

## Typing and Antimicrobial Susceptibilities of Methicillin Resistant *Staphylococcus aureus* (MRSA) Strains Isolated in a Hospital in Korea

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains may cause serious nosocomial infections, including pneumonia and septicemia. The rate of methicillin-resistance among *S. aureus* isolates in Korea is over 50%. In this study, 90 MRSA isolates from Kyung Hee University Hospital were characterized employing bacteriophage typing, pulsed-field gel electrophoresis (PFGE), and antimicrobial susceptibility testing. Eighty percent of the strains could be phage-typed. The largest group or 40% of the strains belonged to lyso group III, followed by 32% of the isolates which produced a reaction with regional additional phages. Phage type 83A was most frequently encountered, followed by phage type D11. PFGE patterns confirmed the presence of two major clusters, which comprise the isolates belonging to lyso group III and the strains that were typable with regional additional phages. The latter group also contained a number of strains that were nontypable with bacteriophages. The resistance rates to ciprofloxacin, erythromycin, tetracycline, gentamicin and clindamycin were over 94%. Strains with intermediate resistance to vancomycin strains or resistance to mupirocin were not found. In conclusion, this study demonstrates that the results of phage typing are confirmed and supplemented by PFGE data.

**Key Words :** *Staphylococcus Aureus*; *Methicillin Resistance*; *Bacteriophage Typing*; *Susceptibility Testing*; *Electrophoresis, Gel, Pulsed-field*

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## INTRODUCTION

Since its first identification, methicillin-resistant *Staphylococcus aureus* (MRSA) has become one of the most challenging nosocomial pathogens. In Korea, MRSA constitutes over 50% of the staphylococcal isolates (1, 3), whereas in Germany, Austria and Switzerland, 12.9% to 15.2% of *S. aureus* isolates are methicillin-resistant (4). MRSA displays a resistance to a wide variety of antimicrobial agents including non- $\beta$ -lactam antimicrobials, which makes it difficult to treat of MRSA infections and leads to a high mortality rate in immunocompromised hosts. In a hospital environment, accurate strain typing is essential for the epidemiological monitoring of the MRSA isolates and for the control of the transmission, accordingly. Previously, the widely-used method for the typing of *S. aureus* was bacteriophage typing, employing the international phage typing set (IPS) and additional regional phages. However, bacteriophage typing fails in a high percentage of MRSA isolates (15-20%). To overcome this, for example, the Center for Disease Control and Prevention has replaced phage typing by pulsed field-gel electrophoresis (PFGE) (5). Other typing

methods for MRSA include biotyping, ribotyping, capsular polysaccharide serotyping, restriction analysis of plasmid DNA, the determination of antibiotic resistance patterns, polymerase chain reaction-mediated genomic fingerprinting, and most recently, sequence analysis of the polymorphic repeat X region of the *protein A* gene (6). However, PFGE is at present the generally recommended method (7). The goal of this study was to investigate and compare the phage types, PFGE patterns, and antimicrobial susceptibility of MRSA from clinical isolates from Kyung Hee University Hospital in Seoul, Korea.

## MATERIALS AND METHODS

### Bacterial isolates

A total of 90 MRSA isolates from patients admitted to Kyung Hee University Hospital during September and October 1996 were used in this study. The specimens were originated from pus, sputum, urine, blood, and tracheal secretions (Table 1, 2). Identification of *S. aureus* was carried

out by testing production of free coagulase, DNase, and growth on mannitol salt agar: all isolates were coagulase positive and DNase positive.

#### Bacteriophage typing

Phage typing was performed with the international phage typing set (IPS) for *S. aureus* at  $\times 100$  routine test dilution (RTD). If the phages of the IPS (group I: 29, 52, 52A, 79 and 80; group II: 3A, 3C, 55, and 71; group III: 6, 42E, 47, 53, 54, 75, 77, 83A, 84, and 85; group V: 94 and 96; group M: 81 and 95) did not provide a lysotype, regional additional phages (D11, 16, 92, 187, and 192) were employed. Phages were typed by demonstrating a strong reaction according to the international rules. Isolates were considered as different phage types if they differed in sensitivity to two or more phages.

#### Pulsed-field gel electrophoresis

Chromosomal DNA for the *Sma*I restriction digest was purified from 31 strains as described previously (8). PFGE was performed with a Bio-Rad CHEF DR III system (Bio-Rad, Munich, Germany) employing Pulsed Field Certified Agarose (1%) (Bio-Rad). Run conditions were 6 V/cm, a field angle of 120°, and with switching times of 5-15 sec for 7 hr to 15-60 sec for further 19 hr. A chromosomal DNA digest of *S. aureus* strain NCTC 8325 served as a mass standard.

#### Antimicrobial susceptibility tests

Antimicrobial susceptibility tests were performed according to the protocols recommended by the National Committee for Clinical Laboratory Standards employing the disk diffusion method. Screening for occurrence of heterogeneous intermediate resistance to vancomycin was performed as recommended (9). Vancomycin minimal inhibitory concentrations were determined by the microbroth dilution method in brain heart infusion broth (9) and read after 24 hr of incubation at 37°C.

## RESULTS

#### Phage typing

The distribution of clinical samples from which the isolates were derived is shown in Table 1 and 2. Seventy-two (80%) of 90 MRSA isolates were identified using bacteriophages of the IPS or regional additional phages, with 40% of the strains belonging to lyso group III. Eighteen isolates (20%) were nontypable and 32% reacted only with regional additional phages (Table 3). None of the strains reacted with phages of group II.

#### Pulsed-field gel electrophoresis

PFGE patterns of 11 MRSA strains representing the major types encountered are shown in Fig. 1. Lanes 2 to 7 show examples of strains that were typable only with regional additional phages or nontypable. Lanes 10 to 12 display the PFGE patterns of strains that belong to lyso group III. The strain in lane 13 was the single isolate that was found to belong to group I. The isolate in lane 14 was nontypable and showed an unrelated PFGE pattern. Corresponding to the results of the phage typing, the isolates in lanes 2-7 and lanes 10-13 formed groups of related strains.

Table 1. MRSA strains isolated by specimens

Type of specimen	No. of strains	(%)
Pus	41	45.6
Sputum	20	22.2
Urine	12	13.3
Blood	4	4.4
Tracheal secretion	2	2.2
Other	8	8.9
Not known	3	3.3
Total	90	100

Table 2. MRSA strains isolated by wards

Ward	No. of specimen	(%)
Internal medicine	14	15.6
MICU	8	8.9
SICU	7	7.8
NICU	7	7.8
3ICU	3	3.3
NS	13	14.4
OS	11	12.2
GS	9	10.0
TS	6	6.7
ENT	7	7.8
Urology	1	1.1
Outpatient	4	4.4
Total	90	100

Abbreviations: MICU, medical intensive care unit; SICU, surgical intensive care unit; NICU, neurosurgical intensive care unit; 3ICU, oriental medicine intensive care unit; OS, orthopedic surgery; GS, general surgery; TS, thoracic surgery; ENT, eye nose throat

Table 3. Major lytic group of MRSA isolates

Group	No. of strains	(%)
I	1	1.1
II	36	40.0
M	2	2.2
I/III	1	1.1
I/M	2	2.2
III/M	1	1.1
Additional phages	29	32.2

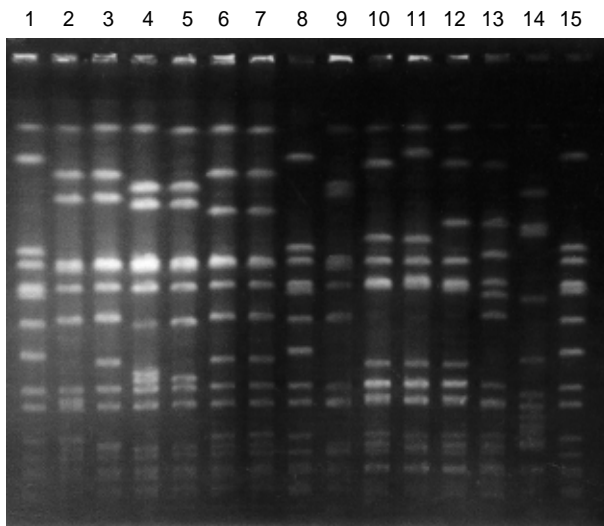


Fig. 1. PFGE pattern of MRSA isolates. The various PFGE patterns on the gel are as follows: lane 1, 8 and 15, *Staphylococcus aureus* NCTC 8325; lane 2, 1304 (D11); lane 3, 1399 (D11); lane 4, 1305 (nontypable); lane 5, 1319 (92); lane 6, 1342 (16,192); lane 7, 1364 (nontypable); lane 8, *Staphylococcus aureus* NCTC 8325; lane 9, *Staphylococcus aureus* Mu 50; lane 10, 1324 (Group III, 42E/54/75/77/83A); lane 11, 1376 (group III, 42E/54/75/77/85); lane 12, 1347 (group III, 47/54/75/ 77/85); lane 13, 1395 (group I, 29); lane 14, 1309 (nontypable).

The first group displayed a similarity to the banding pattern of the Japanese vancomycin intermediately resistant isolate Mu50 (lane 9) which is also nontypable by phages of the IPS. The second group is distinguishable from the first group by its PFGE pattern, but some similarities remain, indicating that the two groups may be related.

#### Antimicrobial susceptibility

The resistance rates to ciprofloxacin, erythromycin, tetracycline, and gentamicin were higher than 94% (Table 4). All strains were screened for the occurrence of subpopulations with intermediate resistance to vancomycin, that is, with an minimal inhibitory concentration (MIC) greater than or equal to 8 mg/L vancomycin on agar plates containing 4 µg/mL vancomycin. No vancomycin-intermediate *S. aureus* (VISA) strains were detected. Two isolates which were able to form colonies on the selection agar harbored less sensitive subpopulations with an MIC of only 4 µg/mL vancomycin and therefore were considered as sensitive. One of these strains (PFGE of in lane 12 in Fig. 1) belonged to the largest group of isolates giving a reaction with group III phages, and the other strain (lane 14 in Fig. 1) was nontypable and displayed an unrelated PFGE pattern. The antibiotic resistance phenotypes and phage types are given in Table 5, showing that the antibiograms of the strains are heterogeneous and vary within a single phage type.

Table 4. Antimicrobial resistance rate of MRSA isolates by disk diffusion method

Antimicrobials	No. of resistant strains	(%)
Methicillin	90	100.0
Imipenem	88	97.8
Ciprofloxacin	87	96.7
Erythromycin	87	96.7
Tetracycline	87	96.7
Gentamicin	85	94.4
Clindamycin	78	86.7
Fusidic acid	11	12.2
Netilmicin	10	11.1
Fosfomycin	8	8.9
Vancomycin	0	0
Mupirocin	0	0

## DISCUSSION

MRSA is one of the most important nosocomial pathogens, causing surgical wound infections, infections at indwelling catheters, abscesses related to injections, etc. The most serious infections include pneumonia and septicemia. The infections are usually acquired during the stay in hospital by direct or indirect contact or airborne transmission. In order to control the transmission of MRSA in a hospital environment, special precautions have to be taken. Typing of the isolates is necessary for epidemiologic monitoring and especially, identification of outbreak strains, which possess a high virulence and often seem to defy infection control measures that successfully inhibit transmission of ordinary *S. aureus* strains.

Phage typing, which has been the classical typing method of *S. aureus*, divided the isolates of this study into three groups, namely, those belonging to lyso group III, those typable with additional phages, and nontypable strains. The 19 isolates that reacted with phage 83A and 12 strains that were typable with phage D11 displayed identical resistance profiles, which indicates at least two clones in the hospital. The group III strains represented the largest proportion of the isolates, and their PFGE patterns also confirmed the close relatedness among the strains. Of notes, lysotype 83A that is rarely encountered in Germany predominated in this group.

Cho et al. (10) and Lee et al. (11) have previously reported on the isolation of lyso group II and lyso group III strains in Korea. Compared with these strains, which were isolated from the skin, the MRSA strains characterized in this paper formed a clearly distinct cluster. Fifty-two percent of the isolates were nontypable with the IPS, and by employing additional regional phages, this proportion could be reduced to 20%. The fact that a high percentage of MRSA strains are nontypable with the IPS is well known and has been reported previously (12). The additional phages that were

**Table 5.** The characteristics of the 90 MRSA strains analyzed

Lyso group	Phage type	Antimicrobial resistance	No. of isolates		
III	83A	GM,EM,CLM,TC,IMP,CTR	19		
		GM,EM,TC,IMP,CTR	5		
		GM,EM,CLM,FA,TC,IMP,CTR	2		
	47,54,75,77,85	GM,EM,CLM,TC,IMP,CTR	1		
		GM,NE,EM,CLM,TC,IMP,CTR	2		
		GM,EM,CLM,TC,IMP,CTR	1		
		53	GM	1	
		47,54,75,77	GM,EM,CLM,TC,IMP,FO,CTR	1	
			GM,NE,EM,CLM,TC,IMP,CTR	1	
		47,53,54	GM,EM,CLM,TC,IMP,CTR	1	
	I	42E,54,75,77,83A	GM,NE,EM,CLM,TC,IMP,CTR	1	
		42E,47,53,54,85	GM,EM,CLM,FA,TC,IMP,FO,CTR	1	
	I/M	29	GM,NE,EM,CLM,TC,IMP,CTR	1	
I/III	52,80,81,95	GM,EM,CLM,TC,IMP,CTR	2		
III/III	52,52A,77	GM,NE,EM,CLM,TC,IMP,CTR	1		
III/M	6,42E,47,54,75,83A/81	GM,NE,EM,CLM,TC,IMP,CTR	1		
M	81	GM,EM,CLM,TC,IMP,CTR	1		
		GM,NE,EM,CLM,TC,IMP,CTR	1		
Additional phage	D11	GM,EM,CLM,TC,IMP,CTR	12		
		GM,EM,CLM,FA,TC,IMP,CTR	4		
		EM,CLM,TC,IMP,CTR	1		
		GM,EM,TC,IMP,CTR	1		
		GM,EM,CLM,TC,IMP,CTR	1		
		D11,16,192	GM,EM,CLM,TC,IMP,CTR	2	
			GM,CLM,TC,IMP,CTR	1	
		D11,192	GM,EM,CLM,TC,IMP,CTR	1	
			GM,EM,CLM,TC,IMP,CTR	3	
		16,192	GM,EM,CLM,TC,IMP,CTR	2	
			GM,EM,CLM,TC,IMP,FO,CTR	2	
		Nontypable	92	GM,EM,CLM,TC,IMP,CTR	1
				GM,EM,CLM,TC,IMP,CTR	6
	GM,EM,TC,IMP,CTR		GM,EM,TC,IMP,CTR	2	
			EM,TC,CTR	2	
	EM,CLM,TC,IMP,CTR		1		
	GM,CLM,FA,TC,IMP,CTR		1		
	GM,EM,CLM,FA,CTR		1		
	GM,EM,CLM,FA,TC,IMP,FO,CTR		1		
	GM,EM,CLM,TC		1		
	GM,EM,CLM,TC,IMP,FO,CTR		1		
	GM,EM,IMP	1			
	GM,EM,LL,FA,TC,IMP,FO,CTR	1			
Total			90		

Abbreviations: GM, gentamicin; NE, Netilmicin; EM, erythromycin; CLM, clindamycin; FA, fusidic acid; TC, tetracycline; IMP, imipenem; FO, fosfomicin; CTR, ciprofloxacin

employed for typing have been selected because of their ability to lyse the local German epidemic strains, among which the Southern German epidemic strain gives a reaction with the regional additional phages D11, 16, and 192. However, the PFGE pattern of this strain did not show similarity to the Korean isolates (data not shown) and rather resembled the Japanese isolate Mu50. *S. aureus* Mu50 belongs to the clonotype IIa MRSA which are prevalent in Japan and U.S.A. (9).

PFGE revealed that many of the strains that were nontypable with the IPS and the strains that reacted with the regional additional phages are closely related to each other.

For example the isolates *S. aureus* 1342 (lane 6, weak reaction with regional additional phages 16 and 192) and *S. aureus* 1364 (lane 7, nontypable) showed identical band patterns and a two-band difference to *S. aureus* 1399 (strong reaction with the phage D11). *S. aureus* 1399 again showed a similarity to *S. aureus* 1304 (lane 2, strong reaction with phage D11 and a weak reaction with phage 192). The isolates *S. aureus* 1305 (lane 4 nontypable) and *S. aureus* 1319 (lane 5, phage 92) were also closely related. Similar variations in PFGE patterns and in phage types were also seen in the Southern German epidemic strains, where a variety of subtypes had been encountered during a long term study in

a university hospital in Germany (Lenz, W., personal communication) and the English E-MRSA 15 and 16 (Morrison, D. et al., Abstract at the 9th International Symposium on Staphylococci and Staphylococcal Infections, Kolding, Denmark, 2000). Therefore, it is possible that a substantial part of the nosocomial infections in Kyung Hee University Hospital were caused by epidemic MRSA strain. The resistance rate to ciprofloxacin, erythromycin, tetracycline, gentamicin and clindamycin were very high. Strains with intermediate resistance to vancomycin or resistance to mupirocin were not discovered.

In conclusion, this study demonstrates that the results of the phage typing are confirmed and supplemented by PFGE data. Phage typing is still be used as an inexpensive screening method in an epidemiological context especially for mass typing. In the meantime, the number of strains nontypable with IPS has been reduced by the introduction of new MRSA phages. PFGE is essential for the examination of nontypable strains, identification and characterization of epidemic strains or confirmation of ambiguous results, although it is more time-consuming, cumbersome and requires expensive equipment.

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