II (654±20 bp); lanes 4-12: coagulase gene type III (735±20 bp); lanes 13-15: coagulase gene type IV (816±20 bp). B) *Alu*I digested coagulase gene PCR products which correspond to figure 1A. Lanes 1-2: coagulase PCR-RFLP pattern A (220 ±20, 220 ±20 bp); lanes 3; coagulase PCR-RFLP pattern B (400±20, 220 ±20 bp); lanes 4-12: coagulase PCR-RFLP pattern C (420±20, 220 ±20 bp) and lanes 13-15: coagulase PCR-RFLP pattern D (510±20, 220 ±20 bp). Lanes M: 100-1,500 bp ladder DNA marker.

Table 2. Coagulase gene typing and PCR-RFLP pattern of MRSA.

Coagulase gene typing / PCR-RFLP pattern	Size of *coa* gene PCR product	Size of *Alu*I fragments	No. of isolates (%)
I / A	492 ± 20	220 ± 20, 220 ± 20	2 (1.5)
II / B	654 ± 20	400 ± 20, 220 ± 20	3 (2.3)
III / C	735 ± 20	420 ± 20, 220 ± 20	106 (82.2)
IV / D	816 ± 20	510 ± 20, 220 ± 20	18 (14)

epidemic and sporadic strains, respectively. Almost all of the antibiotype 1 (n=51) and antibiotype 2 (n=38) isolates had coagulase gene type III and PCR-RFLP pattern C (Table 3).

Discussion

Rapid and accurate differentiation of MRSA associated with nosocomial infection is crucial to epidemiologic surveillance and hospital infection control. This may clarify whether strains from the environment or from staff members are related to those that cause infection, and whether isolates from one patient belong to one genotype. In this study, we have evaluated the use of antibiotype and PCR based typing methods (*coa* gene typing and PCR-RFLP of the *coa*

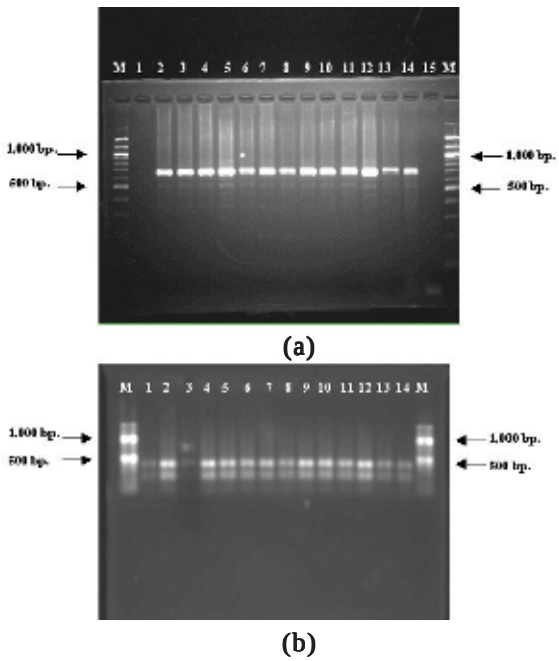

Fig 2. Agarose gel electrophoresis of coagulase gene products obtained from representatives of clinical epidemic MRSA strains from the same hospital. A) Undigested coagulase gene PCR products. Lanes 2-14: coagulase gene type III (735 ±20 bp); lanes 1 and 15: negative controls *S. saprophyticus* and *S. epidermidis*. B) *Alu*I digested coagulase gene PCR products which correspond to figure 2A (lanes 2-14). Lanes 2-14: coagulase PCR-RFLP pattern C (420±20, 220 ±20 bp); lane 1: coagulase PCR-RFLP pattern C from MRSA strain in another hospital. Lanes M: 100-1,500 bp ladder DNA marker.

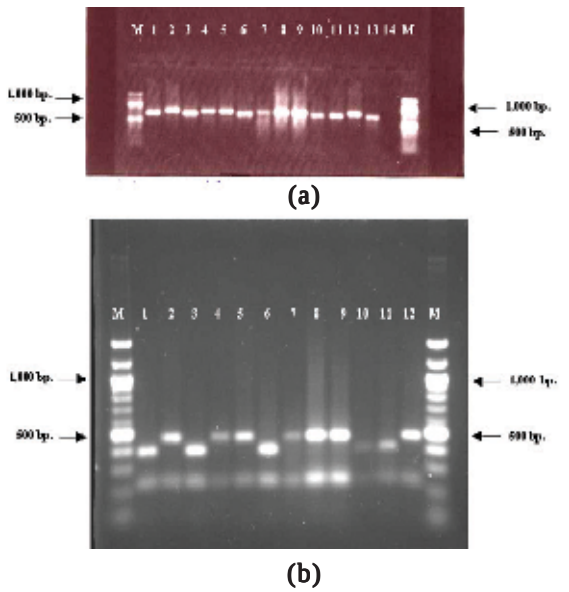

Fig 3. Agarose gel electrophoresis of coagulase gene products obtained from representatives of clinical sporadic MRSA strains from the same hospital. A) Undigested coagulase gene PCR products. Lanes 1, 3, 6, 10-11: coagulase gene type III (735 ±20 bp); lanes 2, 4-5, 7-9, 12: coagulase gene type IV (816 ±20 bp); lane 13 coagulase gene type III from another hospital and lane 14: *E. coli.* negative control B) *Alu*I digested coagulase gene PCR products which compatible to figure 3A (lanes 1-12). Lanes 1, 3, 6, 10-11: coagulase PCR-RFLP pattern C (420±20, 220 ±20 bp); lanes 2, 4-5, 7-9, 12: coagulase PCR-RFLP pattern D (510±20, 220 ±20 bp). Lanes M: 100-1,500 bp ladder DNA marker.

Table 3. Correlations between antibiotypes and PCR based method.

Antibiotypes (n)	Coagulase gene type (n)	Coagulase PCR-RFLP pattern (n)
1 (57)	coa3 (51), coa2 (3), coa4 (3)	RFLP-C (51), RFLP-B (3), RFLP-D (3)
2 (46)	coa3 (38), coa4 (6), coa1 (2)	RFLP-C (38), RFLP-D (6), RFLP-A (2)
3 (12)	coa3 (10), coa4 (2)	RFLP-C (10), RFLP-D (2)
4 (5)	coa3 (5)	RFLP-C (5)
5 (2)	coa4 (2)	RFLP-D (2)
6 (2)	coa4 (2)	RFLP-D (2)
7 (1)	coa4 (1)	RFLP-D (1)
8 (1)	coa4 (1)	RFLP-D (1)
9 (1)	coa4 (1)	RFLP-D (1)

gene) in an epidemiological study of MRSA isolated from four regions of Thailand. Antibiotype was a good epidemiological marker, showing a highly significant correlation with *coa* gene typing and PCR-RFLP of the *coa* gene. We found that antibiotypes 1 and 2 were correlated to *coa* gene type III and PCR-RFLP pattern C. Antimicrobial susceptibility testing is the simplest epidemiologic typing method, the results are easy to interpret and minimal laboratory skills and equipment are required. Nevertheless, its main disadvantage consists of the variability in resistance expression, which is susceptible to instability due to loss of extrachromosomal genetic elements[9,17].

Coagulase enzyme activity is the principle criteria for diagnosis of *S. aureus* from other staphylococci. This enzyme can convert fibrinogen to fibrin. The coagulase gene consists of three distinct regions: (i) the N-terminus containing the prothrombin-binding site, (ii) a central region which is highly conserved, and (iii) a C-terminal region composed of 81-bp tandem repeated units, which each encode 27-amino acid residues. The C-terminal repeated units comprised four, five, six, seven and eight units of the 81-tandem repeat[16]. Therefore, the size of 3′ region of the *coa* gene is variable in MRSA strains. The heterogeneity in the number of tandem repeat units of the *coa* gene would be a potential target for typing of MRSA[13,16,17]. We found that the epidemic strains had *coa* gene type III, in which the PCR product was 735±20 base pairs. The other, sporadic cases had *coa* gene types I, II and IV.

We used *Alu*I restriction endonuclease, which recognizes AT/GC sequences, for RFLP typing, which can be equally well assessed by *Cfo*I or *Hae*III digestion of PCR product[8,10,19]. Four distinct PCR-RFLP patterns were observed among the 129 strains. The number of fragments produced upon *Alu*I digestion were one to two bands. These results differ from previous studies, in which *Alu*I PCR-RFLP fragments varied from one to four bands using the same primer sequences[10,19]. However, the results of epidemic typing of the PCR-based methods are correlated. Both methods have been reported as attractive typing methods for clinical laboratories[21,22]. They require only small quantities of crude DNA and isolates can be compared easily by the number and size of PCR products and their *Alu*I restriction fragments. The low discriminatory power of the PCR-RFLP method makes it less suitable as a single typing method, but this could be overcome by the use of other enzymes to expand the number of bands[23].

In conclusion, antibiotyping is valuable, especially in routine work, as a first-line screening method to determine strain relatedness. It may allow rapid and early recognition of a previously defined epidemic strain in a particular hospital setting. Also, PCR-based method (*coa* gene typing and PCR-RFLP of the *coa* gene) provide easy, inexpensive and reasonable results for epidemiological studies during nosocomial outbreaks and are able to distinguish between the epidemic and sporadic strains.

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References

1. Tambic A, Power E, Anthony R and Franch G (1997) Analysis of an outbreak of non-phage-typable methicillin-resistant *Staphylococcus aureus* by using a randomly amplified polymorphic DNA assay. *Journal of Clinical Microbiology* **35(12)**, 3092-7.
2. Boyce JM (1994) Methicillin-resistant *Staphylococcus aureus*: a continuing infection control challenge. *Europe Journal of Clinical Microbiology Infectious Diseases* **13**, 45-9.
3. Maslow ME and Mulligan ME (1996) Epidemiologic typing system. *Infection Control Hospital Epidemiology* **17**, 595-604.
4. Hartstein AI, Morthland V, Archer G, Schoenknecht F and Rashad A (1989) Restriction enzyme analysis of plasmid DNA and bacteriophage typing of paired *Staphylococcus aureus* blood culture isolates. *Journal of Clinical Microbiology* **27**, 1874-9.
5. Milligan ME, Kwok RY, Citron DM, John JF and Smith PB (1998) Immunoblots, antimicrobial resistance, and bacteriophage typing of oxacillin-resistant *Staphylococcus aureus*. *Journal of Clinical Microbiology* **26**, 2395-401.
6. Blair JE and Willium RE (1961) Phage typing of staphylococci. *Bulletin WHO* **24**, 771-84.
7. Goh SH, Byrne EE, Zhang JL and Chow AW (1992) Molecular typing of *Staphylococcus aureus* on the basis of coagulase gene polymorphisms. *Journal of Clinical Microbiology* **30**, 1642-5.
8. Montesinos I, Salido E, Delgado T, Cuervo M and Sierra A (2002) Epidemiologic genotyping of methicillin-resistant *Staphylococcus aureus* by pulsed-field gel electrophoresis at a university hospital and comparison with antibiotyping and protein A and coagulase gene polymorphism. *Journal of Clinical Microbiology* **40(6)**, 2119-25.
9. Trindade PA, McCulloch JA, Oliveira GA and Mamizuka EM (2003) Molecular techniques for MRSA typing: Current issues and perspectives. *The Brazilian Journal of Infectious Diseases* **7(1)**, 32-43.
10. Hookey J, Richardson JF and Cookson BD (1998) Molecular typing of *Staphylococcus aureus* based on PCR-restriction fragment polymorphism and DNA sequence analysis of the coagulase gene. *Journal of Clinical Microbiology* **36(4)**, 1083-

9.
11. Roberts RB, Lencaster A, Eisner W, Severrina EP, Shopsin B and Kreiswirth BN (1998) Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in 12 New York hospitals. *The Journal of Infectious Disease* **178**, 164-71.
12. Tang Y, Waddington MG, Smith DH, Manahan JM, Kohner PC and Highsmith LM (2000) Comparison of protein A gene sequencing with puled-field gel electrophoresis and epidemiologic data for molecular typing of methicillin-resistant *Staphylococcus aureus*. *Journal of Clinical Microbiology* **38(4)**, 1347-51.
13. Stranden A, Frei R and Widmer AF (2003) Molecular typing of methicillin-resistant *Staphylococcus aureus*: Can PCR replace pulsed-field gel electrophoresis ? *Journal of Clinical Microbiology* **41(7)**, 3181-6.
14. BannermanTL, Hancock GA, Tenover FC and Miller JM (1995) Pulsed-field gel electrophoresis as a replacement for bacteriophage typing of *Staphylococcus aureus*. *Journal of Clinical Microbiology* **33**, 551-5.
15. Cookson BD, Aparcio P, Deplano A, Struelens M, Goering R and Marples R (1996) Inter-centre comparison of pulsed-field gel electrophoresis for the typing of methicillin-resistant *Staphylococcus aureus*. *Journal of Medical Microbiology* **44**, 177-84.
16. Schwarzkope A and Karch H (1994) Genetic variation in *Staphylococcus* coagulase gene: potential and limits for use as epidemiological marker. *Journal of Clinical Microbiology* **32(10)**, 2407-12.
17. Schuermans AH, Peetermans WE, Struelens M, Lierd S and VanEldere J (1997) Clonal analysis and identification of epidemic strains of methicillin-resistant *Staphylococcus aureus* by antibiotyping and determination of protein A gene and coagulase gene polymorphism. *Journal of Clinical Microbiology* **35(10)**, 2514-20.
18. National Committee for Clinical Laboratory Standards (1994) Performance standards for antimicrobial disk susceptibility testing. 5th ed. Approved standard M2-A5. National Committee for Clinical Laboratory Standards, Villanova, Pa.
19. Hookey J, Edwards V, Cookson BD and Richardson J (1999) PCR-RFLP analysis of the coagulase gene of *Staphylococcus aureus*: application to the differentiation of epidemic and sporadic methicillin-resistant strains. *Journal of Hospital Infection* **42,** 205-12.
20. Kaida S, Miyata T, Yoshizawa Y, Igarashi H and Iwanage S (1989) Nucleotide and deduced amino acid sequence of staphylocoagulase gene from *Staphylococcus aureus* strain 213. *Nucleic Acids Research* **17,** 8871.
21. Kobayashi N, Taniguchi K, Kojima K, Urasawa S, Uchara N and Omize Y (1995) Analysis of methicillin-resistant *Staphylococcus aureus* by a molecular typing method based on coagulase gene polymorphisms. *Epidemiology Infection* **115**, 419-26.
22. Nada T, Ichiyama S, Osada Y, Ohta M, Shimokata K, Kato N and Nakashima N (1996) Comparison of DNA fingerprinting by PFGE and PCR-RFLP of the coagulase gene to distinguish MRSA isolated. *Journal of Hospital Infection* **32**, 305-17.
23. Tenover FC, Arbiet R, Archer G, Biddle J, Byrne S, Goering R and Hancock G (1994) Comparison of traditional and molecular methods of typing isolates of *Staphylococcus aureus*. *Journal of Clinical Microbiology* **32**, 407-45.