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Original Article

Molecular epidemiology of community-acquired methicillin-resistant *Staphylococcus aureus* bacteremia in a teaching hospital

Chih-Yu Chi[1], Mao-Wang Ho[1], Cheng-Mao Ho[1], Po-Chang Lin[1], Jen-Hsian Wang[1,2], Chang-Phone Fung[3]

[1]Section of Infectious Diseases, Department of Internal Medicine, China Medical University Hospital, Taichung; [2]Department of Internal Medicine, China Medical University, Taichung; and [3]Department of Internal Medicine, Faculty of Medicine, National Yang-Ming University, Taipei, Taiwan

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Background and Purpose: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a key nosocomial pathogen globally. Community-acquired MRSA (CA-MRSA) infections have become a growing problem in recent years. The purpose of this 4-year retrospective study was to analyze the molecular epidemiology and susceptibility pattern of isolates from adults (≥18 years of age) with CA-MRSA bacteremia in northern Taiwan.

Methods: Molecular epidemiology of CA-MRSA isolates was analyzed by pulsed-field gel electrophoresis. Antimicrobial susceptibility was tested by the disk diffusion method and the minimal inhibitory concentration was determined by Etest.

Results: Thirty eight patients with CA-MRSA bacteremia were enrolled. Thirty one CA-MRSA isolates were available for further molecular typing and susceptibility testing. A total of 13 distinct genotypes were identified and 48.4% (15/31) of the isolates were found to belong to genotype A. Genotype A CA-MRSA isolates were closely associated with the nosocomial strains. All CA-MRSA isolates were multidrug resistant (19.4% susceptible to clindamycin and 25.8% to trimethoprim-sulfamethoxazole) and consistent susceptibility was only observed to glycopeptides, rifampin, and linezolid.

Conclusions: This study demonstrated that although CA-MRSA genotypes were heterogeneous, the predominant genotype that was circulating in our community was genotype A. Also, the multidrug resistance of CA-MRSA might be connected to the spreading of nosocomial strains in the community.

Key words: Bacteremia; Electrophoresis, gel, pulsed-field; Infections, community-acquired; *Staphylococcus aureus*

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a key nosocomial pathogen universally. Community-acquired MRSA (CA-MRSA) infections, which has emerged as a growing problem in recent years, has become a worrying issue for health care systems in western countries [1-4]. MRSA was first documented in Taiwan in the early 1980s, about 10 years after the introduction of oxacillin for clinical use [5,6], and accounts for more than 60% of the *S. aureus* isolates [7] detected in most hospitals in Taiwan. A recent study in Taiwan estimated the incidence of MRSA among patients in an outpatient setting was to be 40% [8]. An earlier study by this group found that 33.7% of community-acquired *S. aureus* (CASA) bacteremia was due to MRSA [9].

Pulsed-field gel electrophoresis (PFGE) is a high reproducible and reliable typing method, and has been widely used in investigating the epidemiology of MRSA infections [10-16]. PFGE was used by Wang et al [7], who found that certain genotypes of MRSA were spreading among the hospitals in Taiwan. However, the dissemination of MRSA between hospitals and the community has not been well studied. We performed a

Corresponding author: Dr. Chang-Phone Fung, Section of Infectious Diseases, Department of Internal Medicine, China Medical University Hospital, No. 2, Yude Rd, North District, Taichung 404, Taiwan.
E-mail: cychyi_123@yahoo.com.tw

retrospective laboratory-based study to identify the MRSA isolates from blood and to evaluate the epidemiology of CA-MRSA bacteremia in northern Taiwan, over a 4-year period between 1999 and 2002. This study aimed to conduct a PFGE analysis of the diversity of strains found in nosocomial and community isolates, and to assess the minimal inhibitory concentration (MIC) of CA-MRSA isolates.

Methods

Case definition

All patients included in this study were adults (≥18 years of age) admitted with *S. aureus* bloodstream infections to the Taipei Veterans General Hospital between January 1, 1999 and December 31, 2002. Patients who had blood cultures performed within 48 h of admission; showed no histories of prior hospitalization in an acute-care setting, renal dialysis (hemodialysis or peritoneal dialysis), residence in a nursing home, or surgery in the year preceding MRSA isolation; and had no permanent indwelling catheter or percutaneous medical device (e.g., Foley catheter, tracheostomy) at the time of admission were considered to have CASA bacteremia. Patients were considered to have significant bacteremia if multiple sets of blood cultures were positive for *S. aureus*, or they had at least one positive set and showed clinical symptoms and signs of the infection. Duplicate isolates from the same patient were excluded and the first one was used for further antimicrobial susceptibility testing, MIC determination, and genomic DNA analysis.

Bacterial isolates

Identification of *S. aureus* was based on the colony morphology on trypticase soy agar supplemented with 5% sheep blood (BBL Microbiology Systems, Cockeysville, MD, USA), Gram stain, and a positive BactiStaph® (Remel Ltd, Lenexa, KS, USA) latex agglutination test. *S. aureus* isolates were screened for methicillin resistance by the disk diffusion method, using Mueller-Hinton agar (BBL Microbiology System), 1 μg oxacillin disk, and incubation for 24 h at 35°C.

Antimicrobial susceptibility test

The susceptibilities of isolates to other drugs were tested by the disk diffusion method in accordance with recent guidelines issued by the Clinical and Laboratory Standards Institute (CLSI; Wayne, PA, USA) M2-A9 [17]. The antibiotic disks (BBL Microbiology Systems) used for susceptibility testing included ampicillin (10 μg), cefazolin (30 μg), oxacillin (1 μg), chloramphenicol (30 μg), gentamicin (10 μg), clindamycin (2 μg), erythromycin (15 μg), trimethoprim-sulfamethoxazole (1.25 μg/23.75 μg), tetracycline (30 μg), vancomycin (30 μg), and teicoplanin (30 μg). MIC values were determined by Etest (AB Biodisk, Solna, Sweden) according to the recommendations proposed by the CLSI M100-S16 [18]. *S. aureus* American Type Culture Collection 29213 was used as a control strain.

PFGE

PFGE was performed using a procedure modified from a previous study [19]. The bacterial DNA was prepared and digested with the restriction enzyme *Sma*I (Takara Bio Inc., Otsu, Shiga, Japan). The PFGE banding patterns were interpreted according to previously described criteria [10,20]. Capital letters (types A, B, C, etc) were used to designate the different PFGE types, which were followed by arabic numerals (A1, A2, etc) to indicate subtypes. The saved tiff file of the photographed gel was further analyzed with Molecular Analyst 1.6 software (Bio-Rad Laboratories, Hercules, CA, USA). Percent similarities were identified on a dendrogram derived from the unweighted pair-group method with arithmetic mean (UPGMA) and based on Jaccard coefficients. Both band position tolerance and optimization were set at 1.0%. PFGE band similarity >80% [21] was used as the criterion for cluster formation.

Results

From January 1999 to December 2002, a total of 11,841 positive blood cultures were obtained in the clinical microbiology laboratory, 2081 (17.6%) of which were *S. aureus*. Excluding the duplicates, 128 of these isolates met the study criteria for CASA bacteremia, and 38 (29.7%) of these CASA isolates were methicillin-resistant strains (CA-MRSA). Thirty one (81.6%) of the CA-MRSA isolates were available for further PFGE typing and MIC determination. Among these 31 isolates, 13 distinct PFGE genotypes (types A to M) and 18 PFGE subtypes were identified (Table 1). One predominant PFGE genotype (designated type A) accounted for 15 (48.4%) of the 31 CA-MRSA isolates and 3 subtypes (genotypes A1 to A3) were characterized within this genotype. Both A1 and A2 genotypes contained 7 isolates (22.6%) each; genotype A3 included one isolate. The other two major DNA genotypes were

Table 1. Pulsed-field gel electrophoresis (PFGE) patterns and minimal inhibitory concentrations (MICs) of community-acquired methicillin-resistant *Staphylococcus aureus* isolates

Isolate number	PFGE type	MIC (μg/mL)									
		OX	CL	CM	EM	GM	RI	TS	TP	VA	LZ
74	A1	>256	6	>256	>256	>256	0.006	>32	2.00	2.0	1.00
73	A1	>256	6	>256	>256	>256	0.004	>32	1.50	1.5	1.00
72	A1	>256	4	>256	>256	>256	0.004	>32	1.00	2.0	0.50
70	A1	>256	6	>256	>256	>256	0.006	>32	1.50	1.5	0.75
64	A1	>256	6	>256	>256	>256	0.006	>32	1.50	1.5	0.75
52	A1	>256	6	>256	>256	>256	0.006	>32	1.50	2.0	1.00
49	A1	>256	6	>256	>256	>256	0.004	>32	2.00	2.0	0.50
75	A2	>256	4	>256	>256	>256	0.006	16	1.50	2.0	0.38
66	A2	>256	3	>256	>256	>256	0.006	>32	1.00	1.5	0.38
48	A2	>256	4	>256	>256	>256	0.004	>32	1.50	1.5	1.00
27	A2	>256	4	>256	>256	>256	0.004	>32	1.00	1.5	0.50
21	A2	>256	4	>256	>256	>256	0.006	>32	1.50	2.0	0.50
9	A2	>256	4	>256	>256	>256	0.004	>32	1.50	1.5	0.50
84	A2	>256	4	>256	>256	>256	0.004	>32	1.00	1.5	0.75
6	A3	>256	3	>256	>256	>256	0.006	>32	1.50	2.0	0.50
40	B1	12	>256	>256	>256	>256	0.006	0.064	0.75	1.5	0.75
4	B2	48	>256	>256	>256	>256	0.006	0.047	0.38	1.0	0.50
25	B3	48	128	>256	>256	>256	0.004	0.064	0.50	1.0	0.50
79	C1	>256	4	0.125	>256	>256	0.006	>32	1.50	2.0	1.00
76	C1	>256	6	0.190	>256	>256	0.006	>32	1.50	2.0	1.00
34	C2	>256	3	0.094	>256	>256	0.004	>32	1.50	1.5	0.75
60	D	96	>256	>256	>256	128	0.006	0.064	1.00	1.5	1.00
63	E	>256	3	0.125	>256	>256	0.750	6	2.00	1.5	0.75
61	F	12	4	1	>256	64	0.008	>32	3.00	1.5	0.75
58	G	6	4	>256	>256	0.75	0.006	0.047	0.75	1.5	0.38
44	H	>256	3	0.125	0.5	>256	0.004	>32	2.00	2.0	0.75
41	I	>256	3	0.125	>256	6	0.750	6	1.00	1.5	1.00
14	J	6	>256	>256	>256	0.5	0.006	0.047	0.50	1.0	0.50
7	K	>256	64	>256	>256	>256	0.004	>32	1.50	1.5	0.75
86	L	128	4	>256	>256	12	0.004	0.19	0.50	2.0	1.00
88	M	32	128	>256	>256	0.5	0.006	0.064	0.75	1.5	0.50

Abbreviations: OX = oxacillin; CL = chloramphenicol; CM = clindamycin; EM = erythromycin; GM = gentamicin; RI = rifampin; TS = trimethoprim-sulfamethoxazole; TP = teicoplanin; VA = vancomycin; LZ = linezolid

genotypes B (3 subtypes) and C (2 subtypes), which had 3 isolates each (9.7%). Ten more PFGE genotypes (genotypes D to M), each having one isolate, were also identified. Band patterns of the major PFGE genotypes and subtypes of CA-MRSA are illustrated in Fig. 1.

During the study period, 9 nosocomial MRSA strains (3 isolates in the first study year, and two isolates in each of the subsequent 3 years) were randomly selected for genomic DNA analysis. PFGE patterns of these nosocomial isolates are presented in Fig. 2. A total of 3 distinct DNA genotypes (genotypes N, O, and P) and 8 subtypes were identified after *Sma*I digestion. Genotype N accounted for 3 isolates, genotype O for 5, and genotype P for 1 isolate. The PFGE patterns of most nosocomial MRSA strains were closely related, with the exception of genotype P (isolate 9). Dendrogram analysis (Fig. 3) revealed 2 clusters (>80% similarity) among the nosocomial strains that were of the genotypes N (isolates 1 to 3) and O (isolates 4 to 8). Genotype A had 74% similarity with genotype N, but less similarity with genotype O. There was a 75% similarity between genotypes O and P. The similarity between genotypes A, N, O and P was greater than 60%. With the exception of genotype K (isolate 7 of community origin), which had 70% similarity with genotype A, PFGE patterns of other CA-MRSA isolates were markedly dissimilar from that of genotype A (Fig. 3).

The disk diffusion test revealed that all 31 CA-MRSA isolates were multidrug resistant (data not shown). The MICs of these isolates are shown in Table 1. All the tested

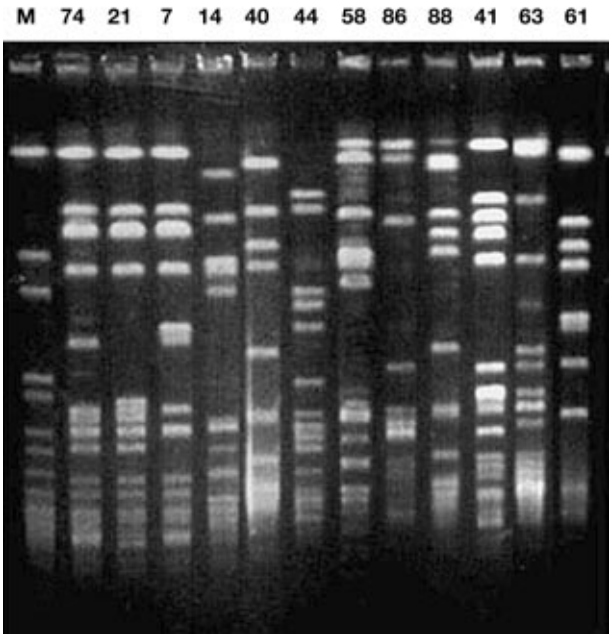

Fig. 1. Pulsed-field gel electrophoresis patterns of selected isolates of community-acquired methicillin-resistant *Staphylococcus aureus*. M: molecular weight marker (American Type Culture Collection 25923).

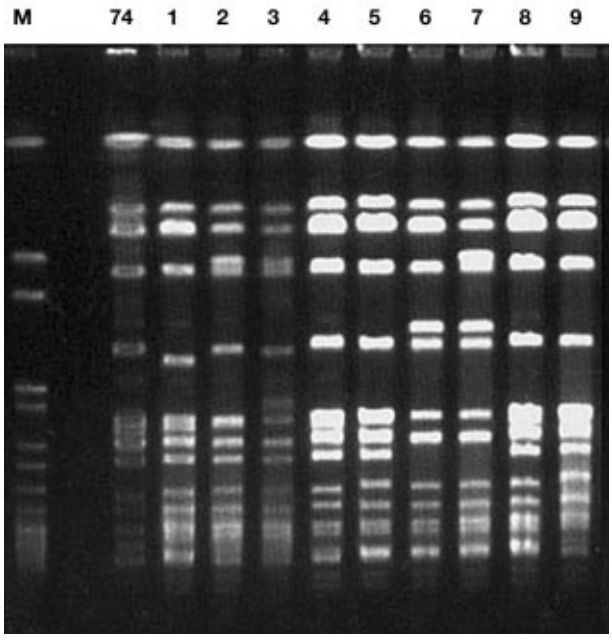

Fig. 2. Comparison of the pulsed-field gel electrophoresis patterns of nosocomial methicillin-resistant *Staphylococcus aureus* (MRSA) [isolate no.s 1-9] and genotype A community-acquired MRSA (isolate no. 74). M: molecular weight marker (American Type Culture Collection 25923).

strains were susceptible to vancomycin, teicoplanin, linezolid, and rifampin. With the exception of isolates 63 and 41, all CA-MRSA isolates had extremely low MIC values to rifampin (range, 0.004-0.008 μg/mL). The susceptibility rate to erythromycin, gentamicin, trimethoprim-sulfamethoxazole, and chloramphenicol was 3%, 9.7%, 25.8%, and 77.4%, respectively. With regard to clindamycin, only 19.4% of the CA-MRSA isolates were susceptible to the drug. High clindamycin MIC values (>256 μg/mL) were found among the isolates belonging to the genotypes A, B, D, G, and J to M. Isolates of genotype C, E, H and I had low MIC values to clindamycin (range, 0.094-0.125 μg/mL). Most of the CA-MRSA isolates (71%) were highly resistant to oxacillin (MIC >256 μg/mL), but some of them were less resistant, especially those that belonged to genotypes G and J (MIC value range, 6-96 μg/mL).

Discussion

We used PFGE to demonstrate that CA-MRSA blood isolates in Taiwan belonged to multiple clones. Similar observations have been reported by previous studies in other populations [11,12]. A total of 13 distinct genotypes were identified. Among these, genotype A was the predominant clone and constituted 48.4% of all CA-MRSA. Strains of genotype A were multidrug resistant and were only susceptible to chloramphenicol, rifampin, glycopeptides, and linezolid.

Most patients with genotype A CA-MRSA bacteremia had at least one risk factor and were repeatedly in contact with health care facilities [9]. In comparison with the genomic similarity between CA-MRSA isolates of genotype A and nosocomial MRSA strains, a high similarity was seen between the two groups, especially those nosocomial isolates from genotype N. Charlebois et al [14] described that there was a close genomic similarity between CA-MRSA and hospital-acquired MRSA strains. They also reported that these infected individuals were usually more frequently in contact with the health care system. Chen et al [22] similarly found a close association between CA-MRSA and nosocominal isolates. There were 3 major genotypes in their study and one of them (genotype A) had a high similarity to the PFGE pattern of genotype A found in the present study. Chambers [23] postulated that the increasing percentage of MRSA in nosocomial infections (more than 60% in Taiwan) might facilitate the dissemination of MRSA into the community. The intimate relationship between CA-MRSA isolates of genotype A and nosocomial MRSA strains indicate that isolates belonging to this genotype might originate from health care facilities [14,22,23].

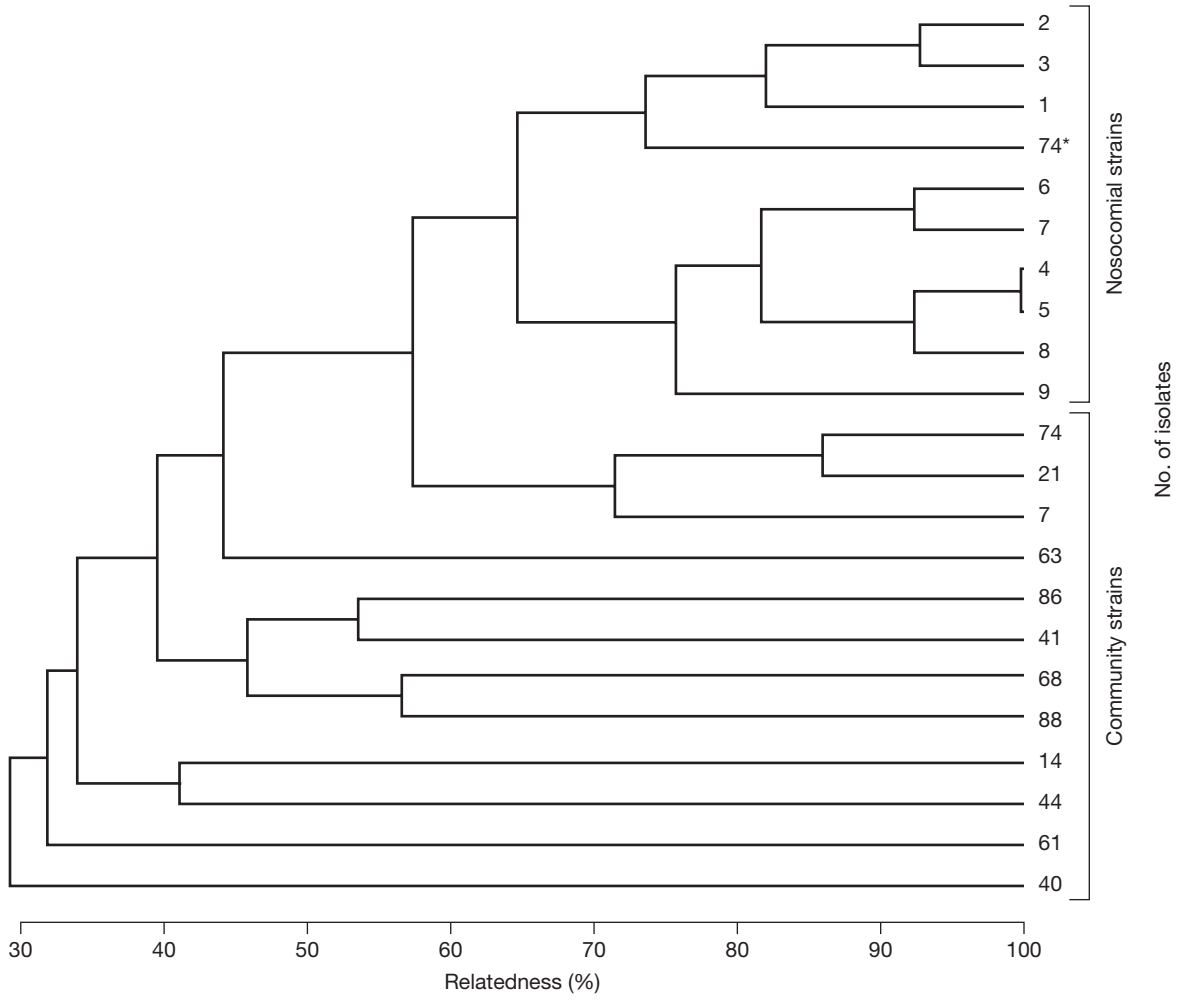

Fig. 3. Unweighted pair-group method with arithmetic mean dendrogram showing percentage similarity between selected isolates of community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) and nosocomial MRSA strains. *Community strain.

The PFGE patterns of all other non-genotype A CA-MRSA (types B to M) isolates were markedly different from those of nosocomial strains, except for genotype K, and further studies will be needed to elucidate whether these strains arose de novo in the community or merely represent minor nosocomial clones that were subsequently disseminated into the community.

In contrast to reports from western countries [1,13, 14,24-26], all 31 CA-MRSA isolates in this study were multidrug resistant, especially those of genotype K. With the exception of rifampin, glycopeptides, and linezolid, the isolates showed no consistent susceptibility to other antibiotics. Resistance to non-beta-lactam antibiotics was common, especially to erythromycin (96.8%), gentamicin (90.3%) and trimethoprim-sulfamethoxazole (74.2%). With regard to clindamycin, CA-MRSA isolates with high susceptibility rates (up to 100%) have been reported in the United States [13,14,16], but this was not so in the present study. Only 19.4% of the CA-MRSA isolates were susceptible to clindamycin. Such high resistance rates to clindamycin (>90%) were also observed by Lu et al [27]. The excessive use, and possibly overuse, of antibiotics by the primary care units in Taiwan, especially of the penicillins, cephalosporins, and macrolides [28,29], may be the reason behind the observed multidrug resistance of isolates. Excessive antibiotic use, especially of macrolides, might partially explain the low susceptibility rate of CA-MRSA to clindamycin and erythromycin in this study [5,30]. Similar to the findings of Naimi et al [13,26], all CA-MRSA isolates in this study had high susceptibility rates to rifampin. The mechanism of resistance in MRSA is mainly mediated by the production of a unique penicillin-binding protein, PBP2a. Although all of isolates in this study were resistant to oxacillin, their MIC values ranged widely from 6 μg/mL to >256 μg/mL. However, whether

this novel protein actually explains the resistance patterns observed in the isolates, especially those with borderline resistance, remains to be determined.

This study has several limitations. Firstly, only isolates from bloodstream infections were included, and their characteristics might not be representative of isolates from other infection sites. Secondly, we only studied cases from a single medical center, and so, the findings may not reflect the true situation in Taiwan. Finally, this retrospective study did not consider the impact of outpatient visits other than those to our hospital, or the risk of intra-family transmission. An exhaustive study, with more extensive specimen collection, will be needed to account for these factors.

In conclusion, this study demonstrated that the genotypes of CA-MRSA were heterogeneous, but that there was a predominant clone circulating in the community. All CA-MRSA isolates were multidrug resistant and this phenomenon might be explained partly by the spreading of nosocomial strains in the community.

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