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Original Article

Molecular pattern and antimicrobial susceptibility of methicillin-resistant *Staphylococcus aureus* isolates at a teaching hospital in Northern Taiwan

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Background and Purpose: Methicillin-resistant *Staphylococcus aureus* (MRSA) infection has progressively increased worldwide. Knowledge of the specific epidemiological pattern of isolates at individual hospitals is important.

Methods: MRSA bacteremia was diagnosed in a total of 68 patients from January 2002 through December 2003, stratified for drug susceptibility and molecular pattern (staphylococcal cassette chromosome *mec* element [SCC*mec*] typing and genotypes).

Results: SCC*mec*-A-positive isolates were found on polymerase chain reaction in 58 patients. The most frequent SCC*mec* types were III (40 cases) of which less than 5% were susceptible to other beta-lactam antibiotics and most were health care-associated, followed by SCC*mec* type IV (15 cases), that were demonstrated to be community-acquired. SCC*mec* type IV MRSA isolates were more likely to be susceptible to ciprofloxacin (93.3%), gentamicin (46.7%) and trimethoprim-sulfamethoxazole (93.3%) than type III isolates. All MRSA isolates were susceptible to glycopeptides and vancomycin (minimum inhibitory concentrations <2 μg/mL). Pulsed-field gel electrophoresis with *SmaI* digestion was used to fingerprint these isolates. A total of 9 genotypes with 26 type-subtypes were identified. Genotype A was the most frequent (9 subtypes) indicating that it is epidemic in this hospital.

Conclusion: After analysis, SCC*mec* typing could be used to predict drug susceptibility. Specific clones of *S. aureus* are circulating in hospital and communities in Taiwan.

Key words: Bacterial typing techniques, epidemiology, methicillin resistance, microbial sensitivity tests, *Staphylococcus aureus*

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) has become increasingly prevalent worldwide, as is evident from many surveillance studies [1-5]. MRSA is associated with community-acquired and nosocomial infection. These infections are associated with longer duration of hospital stay, greater use of health resources, and higher treatment cost [6-8]. The emergence of MRSA strains in many countries and in a diversity of hospital settings is a result of selection by exposure to antibiotics and by cross-infection. Inter-hospital, inter-city, inter-country or even inter-continental spread has occurred by transfer of infected or colonized patients or staff. In addition, MRSA-infected patients require strict isolation and hygiene to limit spreading.

MRSA infection was first documented in Taiwan in early 1980. Recent overall prevalence rates of MRSA infections in Taiwan ranged from 50% to 80% [9,10]. The proportion of staphylococcal infections caused by MRSA is approximately 50% in our hospital. The increased prevalence of MRSA in a hospital could be due to increased transmission of MRSA strains among patients and/or admission of patients who are already

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colonized. The purpose of this study was to investigate the drug susceptibility and molecular pattern of MRSA isolates in our hospital.

Methods

Study population

Cathay General Hospital is an 800-bed tertiary care teaching hospital in Northern Taiwan, with an average of 30,000 patient discharges per year. From January 2002 to December 2003, records of patients with MRSA bacteremia (n = 68) with at least 1 blood culture were extracted from the computerized microbiology record database. Multiple sets of isolates in the same patients were counted as a single set. The MRSA strains were stored at –80°C in the infectious disease laboratory and were inoculated onto blood agar plate with 5% carbon dioxide for 24 h. *S. aureus* isolates were identified by standard methods that included Gram staining, tests for colonial morphology and coagulase production (BBL staphyloslide latex test; Becton Dickinson, NJ, USA). A 0.5 McFarland bacterial suspension was inoculated onto Muellar-Hinton agar with 4% sodium chloride, and 6 μg/mL oxacillin for resistance testing in accordance with National Committee for Clinical Laboratory Standards (NCCLS) guidelines [11]. Agar plates were incubated at 35°C for 24 h and examined for evidence of growth.

Antimicrobial susceptibility testing

The MRSA isolates were tested for susceptibility and minimum inhibitory concentration (MIC) by the Phoenix system (Becton Dickinson) with Gram-positive test panels, according to the manufacturer's instructions. The results were interpreted in accordance with NCCLS guidelines [11].

Polymerase chain reaction assay for typing of major SCC*mec*

Four main loci (A, B, D, E) were chosen for multiplex polymerase chain reaction (PCR), as described by Oliveira and de Lencastre [12] to type the staphylococcal cassette chromosome *mec* element (SCC*mec*) in MRSA. The 4 loci are shown in Table 1. The *mec*-A gene was used for internal positive control.

Pulsed-field gel electrophoresis

Pulsed-field gel electrophoresis (PFGE) with *SmaI* digestion was used for genotyping according to a previously described procedure [13] and the criteria proposed by Tenover et al were employed to analyze the DNA fingerprints generated by PFGE [14].

Results

Of the 68 MRSA isolates, 58 were *mec*-A-positive and 10 were *mec*-A-negative by PCR assay. The oxacillin agar screen test for *S. aureus* identified all of the 58 *mec*-A-positive isolates, resulting in a sensitivity of 100%. However, it also yielded 10 false-positive results in *mec*-A-negative *S. aureus* isolates tested in this study, resulting in a specificity of <60%. Subculture of these isolates to obtain single colonies yielded oxacillin-resistance isolates that were negative for *mec*-A by PCR. Oxacillin MICs were >2 μg/mL for 2 isolates, and equal to 0.5 μg/mL for 8 of these isolates.

The susceptibility testing results for the 60 MRSA isolates are shown in Table 2. All isolates were susceptible to glycopeptides and linezolid and 100% were resistant to penicillin. Thirty nine isolates (65.0%) were resistant to ciprofloxacin, 44 (73.3%) were resistant to gentamicin, and 38 (63.3%) were resistant to trimethoprim-sulfamethoxazole. The majority of the

Table 1. Primers used in the polymerase chain reaction to type the staphylococcal cassette chromosome *mec* element (SCC*mec*) in methicillin-resistant *Staphylococcus aureus*

Locus	Primer	Oligonucleotide	Specificity (SCC*mec* type)
A	CIF2 F2	TTCGAGTTGCTGATGAAGAAGG	I
	CIF2 R2	ATTTACCACAAGGACTACCAGC	
B	KDP F1	AATCATCTGCCATTGGTGATGC	II
	KDP R1	CGAATGAAGTGAAAGAAAGTGG	
D	DCS F2	CATCCTATGATAGCTTGGTC	I, II, IV
	DCS R1	CTAAATCATAGCCATGACCG	
E	RIF F3	GTGATTGTTCGAGATATGTGG	III
	RIF4 F3	CGCTTTATCTGTATCTATCGC	
Mec-A	MECA P4	TCCAGATTACAACTTCACCAGG	Internal control
	MECA P7	CCACTTCATATCTTGTAACG	

Table 2. Antibiotic susceptibility of 60 methicillin-resistant *Staphylococcus aureus* isolates

Antibiotic	MIC (μg/mL)	No. (%) of isolates	
		Susceptible	Resistant
Penicillin	4-128	0	60 (100)
Oxacillin	4->128	0	60 (100)
Erythromycin	4->128	2 (3.3)	58 (96.7)
Clindamycin	0.06->128	3 (5.0)	57 (94.0)
Gentamicin	0.06->128	16 (26.7)	44 (73.3)
Vancomycin	0.06-4	60 (100)	0
Teicoplanin	0.12-8	60 (100)	0
Linezoid	1-2	60 (100)	0
TMP-SMX	2->128	22 (36.7)	38 (63.3)
Ciprofloxacin	0.06->128	21 (35.0)	39 (65.0)

Abbreviations: MIC = minimum inhibitory concentration; TMP-SMX = trimethoprim-sulfamethoxazole

isolates were also resistant to erythromycin (96.7%) and clindamycin (94.0%). Multidrug-resistant MRSA, defined as isolates with resistance to 3 or more drug classes other than beta (β)-lactam antibiotics, accounted for 82% of MRSA isolates, a lower percentage than previously reported [15,16]. MICs of vancomycin were measured for all 60 isolates and none were greater than 2 μg/mL.

SCC*mec* typing (Fig. 1) showed that most isolates were type III (40 cases), followed by type IV (15 cases) and type II (3 cases). Another 10 cases of *mec*-A-negative MRSA were not subjected to further identification tests. The relationships between susceptibility and SCC*mec* typing are shown in Table 3. SCC*mec* type IV MRSA isolates were more likely than SCC*mec* type III MRSA isolates to be susceptible to all 3 of the following antimicrobial agents: ciprofloxacin (93% vs 7.5%), gentamicin (46.7% vs 5.0%) and trimethoprim-sulfamethoxazole (93.3% vs 5.0%).

Among the 58 MRSA isolates, a total of 9 genotypes with 26 type-subtypes were identified by PFGE. Subtypes could be identified in 3 genotypes (A, B, C). The banding patterns of most of these genotypes are shown in Fig. 2. There were a total of 9 subtypes in genotype A, 8 subtypes in genotype B, and 3 subtypes in genotype C.

Discussion

In most MRSA isolates tested in this study, the methicillin resistance determinant was carried on an SCC*mec* type III (68%), which tended to be multi-resistant and in most cases health care-associated.

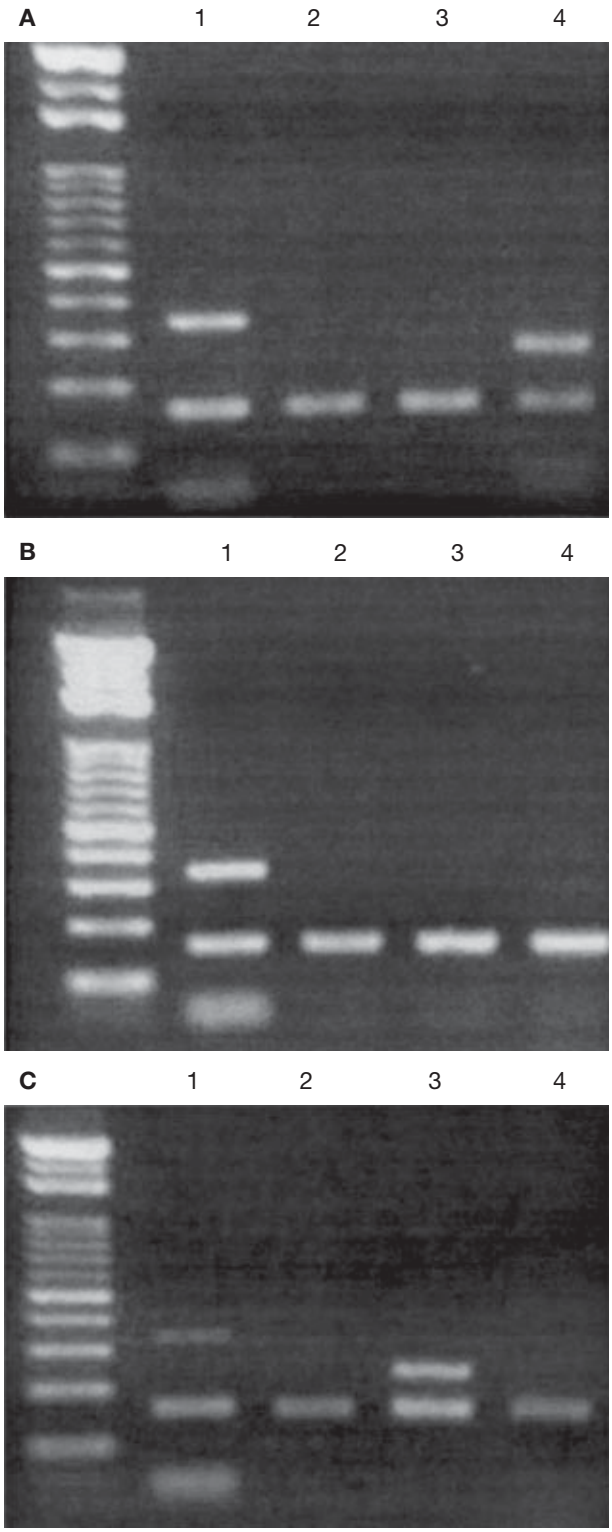

Fig. 1. Validation of the staphylococcal cassette chromosome *mec* element (SCC*mec*) polymerase chain reaction strategy. Lane: 1, loci D, present in SCC*mec* type I, II, and IV; 2: loci A, specific for SCC*mec* type I; 3: loci E, specific for SCC*mec* type III; 4: loci B, specific for SCC*mec* type II. (A) SCC*mec* type II. (B) SCC*mec* type IV. (C) SCC*mec* type III.

Table 3. Drug susceptibility and staphylococcal cassette chromosome *mec* element (SCC*mec*) typing of 58 methicillin-resistant *Staphylococcus aureus* isolates

Drug	SCC*mec* type IV n =15, (%) of isolates		SCC*mec* type III n = 40, (%) of isolates		SCC*mec* type II n = 3, (%) of isolates	
	S	R	S	R	S	R
Penicillin	0	15 (100)	0	40 (100)	0	3 (100)
Oxacillin	0	15 (100)	0	40 (100)	0	3 (100)
Erythromycin	0	15 (100)	0	40 (100)	0	3 (100)
Clindamycin	0	15 (100)	1 (1.1)	39 (98.9)	0	3 (100)
Gentamicin	7 (46.7)	8 (53.3)	2 (5.0)	38 (95.0)	0	3 (100)
Vancomycin	15 (100)	0	40 (100)	0	3 (100)	0
Teiclopanin	15 (100)	0	40 (100)	0	3 (100)	0
Linezoid	15 (100)	0	40 (100)	0	3 (100)	0
TMP-SMX	14 (93.3)	1 (6.7)	2 (5.0)	38 (95.0)	1 (33.3)	2 (66.7)
Ciprofloxacin	14 (93.3)	1 (6.7)	3 (7.5)	37 (92.5)	0	3 (100)

Abbreviations: S = susceptible; R = resistant; TMP-SMX = trimethoprim-sulfamethoxazole

SCC*mec* type IV (25.8%) was the second most dominant; this type demonstrated an origin from community-acquired MRSA in previous studies [17,18], and was susceptible to many other non-β-lactam antibiotics (46% vs <5%, respectively). This study did not examine the timeline of the exposure to MRSA and/or whether the patients were exposed to nosocomial settings prior to acquiring MRSA. We did or did not limit the definition of community-acquired or health care-acquired MRSA according to the drug susceptibility test results and previous molecular characteristics [19]. No MRSA isolate belonged to SCC*mec* type I. Due to the small number of cases in this study, further study is needed to confirm the SCC*mec* type and susceptibility patterns of MRSA isolates in this hospital.

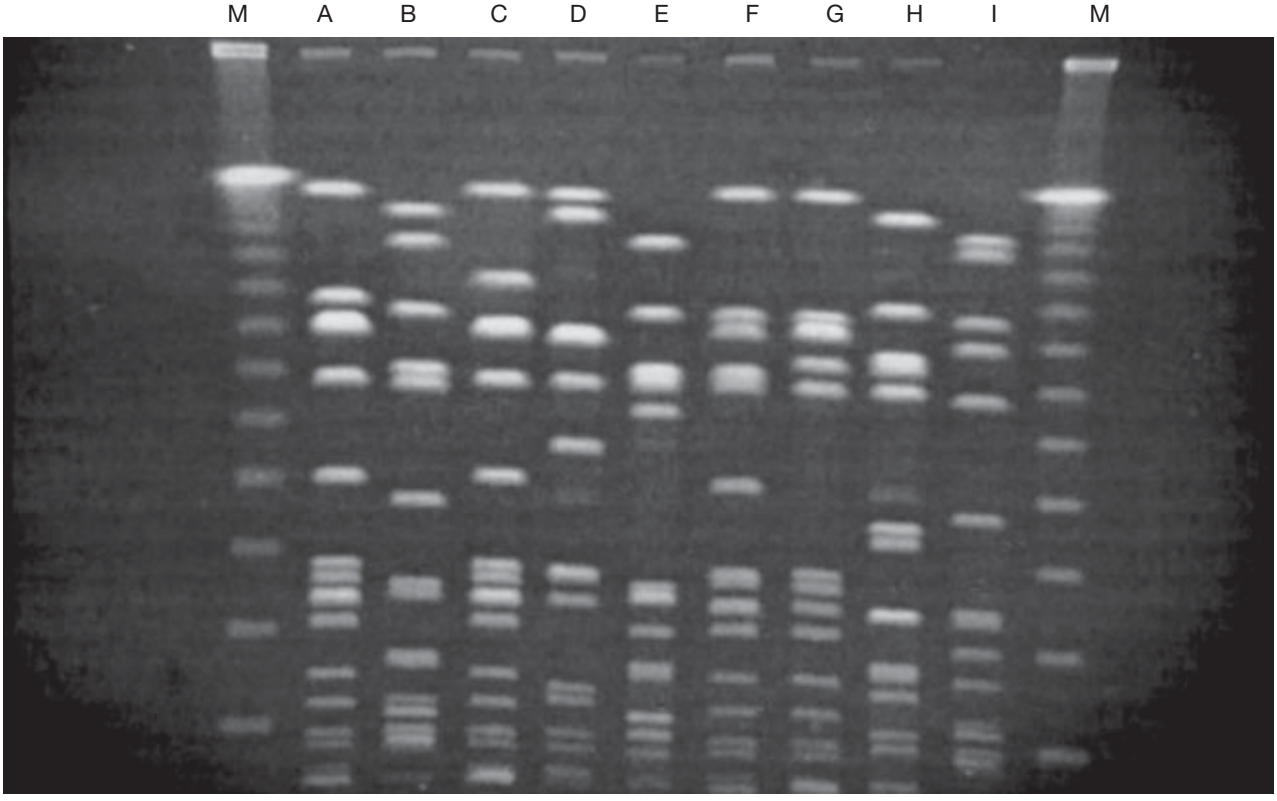

Fig. 2. Pulsed-field gel electrophoresis patterns of *SmaI*-digested genomic DNA from methicillin-resistant *Staphylococcus aureus* isolates. M, lambda DNA concatemer standard; A to I, 9 genotypes.

Standard methods for susceptibility testing of MRSA according to recommendations of the NCCLS in this study revealed that 10 MRSA isolates that did not carry *mec*-A were phenotypically resistant to methicillin according to the oxacillin agar screen test. Phenotypic expression of resistance can vary depending on the growth conditions such as the temperature or osmolarity of the medium, making susceptibility testing of MRSA by standard methods potentially problematic [20]. No phenotypic system is completely reliable for the detection of oxacillin resistance in *S. aureus* [21]. Although PCR may be considered the gold standard diagnostic tool for detection of MRSA, it could not serve as a conclusive test in this study as it was not preformed routinely in the laboratory.

The accuracy and rapidity by which PCR assay results can be obtained to detect MRSA infection are especially important for the control of MRSA in hospitals and to guide the appropriate use of antimicrobial agents in critically ill patients. This method may be used with sterile or non-sterile clinical samples [22-24] for the screening of high-risk infected patients or health care workers to provide information about spread within hospitals and circulation in the community.

This study demonstrated the spread of a major PFGE genotype (genotype A, with 9 subtypes) of MRSA isolates in our hospital, which is similar to that previously reported in MRSA isolates from Taiwan [18, 25]. These findings indicate that the same genetic lineages of *S. aureus* have spread widely within hospitals and are circulating in communities in Taiwan. Increased awareness and efforts by individual hospitals are needed to control this spread.

Maintenance of a database of isolates at individual hospitals is important in establishing effective antimicrobial drug policy, identifying endemic strains in each hospital, and assessing the degree of compliance with hygiene protocols by medical staff. Such data would provide valuable information on endemic strains in each hospital and would be helpful for epidemiological studies.

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