

## Epidemiological Typing of Methicillin-Resistant *Staphylococcus aureus* Outbreak Isolates by Pulsed-Field Gel Electrophoresis and Antibigram

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most common nosocomial pathogens. In April 1997, there were five MRSA-infected patients among 16 patients in the Neonatal Intensive Care Unit (NICU), Seoul National University Hospital, which is a tertiary-care hospital with 1,500 beds. The infections had spread from twin patients with MRSA who had transferred from Hospital C. MRSA was isolated from the axilla of 15 (94%) of the 16 patients, including the two patients with obvious infections. Three (19%) of 16 doctors and nine (30%) of 30 nurses had MRSA colonization of the anterior nares. Six different PFGE patterns (A through F) were identified in the 53 isolates of MRSA tested. Twelve of 13 isolates from infected sites of five patients showed pattern F. Three MRSA strains obtained from hospital C showed closely or possibly related pattern F. MRSA of type F was isolated from three of 16 patients' axilla, and one of 3 doctors' and three of 30 nurses' nasal swabs. The antibiogram code for 12 of 13 MRSA isolates from five infected patients was 66754. PFGE patterns of these isolates were either F, F1, F2 or Fa. Only one of three strains isolated from clinical specimens of patients in Hospital C showed the antibiogram code 66754, although they were all PFGE types F1 and Fa. In conclusion, the presumptive sources of the outbreak of MRSA infection in NICU were the twin patients transferred from hospital C. Antibiogram correlated reasonably well to the PFGE type. An effective notification system is needed when a MRSA-infected patient is transferred to another hospital to control the spread of the infection.

**Key Words:** Methicillin-resistant *Staphylococcus aureus*, pulsed-field gel electrophoresis, antibiogram

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most common causative agents of nosocomial infections. The proportion of MRSA varies greatly from country to country. Over 70% of clinical isolates of *S. aureus* are resistant to methicillin in large hospitals in Korea (Kim *et al.* 1997),

indicating the endemic nature of the infection. Nosocomial outbreaks of MRSA infection have also been reported from several hospitals in Korea (Kim *et al.* 1996; Kim *et al.* 1998). As MRSA is often multiresistant and the infection is difficult to treat, prevention of the infections is very important.

Indications for the eradication of MRSA are elimination of an outbreak in a health care setting and prevention of recurrent infections in an individual (Mulligan *et al.* 1993). In settings where MRSA is endemic, elimination of carriage has not been found to be cost-effective and is therefore considered to be not indicated by these authors.

In an outbreak situation, the first goal is to identify all carriers, including patients and health

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care workers (Kluytmans *et al.* 1997). Then, elimination of carriage can be achieved in all identified carriers. Other interventions such as extensive infection control measures are considered to play a significant role in controlling these outbreaks. However, the effect of elimination strategies in settings where MRSA is endemic has not been studied extensively.

When MRSAs are detected from patients and carriers, it is necessary to determine their relation. Various typing methods are used with varying degrees of discrimination to distinguish strains carried by hospital staff and patients (Tenover *et al.* 1994). Pulsed-field gel electrophoresis of restriction endonuclease-digested genomic DNA is known to be very useful for typing, however it requires expensive instruments and expert technique. Of the two traditional typing techniques, biotyping and antibiogram determination, the latter is the most accessible to clinical laboratories and works reasonably well, but only when zone diameters are used as strain markers. The discriminating value may also differ depending on the kind of antimicrobial agents used.

In this study the MRSA strains isolated from outbreak cases and carriers were subjected to PFGE and antibiogram typing to determine the sources of infection and to evaluate the usefulness of antibiogram typing in the differentiation of MRSA strains.

## MATERIALS AND METHODS

### MRSA-infected patients and carriers

In April 1997, five of 16 patients in the Neonatal Intensive Care Unit (NICU), Seoul National University Hospital (SNUH) were found to be infected with MRSA. The types of infections were: one patient with bacteremia, three with wound infections, and one with eye infection. Among the patients, twin patients had been transferred from Hospital C. The isolate from one patient was not available at the time of this study. Another isolate taken from the blood of a patient in May 1997 was added for the molecular analysis of strain relatedness. Doctors and nurses assigned during the outbreak were subjected to the carrier study.

### Bacterial culture

Specimens of blood and discharges from wounds and eyes were taken from the 6 NICU patients and processed according to the routine procedure for the isolation and identification of MRSA. Three MRSA strains isolated from NICU patients in Hospital C were obtained for comparison.

MRSA carriage surveillance was performed by a procedure modified from that of Cookson *et al.* (1989). Swab specimens were collected from the anterior nares of 16 doctors and 30 nurses and from the axilla of 16 patients of NICU. The specimens were inoculated into a staphylococcal broth which contained 7.5% NaCl, and incubated at 35°C overnight. The broth cultures were subcultured using mannitol-salt agar with 6 µg/mL of oxacillin and incubated at 35°C overnight. The colonies were selected and used for species identification with the Vitek Gram Positive Identification card system (bioMerieux, Marcy l'Etoile, France). Methicillin-resistance was confirmed by the method of the National Committee for Clinical Laboratory Standards (NCCLS, 1997).

### PFGE

PFGE was carried out by a procedure modified from that of Back *et al.* (1993) and Udo and Grubb (1993). Briefly, 0.35 mL of overnight brain heart infusion broth culture of MRSA isolates were centrifuged at 7,000 rpm for 2 min. Pelleted cells were washed in 1 mL of TEN buffer (0.1 M Tris, 0.15 M NaCl, 0.1 M EDTA) and centrifuged again. The washed cells were resuspended in 0.2 mL of EC buffer (6 mM Tris, 1 M NaCl, 0.1 M EDTA, 0.5% Brij 58, 0.2% deoxycholate, 0.5% sarkosyl) and 10 U/mL of lysostaphin. Two hundred microliters of molten 1.3% Incert agarose (FMC BioProducts, Rockland, Maine, U.S.A.) in EC buffer were added to the cell suspension, briefly vortexed and quickly pipetted into a plug mold. After solidification, the plug was placed in a test tube containing 1 mL of EC buffer with 5 U/mL of lysostaphin and 20 µg/mL of RNase. The cells in the plug were lysed for 6 hours at 37°C and the plugs were then incubated for 3 hours in 1 mL of ESP buffer (0.25 M disodium

EDTA, 1% N-lauroyl sarcosine, and 100  $\mu$ g of proteinase K per mL) at 50°C. The ESP buffer was replaced with 1 mL of new ESP buffer and incubated at 50°C overnight. After proteinase K treatment, the ESP buffer was removed and replaced with 5 mL of TE buffer (10 mM Tris, 5 mM EDTA). The plug was incubated twice for 2 hours at 50°C.

The plug was cut into small slices (2  $\times$  5 mm) and placed in 125  $\mu$ L of restriction buffer containing 20 U of *Sma* I. After a 6-hour incubation at 25°C with shaking at 140 rpm, the plug was loaded into the well of a 1% SeaKem agarose gel. Electrophoresis was performed with CHEF Mapper Electrophoresis System (Bio-Rad Laboratories, Hercules, California, U.S.A.). Concatemers of bacteriophage lambda DNA were used as a molecular weight standard. The running parameters were as follows: initial pulse, 5 sec; final pulse, 40 sec; 200 volts; time, 20 h; temperature, 14°C. After electrophoresis, the gels were stained with ethidium bromide and photographed. The PFGE patterns were interpreted with the criteria suggested by Tenover *et al.* (1995). Different types were expressed with upper case Roman letters, while closely related and possibly related types were expressed with arabic numerals and lower case Roman letters, respectively.

### Antimicrobial susceptibility pattern

Antimicrobial susceptibility was tested by the disk diffusion method (NCCLS, 1997). Antibiotic disks used were: rifampin (5  $\mu$ g), clindamycin (2  $\mu$ g), erythromycin (15  $\mu$ g), trimethoprim/sulfamethoxazole (1.25/23.75  $\mu$ g), ciprofloxacin (5  $\mu$ g), tetracycline (30  $\mu$ g) ampicillin/sulbactam (10/10  $\mu$ g), cephalothin (30  $\mu$ g), cefotaxime (30  $\mu$ g), cefoperazone/sulbactam (75/30  $\mu$ g), amikacin (30  $\mu$ g), gentamicin (10  $\mu$ g and 120  $\mu$ g), streptomycin (300  $\mu$ g), and tobramycin (10  $\mu$ g). The antibiogram of 5 digits, each consisting of results of 3 antimicrobial resistance was constructed. Values of 1, 2 and 4 were assigned when resistant to the 1st, 2nd and 3rd antibiotic, respectively.

## RESULTS

### MRSA isolated from clinical samples

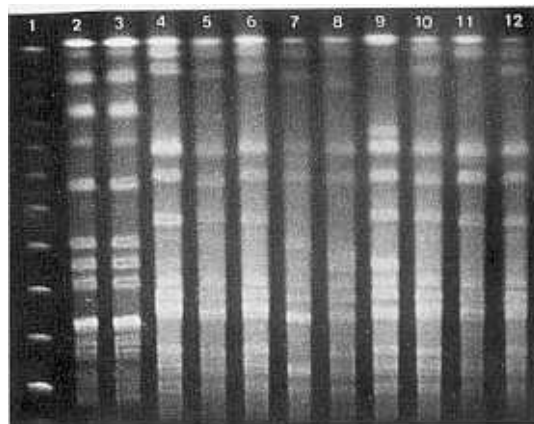
Eye discharges and blood specimens which were

collected on April 7th and 14th from the transferred patients Nos. 4 and 5, respectively, yielded MRSA. In the same period, MRSA was isolated from blood and various sites taken from two other patients (No. 23, 24). On May 12th, MRSA was isolated from the blood of another patient (No. 16).

MRSA strains obtained from hospital C included those isolated from urine specimens of two patients and one from the intravenous catheter tip of another patient.

### MRSA carriage

Fifteen (94%) of 16 NICU patients, including 2 infected ones, yielded MRSA from the specimens of axilla. Three (19%) of 16 doctors and 9 (30%) of 30 nurses were found to have MRSA colonization on the mucous membrane of the anterior nares. MRSA was isolated from all of the three consecutive weekly samples from one of three doctors (Doctor No. 3) and two of nine nurses (Nurse No. 4 and No. 6) (Table 1), who could be defined as the persistent carriers. The doctor had MRSA in his anterior nares



**Fig. 1.** Pulsed-field gel electrophoresis (PFGE) patterns of genomic DNA of methicillin-resistant *Staphylococcus aureus* (MRSA) after *Sma* I restriction. Lane 1 is molecular weight marker. Lanes 2 and 3 are isolates from nasal swab of Nurse No. 4 and No. 9, showing PFGE pattern A. Lanes 4, 5, and 10 are MRSA of two persistent carriage medical personnel (Nurse No. 6 and Doctor No. 3), showing PFGE pattern F. Lanes 6, 11, and 12 are isolates from infected sites of Patient No. 16 and No. 23, showing PFGE pattern F. Lanes 7, 8, and 9 are isolates obtained from Hospital C, showing PFGE pattern closely or possibly related with type F.

**Table 1. PFGE type of MRSAs isolated from patients and carriers**

Subjects	Date isolated	Specimen	Number of isolates according to PFGE type																	
			A	Aa	Ab	Ac	B	B1	C	Ca	D	E	E1	Ea	F	F1	F2	Fa	Fb	
Patient 4	Apr 4	Eye discharge															1			
	Apr 17	E tube												1						
Patient 5	Apr 14	Blood																1		
	Apr 16	Blood													1					
	Apr 18	Blood													1					
	Apr 19	IV catheter tip														1				
Patient 16	May 12	Blood												1						
Patient 23	Apr 9	Chest tube												1						
	Apr 11	Wound pus												1						
	Apr 14	Chest tube												1						
	Apr 21	IV catheter tip												1						
Patient 24	Apr 6	Eye discharge												1						
	Apr 12	Blood								1										
Hospital C-1	Apr 2	Urine														1				
Hospital C-2	Apr 3	Urine																1		
Hospital C-3	May 16	IV catheter tip															1			
Patients 1-3	Apr 23	Axilla swab	3																	
Patient 4	Apr 23	Axilla swab	1																	
Patient 5	Apr 23	Axilla swab										1								
Patients 6-15	Apr 23	Axilla swab	5									1	1		2	1				
Nurses 1, 2	Apr 23	Nasal swab	1		1															
Nurse 3	Apr 23	Nasal swab				1														
	Apr 29	Nasal swab					1													
Nurse 4	Apr 23	Nasal swab						1												
	Apr 29	Nasal swab							1											
	May 7	Nasal swab	1																	
Nurse 5	Apr 23	Nasal swab													1					
	Apr 29	Nasal swab													1					
Nurse 6	Apr 23	Nasal swab													1					
	Apr 29	Nasal swab													1					
	May 7	Nasal swab													1					
Nurse 7	Apr 23	Nasal swab													1					
	May 7	Nasal swab													1					
Nurses 8, 9	Apr 23	Nasal swab	1												1					
Doctors 1, 2	Apr 23	Nasal swab		1		1														
Doctor 3	Apr 23	Nasal swab													1					
	Apr 29	Nasal swab													1					
	May 7	Nasal swab													1					
	Jun 30	Nasal swab													1					
Total			12	1	1	1	1	1	1	1	1	1	2	1	1	20	4	2	2	1

E tube: endotracheal tube aspirate.

for more than two months. Their carriage was subsequently cleared with topical application of mupirocin.

#### PFGE type

Six different PFGE patterns (A through F) of *Sma*

I-digested genomic DNA were identified with 53 isolates of MRSA tested (Table 1).

Fig. 1 shows representative PFGE patterns of A and F. Twelve of 13 isolates from infected sites of 5 patients showed pattern F, while one isolate showed pattern D. Three MRSA strains obtained from hospital C showed PFGE patterns F1, Fa, and Fb,

which were closely or possibly related to pattern F.

At the first time of surveillance, MRSA of type F was isolated from three of 16 patients' axilla, and one of 3 doctors' and three of 30 nurses' nasal swabs. Doctor No. 3 and Nurse No. 6 showed persistent carriage of the type F MRSA. One MRSA isolate obtained from the anterior nares of Doctor No. 3 after 2 months showed the same PFGE type F. The majority of isolates from axilla and some from nasal swab were type A, which was not a type detected in isolates from infected sites. Isolates from the anterior nares of Nurse No. 4, who was a persistent carrier, showed various patterns: C, Ca, and A.

### Antibiogram

Preliminary tests showed that all of the MRSA isolates were resistant to penicillin and oxacillin, and

susceptible to vancomycin, teicoplanin and chloramphenicol. Therefore, these antibiotics were not included for the determination of an antibiogram.

The antibiotic susceptibility phenotype code for 12 of 13 MRSA isolates from 5 infected patients was 66754 (Table 2). All of these isolates were either PFGE type F, F1, F2 or Fa. The remaining one isolate showed antibiogram 60444 and PFGE type D. Only one of three strains isolated from clinical specimens of patients in Hospital C showed antibiogram 66754 and the remaining two were more susceptible types, although they were PFGE types F1 and Fa.

The antibiogram for the strains from surveillance culture of axilla of the NICU patients were quite heterogeneous and among the 15 strains, only 2 which were isolated from the infected patients showed the pattern 66754, PFGE type F. Seven of 16 isolates from nasal swabs of the NICU nurses had

Table 2. Correlation of PFGE type and antibiogram

Number of isolates according to source and PFGE type																									
Antibio-gram	Patient (5) Clinical (13)					Patient (15) Axilla (15)					Hospital C (3) Clinical (3)				Nurse (9) Nasal (16)						Doctor (3) Nasal (6)				Total
	D	F	F1	F2	Fa	A	E	E1	F	F1	F1	Fa	Fb	A	Ab	B	B1	C	Ca	Ea	F	Aa	Ac	F	
	(1)	(7)	(2)	(2)	(1)	(9)	(2)	(1)	(2)	(1)	(1)	(1)	(1)	(3)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(7)	(1)	(1)	
00440																	1						1		2
04044											1														1
04754												1													1
20440																1									1
46754																								4	4
55544																						1			1
60404															1										1
60444	1														1										2
61444						1														1					2
62544											1														1
64004						3																			3
64144						1																			1
64404						2									1										3
65400						1																			1
65444						1																			1
66444															1										1
66754	7	2	2	1					2				1								7				22
72544								1																	1
72575							1																		1
73544							1																		1
73776																				1					1
74444																			1						1

the antibiogram 66754 and were PFGE type F. None of the 6 isolates from the three NICU doctors showed this antibiogram, but 4 of the isolates were PFGE type F.

## DISCUSSION

MRSA is a typical nosocomial pathogen. The majority of MRSA infections are nosocomial and less than 5% of all *S. aureus* isolates from the community were methicillin-resistant in Korea (Kim, 1998). A methicillin resistance rate of over 70% of clinical isolates of *S. aureus* indicates that nosocomial infection is very prevalent in tertiary-care hospitals in Korea (Kim *et al.* 1997).

In surgery, cross infection was the primary focus of attention, and much was learned about the relative importance of patients, health care workers, and the environment in the transmission of *S. aureus*. In the presence of increased infection rates, either epidemic or endemic, typing of the isolates cultured from patients is needed. If typing shows there is no relatedness among the strains, an investigation is recommended to detect the presence of breaks in hygiene (Kluytmans *et al.* 1997).

Depending on the situation, relative prevalences of cross infection and endogenous infection vary (Kluytmans *et al.* 1997). It was considered that the proportion of endogenous *S. aureus* infection as opposed to cross infection may even be higher today than formerly because the risk of cross infection in the setting of a modern hospital may be lower than that in previous investigation.

A recent review (Kluytmans *et al.* 1997) showed that the nasal carriage rate of *S. aureus* is very high for both the general population and patients: 19% to 55% for general population, 17% to 56% for health care workers, and 14% to 53% for hospitalized patients. The rate was reported as high as 42% to 100% for patients with *S. aureus* skin infection. Among the three patterns of carriage, acquisition and transmission of antibiotic-resistant *S. aureus* in the hospital mainly concern intermittent carriers and persistent carriers treated with antibiotics.

Carriage of *S. aureus* has been identified as a risk factor for the development of infection (Kluytmans

*et al.* 1997). *S. aureus* infection can be either endogenous or exogenous. MRSA strains have often been recovered from the hands of medical personnel during outbreaks indicating medical personnel can be the source or transmitter of MRSA (Boyce *et al.* 1993; Byun *et al.* 1997). Therefore, it is important to determine the source of MRSA to control the spread of infection.

The nose is the main site of *S. aureus* colonization from where the strain spreads to the skin, subsequently causing infection when the patient has an impaired skin site (Kluytmans *et al.* 1997). Therefore, skin carriage of *S. aureus* in patients who are nasal carriers could be an explanation of endogenous infection.

It was reported that the carriage rate of *S. aureus* on nares was much higher than that on axilla, 43% vs 4% (Kloos and Schleifer, 1975). In this study, we used swab specimens of axilla of NICU patients, although anterior nasal swab is the most sensitive method (Cookson *et al.* 1989; Sautter and Wells, 1990), because hospital personnel often touch the axilla of neonates. Studies that use a very sensitive culture technique will identify more carriers who are not at increased risk. As to the culture method, we used Cooksons enrichment method as it is the most sensitive method for the epidemiologic surveillance of MRSA carriage.

PFGE is a useful typing method for various bacterial species, although it requires technical skill and a long processing time, as well as requiring expensive instrument (Tenover, 1994). In this study, PFGE was used to determine epidemiological relatedness of the MRSA strains isolated from NICU patients and medical personnel in the service and as a standard to evaluate the usefulness of antibiogram for strain typing.

The PFGE type of the MRSA isolates from various infected sites of 5 patients was only F, except one isolate which was D. The strains isolated from the blood and eye discharge of the twin patients (Patient No. 4 and No. 5) who had been transferred from hospital C showed type F. Also the strains isolated from patients in NICU of hospital C showed a pattern closely or possibly related to pattern F, suggesting a probable related clone of the outbreak (Table 1). Three patients (Patient No. 12, No. 13, and No. 14) were colonized by MRSA of

PFGE type F, but were not infected. Original source of the type F strain may have been the twin patients transferred from hospital C, but health care personnel may have directly or indirectly transferred the strain to other patients.

During the NICU outbreak, MRSA was isolated from 15 axilla specimens of 16 patients. MRSA isolates from axilla of nine (60%) of 15 patients and some of the strains from health care personnel showed PFGE type A, while none of the isolates from infected sites were type A, indicating that the clone had been the one colonized in hospital personnel for sometime.

Biotyping and antimicrobial susceptibility pattern are two traditional techniques readily available to clinical laboratories (Tenover *et al.* 1994). Biotyping was reported to not correlate well with epidemiologic data of *S. aureus*, although it worked well with coagulase-negative staphylococci. For many epidemiologic studies, antibiogram has a relatively limited utility because antibiotic resistance is under extraordinary selective pressure in hospitals. There are multiple genetic mechanisms by which a given strain can become abruptly resistant to a particular antibiotic-spontaneous mutation to quinolones, acquisition of plasmids or transposons. A single plasmid or compound transposon can carry multiple resistant determinants. On the other hand in the absence of specific pressure, such elements may be lost (Mickelsen *et al.* 1985). Therefore, different strains may show similar resistance patterns and, conversely, the susceptibility patterns of sequential clinical isolates representing the same strain may differ for one or more antibiotics (Tenover *et al.* 1994).

However, antibiogram typing is the least expensive typing method and could be considered, especially in small laboratories, as an initial screening to determine strain relatedness. It was reported that antibiogram typing worked reasonably well only when zone diameters were used as strain markers (Tenover *et al.* 1994). They reported that 11 antimicrobial agents including amoxicillin/clavulanic acid, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, minocycline, tetracycline, oxacillin, penicillin, and rifampin were helpful in discriminating among strains. In our study we excluded penicillin and oxacillin since the strains we typed were MRSA, as well as chloramphenicol since all of the

strains were susceptible. We did not compare the inhibition zone diameter, instead we tried to determine the effect of using a high-level gentamicin disk as it was commercially available.

Change in the disk diffusion zone size for a single antimicrobial agent was not considered significant. Therefore, changes in the zone sizes around disks for two or more antimicrobial agents must be observed before two isolates can be considered to be different (Tenover *et al.* 1994). In this study most of PFGE type F strains showed the same antibiogram, 66754. The problem with the MRSA antibiogram is that many strains are multiresistant and therefore an unrelated strain could show the same pattern. In this study it has been shown that some agents in the  $\beta$ -lactams and aminoglycosides can be helpful: i.e., ampicillin/sulbactam, cephalothin, cefotaxime, ceftoperazone/sulbactam, gentamicin and streptomycin. Some of the strains resistant to low-level gentamicin were susceptible to high-level gentamicin. Testing against different concentrations of antimicrobial agents may be analogous to using a zone diameter reading rather than NCCLS category determination in the antibiogram typing.

It is well recognized that the control of MRSA infection is not a simple task. It was concluded by Kluytmans *et al.* that the ability to control staphylococcal infections in the future will depend on many factors, such as the development of effective therapeutic agents, optimization of infection control measures, and use of new medical devices with a low risk of infection (Kluytmans *et al.* 1997).

In conclusion, as the antibiogram correlates reasonably well to the PFGE type of *Sma* I-digested genomic DNA of MRSA isolates, the test may be useful for clinical laboratories to determine the presumptive source of MRSA epidemic infection. However, further study is required to determine the kind of antimicrobial agents and the disk content which can give the best results. In this study the source of the outbreak of MRSA infection in NICU was considered to be the twin patients who were transferred from hospital C, from whom the strains rapidly spread to other patients and hospital personnel. An effective notification system is needed when a MRSA-infected patient is transferred to another hospital to control the spread of infection.

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