Methicillin

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Molecular Epidemiological Typing of Clinical Strains of
Methicillin-Resistant *Staphylococcus aureus*

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Background Meicillin-resistant *Staphylococcus aureus*(MRSA) is a common cause of nosocomial infections worldwide. Identification of strains by molecular typing facilitates epidemiological studies and improves disease control. This study was performed to determine the usefulness of *mec*A-associated hypervariable region(HVR) polymerase chain reaction (PCR) and random amplified polymorphic DNA(RAPD) analysis in the investigation of a nosocomial MRSA infections.

Methods Methicillin-resistance was identified by NCCLS disk diffusion method using the oxacillin disk. And PCR was done for detection of *mec*A gene. Antimicrobial susceptibility test, HVR-PCR and RAPD using 3 primers were performed for epidemiological analysis on isolates of MRSA.

Results During the period from 1997 Dec. to 1998 May, 120 strains of *S. aureus* were isolated from clinical specimens. Among them, 78 strains were MRSA, and 72 strains were *mec*A positive. The strains of *mec*A positive MRSA were classified into four types by antibiogram, six genotypes by HVR-PCR, and 29 groups by RAPD using three primers. The combination of HVR genotypes and RAPD analysis showed 43 different types in 72 *mec*A positive MRSA isolates. The five strains which were repeatedly isolated from the same patients showed the same HVR genotypes and RAPD analysis.

Conclusions Antibiogram, HVR-PCR, and RAPD could classify MRSA isolates into only 4-6 types, respectively, but combination of these methods could improve the typability. And combination of results of RAPD analysis using three primers were better than that using one primer in epidemiological studies of MRSA because of same reasons. It can be concluded

that molecular typing of MRSA using HVR-PCR and RAPD assay is useful in epidemiolgical investigation of nosocomial infections caused by MRSA, because of its simplicity and reproducibility. (Korean J Clin Microbiol 1999;2:40~48)

Key words Methicillin-resistant *Staphylococcus aureus*, Hypervariable region, *mec*A, Polymerase chain reaction, Genotype, Random amplified polymorphic DNA

Methicillin-resistant *Staphylococcus aureus* (MRSA)

. Methicillin

2 1961

[1], 1970

,

[2,3]. 1990

S. aureus MRSA

59.1~67.3% [4].

methicillin

1980 23.3~48% [5,6],

70~80% [7].

MRSA

, (,),

.

, ,

, [4].

MRSA

,

MRSA .MRSA

antibiogram, phage , coagulase , plasmid

[8~16] .

. rare-cutting endonuclease

DNA pulsed-field gel electrophoresis (PFGE)

[17~20]. PFGE

,

.

*mec*A MRSA

,

mec (*mec*-associated hypervariable region, HVR) [21] random amplified polymorphic DNA (RAPD)

.

1.

1997 12 1998 5

120 *S. aureus* .

[22] ,

Vitek GPI card (bioMeriux, Hazelwood, Mo., USA)

.

2. Oxacillin

NCCLS [23] oxacillin (BBL, Cockeysville, MD, U.S.A.) .

Trypticase soy broth (TSB, BBL)

McFarland No. 0.5 Mueller-Hinton (BBL) .

35 24 ,

mm . *S. aureus* ATCC 25923 .

3. *mec*A

Oxacillin

100μL McFarland No. 4 .

1 mg/mL lysostaphin (Sigma, St. Louis, U.S.A.) 10μL , 37 95 5

. 10,000 rpm 5

DNA extract .

Primer 5'-AAAATCGATGGTAAAGGTTGGC-3' 5'-AGTTCTGCAGTACCGGATTTGC-3' .

20μL, Taq 2.5μL, dNTP 0.25μL, primer (25μM) 0.5μL DNA extract 1 μL

25.1μL premix . Gene Amp PCR system 9600 (Perkin Elmer Co., Norwalk, U.S.A.)

94 1 predenaturation , 94 30 denaturation, 50 30 annealing 72 1 extension cycle 40 , postextension 72 5 . 10μL DNA marker

1.3% agarose gel ethidium bromide 533 bp .

*mec*A *S. aureus* ATCC 25923, E. coli ATCC 25922 .

4. Antibiogram

*mec*A MRSA . NCCLS

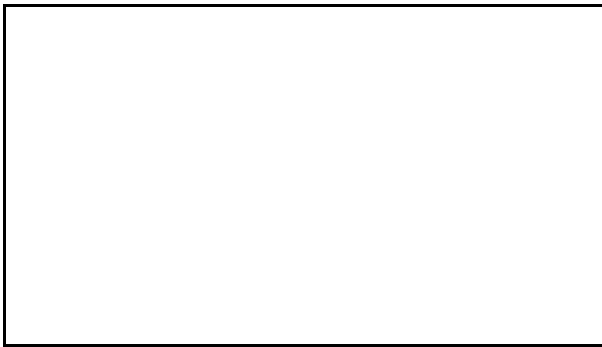[23] cephalothin, ciprofloxacin, clindamycin, erythromycin, gentamicin, imipenem, oxacillin, penicillin G,

Fig. 1. *mec*A-PCR products (533bp) amplified from clinical isolates. Lanes: M, size marker; 1 to 5, MRSA; 6, MSSA; 7, *S. aureus* ATCC 25923; 8, E. coli ATCC 25922.

teicoplanin   vancomycin (BBL)
         .

5. HVR-PCR

*mec*A   MRSA   . Primer
5'-ACTATTCCCTCAGGCGTC-3'   5'-GGAGTTAATCTA
CGTCTCATC-3'   . Premix   *mec*A
                , PCR   denaturation
94   1 , annealing 55   1 , extension 72   2   1
cycle   30   .   10μL   DNA marker
        1.3% agarose gel   ethidium
bromide   .

6. RAPD

*mec*A   MRSA   Slots   [24]
                    . Primer   OPA-3 (5'-AGTCAGCC
AC-3'), OPA-13(5'-CAGCACCCAC-3')   OPA-14 (5'-T
CTGTGGGG-3')   RAPD
    . Premix   10 mM Tris-HCl (pH 8.3), 50 mM KCl, 4 mM
MgCl₂, 0.1 mM dNTP, 10 pmol primer   2.5 unit   taq
polymerase (Takara Shuzo Co. LTD, Japan)   DNA extract
1.5μL   . PCR   94   5
predenaturation   94   1 , 42   2   72   2   1
cycle   31   , 72   10
postextension   . PCR   2% agarose gel
                ethidium bromide   ,

.   scanner
1 D Image Analysis Software (Eastman Kodak Co.,
Rochester, NY, USA)
            .

1. Oxacillin   *mec*A

                    120   *S. aureus*   78
(65%)   oxacillin   , 42   .
MRSA   72 (92%)   *mec*A
        (Fig. 1).

2. Antibiogram

MRSA   4
    . 58   vancomycin   teicoplanin
    ,   14   ciprofloxacin
clindamycin
    (Table 1).

3. HVR   RAPD

*mec*A   MRSA   HVR   640, 590,
550, 500, 420, 320 bp   6   (Fig. 2, 3).
                                -
    ,   10 ,   8 ,
15 ,   1 ,   35 ,   3 .
RAPD   primer OPA-3   5
(1-5), OPA-13   5   (a-e), OPA-14
      4   (A-D)   (Fig. 4, 5).
    1   (42), e   (36)   A   (30)   .
    primer   MRSA   29
            (Table 2).

HVR   RAPD   *mec*A
    MRSA   43   . HVR
            OPA-3 2   (7/10) , HVR
OPA-3 1   (12/15, 26/35), OPA-13 e   (7/15, 22/35)
OPA-14 A   (7/15, 16/35)   (Table 3).

Table 1. Antimicrobial susceptibility patterns of MRSA

Patterns	Antimicrobial agents										
	CF	CIPR	CC	EM	GM	IPM	OX	P	TEC	VA	N
	R	R	R	R	R	R	R	R	S	S	58
	S	R	R	R	R	R	R	R	S	S	10
	R	S	R	R	R	R	R	R	S	S	2
	S	S	R	R	R	R	R	R	S	S	2

Abbreviations: CF, cephalothin; CIPR, ciprofloxacin; CC, clindamycin; EM, erythromycin; GM, gentamicin; IPM, imipenem; OX, oxacillin; P, penicillin G; TEC, teicoplanin; VA, vancomycin; R, resistant : S, susceptible.
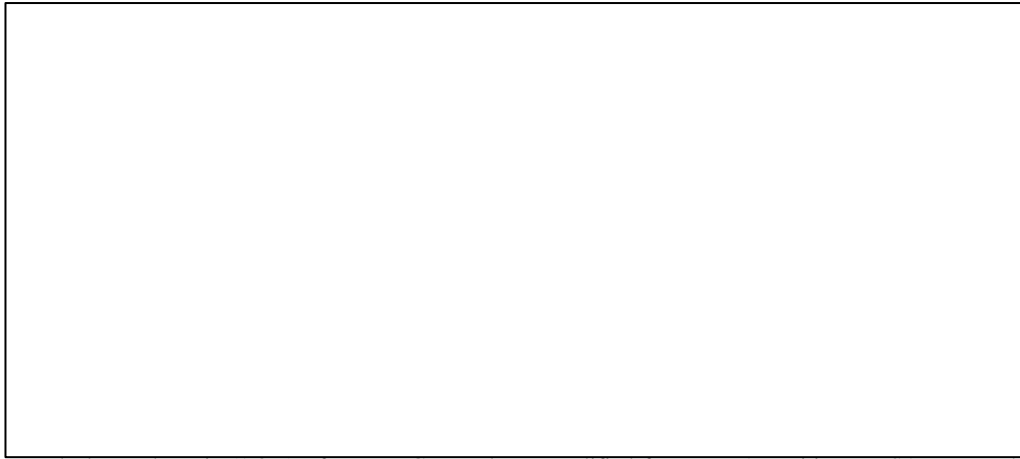
Fig. 2. Agarose gel electrophoresis (1.3%) of HVR-PCR products amplified from *mec*A positive MRSA. Lanes show the band patterns of various sizes. Lanes: M, size marker; 1 to 18, clinical isolates of *mec*A positive MRSA.
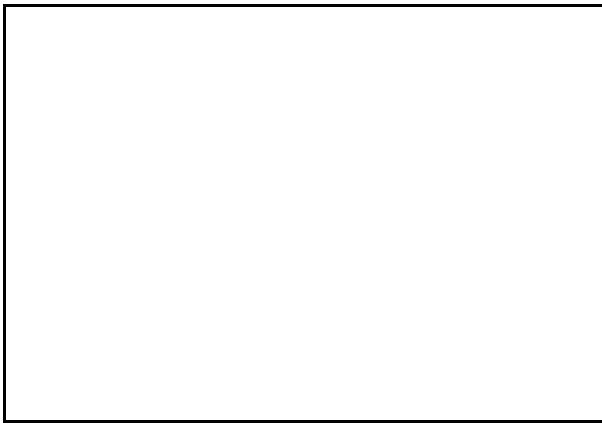

Fig. 3. Schematic diagram of HVR-PCR products amplified from *mec*A positive MRSA. Lane M, size marker; lane 1, type (640bp); 2, type (590bp); 3, type (540bp); 4, type (500bp); 5, type (420bp); 6, type (320bp). Approximate molecular weight of fragments was calculated against DNA standard marker(Lane M).
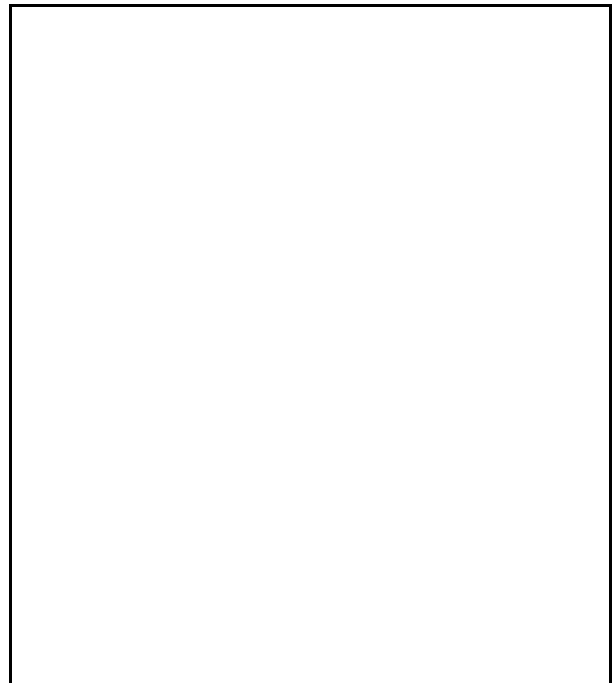

Fig. 4. DNA fingerprint patterns produced by RAPD. Lanes show the various band patterns among a collection of 72 MRSA strains. Different primers were used; a) primer OPA-3, b) primer OPA-13, c) primer OPA-14. Lanes: M, size marker; 1 to 19, clinical isolates of *mec*A positive MRSA.

4. MRSA

MRSA

Table 4 .

, 7 , 8 , 9 antibiogram-I ,
HVR- , OPA-3 1 , OPA-13 e OPA-14 A B
, I , , 1 ,
e A . I ,
2 .

MRSA ,
MRSA

. MRSA MSSA
-lactam

,

[4].
MRSA -lactam *mec*A
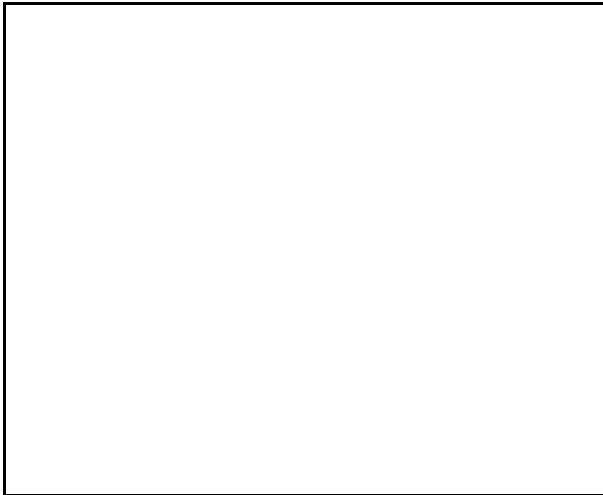penicillin-binding protein 2'(PBP2')
. PBP 2' -lactam

Fig. 5. Schematic diagram representig 2% agarose gel electrophoresis patterns of MRSA fingerprints by RAPD. Individual profiles were assigned as an arbitrary number from 1 to 5 for primer OPA-3, a to e for primer OPA- 13, and A to D for primer OPA-14, respectively. Molecular weight of fragments was calculated against DNA standard marker(Lane M).

Table 2. Comparison of RAPD patterns of MRSA by arbitrary primers

Arbitrary primers					
OPA-3		OPA-13		OPA-14	
Type	No. isolates	Type	No. isolates	Type	No. isolates
1	42	b	1	A	1
		c	13	A	3
				B	5
				D	5
		d	9	A	1
				B	6
				C	1
				D	1
		e	19	A	11
				B	7
				C	1
2	12	a	2	B	1
				C	1
		b	1	B	1
		c	4	A	2
				C	1
				D	1
		d	3	A	2
				B	1
		e	2	A	2
3	8	c	1	A	1
		e	7	A	6
				C	1
4	9	c	1	D	1
		d	1	B	1
		e	7	A	1
				C	3
				D	3
5	1	e	1	B	1

, -lactam

. *S. aureus*

65% methicillin ,

.

MRSA ,

. MRSA

92% *mec*A , MRSA *mec*A

. *mec*A MRSA PBP

-lactamase

.

MRSA *mec* operon [25], *mec*A

mec operon [26,27]. *mec*A

2.5 Kb transposon code

IS 431 ,

[28]. *mec* A IS 431 2 Kb

, Ryffel [29] HVR .

Nishi [21] HVR MRSA 106

5 ,

MRSA 6

[16]. MRSA HVR 6

(-) .

, ,

,

MRSA

.

RAPD 1990 , Polymorphic

DNA random amplification

,

,

[30,31]. [32]

MRSA RAPD DNA ,

MgCl$_2$, buffer pH PCR cycle

.

,

band RAPD

Table 3. Comparison of hypervariable region genotypes with RAPD patterns

HVR genotype (No. isolates)	RAPD pattern (No. isolates)		
	OPA-3	OPA-13	OPA-14
(10)	2 (7)	a (2)	B (1)
			C (1)
		b (1)	B (1)
		c (1)	C (1)
		d (2)	A (2)
		e (1)	A (1)
	4 (3)	c (1)	D (1)
		e (2)	C (1)
			D (1)
(8)	1 (4)	c (2)	D (2)
		d (1)	D (1)
		e (1)	B (1)
	2 (2)	c (1)	A (1)
		d (1)	B (1)
	4 (1)	e (1)	A (1)
	5 (1)	e (1)	B (1)
(15)	1 (12)	c (6)	A (2)
			B (3)
			D (1)
		d (2)	B (2)
		e (4)	A (3)
			B (1)
	3 (2)	e (2)	A (2)
	4 (1)	e (1)	D (1)
(1)	4 (1)	d (1)	B (1)
(35)	1 (26)	b (1)	A (1)
		c (5)	A (1)
			B (2)
			D (2)
		d (6)	A (1)
			B (4)
			C (1)
		e (14)	A (8)
			B (5)
			C (1)
	3 (6)	c (1)	A (1)
		e (5)	A (4)
			C (1)
	4 (3)	e (3)	C (2)
			D (1)
(3)	2 (3)	c (2)	A (1)
			D (1)
		e (1)	A (1)

.   RAPD   van Belkum   [33]   6   primer   MRSA
      MRSA 2   3   ,   48   23   , Fang   [34]   3   primer
                                                    .   MRSA 45   5   .
                      5   [35]   4   primer   *S.*
                                      aureus 83   10   ,   MRSA
  ,   RAPD
                    .   .   3   primer   ,

Table 4. Distribution of MRSA by antibiogram, HVR genotypes and RAPD patterns

Ward/Department		Antibiogram or genotype*				No. isolates
Strains isolated from inpatients						
7W	NS	- -2-a-B	- -1-c-B	- -1-e-A	- -1-e-B	5
		- -4-e-A				
	UR	- -1-c-D	- -3-e-A	- -1-c-B		3
	DT	- -3-e-A				1
8W	CS	- -1-e-B	- -1-c-A	- -1-d-B	- -1-e-A	5
		- -4-e-C				
	GS	- -2-a-C	- -4-e-D	- -1-c-D	- -1-e-B	4
	IM	- -3-e-A				1
9W	OS	- -2-c-C	- -2-d-A	- -4-e-D	- -1-c-B	13
		- -1-c-D	- -1-d-C	- -1-e-A	- -4-e-C	
		- -1-d-B	- -1-e-A	- -1-e-B	- -1-e-C	
		- -3-e-C				
	PS	- -1-d-D	- -1-b-A	- -3-e-A		3
10W	NU	- -1-e-B	- -1-c-A			2
	OL	- -4-e-A				1
11W	IM	- -1-d-B	- -1-e-A			2
12W	NS	- -1-e-A				1
CCU		- -1-c-A	- -1-d-B	- -1-e-A	- -3-e-A	8
		- -1-e-A	- -3-e-A	- -1-e-A	- -2-c-D	
ICU	IM	- -1-c-B				1
	NS	- -1-c-D	- -5-e-B	- -1-c-B	- -1-c-D	10
		- -1-d-B	- -1-e-A	- -1-d-A	- -1-d-B	
		- -1-e-A	- -1-e-B			
NICU		- -2-c-A	- -2-e-A			2
Strains isolated from outpatients						
OPD	OL	- -2-b-B	- -2-d-A	- -2-e-A	- -4-c-D	6
		- -4-e-C	- -2-d-B			
	OS	- -2-c-A				1
	DM	- -4-d-B				1
ER		- -1-e-B	- -3-c-A			2

Abbreviations: NS, neurosurgery; UR, urology; DT, dentistry; CS, chest surgery; GS, general surgery; IM, internal medicine; OS, orthopedic surgery; PS, plastic surgery; NU, neurology; OL, otolaryngology; NS, neurosurgery; CCU, cardiac care unit; ICU, intensive care unit; NICU, neonatal intensive care unit; OPD, outpatient department; ER, emergency room.

*antibiogram I~IV, HVR Genotype ~ , RAPD Pattern 1~5, a~e, A~D

primer *mec*A MRSA 72 5, 5
4 . .
100 MRSA antibiogram 4 ,
29 . HVR 6 , RAPD
primer 4-5 .
MRSA MRSA
. ,
MRSA , ,

MRSA

antibiogram

HVR , RAPD 1-e-A

B . HVR

RAPD

. HVR RAPD

. HVR OPA-3

2 , OPA-3 1 , OPA-13 e

OPA-14 A .

MRSA

PFGE gold standard

, , ,

. HVR-PCR RAPD

,

primer

, MRSA

.

Methicillin-resistant *S. aureus* (MRSA)

.

.

*mec*A MRSA

mec RAPD .

1997 12 1998 5

S. aureus methicillin

*mec*A . *mec*A MRSA

, *mec*-associated HVR-PCR

RAPD .

MRSA .

S. aureus 120 78 MRSA

72 *mec*A . *mec*A MRSA 72

antibiogram 4 , HVR-PCR

6 3 primer

RAPD 29 .

HVR RAPD 43

. 5

MRSA HVR RAPD

.

Antibiogram, HVR RAPD

*mec*A MRSA 4-6

,

,

RAPD primer

.

, PFGE

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    .
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15.   ,   ,   .   coagulase
                                    .
        1996;16:928-34.

16.   ,   ,   ,   ,   ,   .
Methicillin
                        mec   coagulase
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