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ORIGINAL ARTICLE

An investigation of vancomycin minimum inhibitory concentration creep among methicillin-resistant *Staphylococcus aureus* strains isolated from pediatric patients and healthy children in Northern Taiwan

Chia-Ning Chang [a], Wen-Tsung Lo [a,d], Ming-Chin Chan [b], Ching-Mei Yu [c], Chih-Chien Wang [a,*]

[a] *Department of Pediatrics, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan*
[b] *Infection Control Office, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan*
[c] *Clinical Microbiology Laboratory Room, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan*
[d] *Department of Pediatrics, St. Joseph Hospital, Kaohsiung, Taiwan*

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Abstract *Background and purpose:* The phenomenon of vancomycin minimum inhibitory concentration (MIC) creep is an increasingly serious problem in the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) infections. In this study, we investigated the vancomycin and daptomycin MIC values of MRSA strains isolated from pediatric patients and MRSA colonized healthy children. Then, we assessed whether there was evidence of clonal dissemination for strains with an MIC to vancomycin of $\geq$ 1.5 μg/mL.
Methods: We collected clinical MRSA isolates from pediatric patients and from healthy children colonized with MRSA during 2008–2012 at a tertiary medical center in northern Taiwan and obtained vancomycin and daptomycin MIC values using the Etest method. Pulse-field gel electrophoresis (PFGE) and staphylococcal cassette chromosome (SCC*mec*) typing were used to assess clonal dissemination for strains with an MIC to vancomycin of $\geq$ 1.5 μg/mL.
Results: A total 195 MRSA strains were included in this study; 87 were isolated patients with a clinical MRSA infection, and the other 108 strains from nasally colonized healthy children.

* Corresponding author. Department of Pediatrics, Tri-Service General Hospital, National Defense Medical Center, 325 Cheng-Kung Road, Sector 2, Neihu, Taipei 114, Taiwan, ROC.
E-mail address: ndmcccw@yahoo.com.tw (C.-C. Wang).

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Vancomycin MIC $\geq$ 1.5 μg/mL was seen in more clinical isolates (60/87, 69%) than colonized isolates (32/108, 29.6%), $p < 0.001$. The PFGE typing of both strains revealed multiple pulsotypes. *Conclusion:* Vancomycin MIC creeps existed in both clinical MRSA isolates and colonized MRSA strains. Great diversity of PFGE typing was in both strains collected. There was no association between the clinical and colonized MRSA isolates with vancomycin MIC creep.

Introduction

Staphylococcus aureus often results in serious infections, including pneumonia, bacteremia, skin and soft tissue infections, and endocarditis. These infections can occur in hospitals, health care institutions, as well as in the community. *S. aureus* has the ability to colonize humans, especially the nose.[1] Colonization plays an important role in the pathogenesis and epidemiology of infections caused by *Staphyloccocus aureus*, including both methicillin-sensitive and methicillin-resistant (MRSA).[1–3] Safdar and Bradley[4] found that there is a greater risk for infection including invasive infections following colonization with MRSA.

Vancomycin has been a front-line drug of choice for treating infections caused by MRSA for the past 60 years.[5] Nevertheless, the association of vancomycin treatment failures with increased vancomycin minimum inhibitory concentration (MIC) is a recent challenge in the current medical circumstances.[6] Even when MRSA isolates are susceptible to vancomycin, treatment failure is frequently seen. In recent years, progressive elevation in glycopeptides MICs for *S. aureus* strains, a phenomenon recognized as vancomycin MIC creep has been discussed. Investigators have reported that an increase in vancomycin MIC for MRSA isolates poses a substantial risk of failure. Sakoulas et al[7] reported that the possibility of treatment success is lower in patients with MRSA infections with a vancomycin MIC of 1–2 mg/mL compared with patients infected with isolates with a vancomycin MIC $\leq$ 0.5 mg/mL. The Infectious Diseases Society of America recommended that the dosage of vancomycin should be at least 60 mg/kg/d to achieve the target vancomycin trough level of 15–20 mg/mL for serious MRSA infections in adults in 2009.[5,8] The guideline for targeting vancomycin trough level for pediatric patients was not discussed. As a result, we obtained more information on MRSA treatment in children. During recent years, community-associated (CA) MRSA infection has become increasingly common. According to reports from Taiwan,[9–11] compared with those reported from America and other nations, CA-MRSA isolates in Taiwan did not always contain type IV staphylococcal cassette chromosome (SCCmec) and were resistant to multiple non-β-lactam antibiotics, including clindamycin and macrolides. There has been no similar study investigating vancomycin and daptomycin MIC values of MRSA strains in children to date.

The aim of this study was to investigate the vancomycin and daptomycin MIC values of MRSA strains isolated from pediatric patients and MRSA-colonized healthy children from 2008 to 2012 at a tertiary medical center using the Etest method. If the vancomycin MIC $\geq$ 1.5 μg/mL, further evaluation with pulsed-field gel electrophoresis (PFGE) and staphylococcal cassette chromosome mec (SCC*mec*) elements typing were used to assess for clonal dissemination.

Methods

Study design and specimen collection

This retrospective study was conducted from 2008 to 2012 at Tri-Service General Hospital, a 1400-bed tertiary medical center in northern Taiwan. All patients were aged $\leq$ 18 years and hospitalized with an MRSA infection identified from medical records and the clinical microbiology laboratory. Eligible healthy children aged $\leq$ 14 years without acute medical problems were enrolled who either visited a healthcare facility for a regular well-child checkup or attended kindergartens in Taipei, Taiwan; this was reviewed and approved by the National Defense Medical Center Institutional Review Board, Taipei, Taiwan. We obtained written informed consent from each child's parents or legal representative before nasal specimen collection or interviews. During the 5-year study period, all children who presented for regular health maintenance visits to our hospital were invited to take part in this study. The kindergartens were chosen based on support for the surveillance investigation by the kindergartens' principals.

The definition of CA-MRSA infection as any MRSA infection which is diagnosed in an outpatient or within 48 hours of admission to hospital, of which the patient has none of the following risk factors for health-care-associated MRSA: hemodialysis; operation; residence in a long-term care facility or treatment in hospital during the previous year; presence of a lasting catheter or percutaneous instrument at the time of culture; or prior isolation of MRSA.[12,13]

Bacterial strains and antimicrobial susceptibility testing

Nasal samples were acquired with a sterile cotton swab, placed in transport medium (Venturi Transystem; Copan Diagnostics, Corona, CA, USA); transported to and processed in our microbiology laboratory within 4 hours. Cotton swabs were plated on mannitol salt agar (BBL Microbiology Systems, Becton Dickinson, Company, Sparks, MD, USA). Distinctive morphotypes of mannitol-fermenting colonies were selected from a mannitol salt agar plate,

subculture to trypticase soy agar with 5% sheep blood agar plate (BBL Microbiology Systems; Becton Dickson); incubated at 37°C in a humidified incubator with 5% CO_2. Cultures on blood agar plates were screened using Slidex Staph Plus (bioMérieux, Marcy l'Etoile, France). MRSA identification and antimicrobial susceptibility testing were executed according to Clinical Laboratory Standards Institute guidelines.[14] All MRSA isolates were frozen at −70°C for additional testing. Laboratory records of susceptibility data for all MRSA isolates were used to define the subset of MRSA. Duplicate isolates from the same patient were excluded.

Vancomycin and daptomycin MIC Etest

The MIC was performed using the Etest method, according to the manufacturer's guidelines. A suspension of saline calibrated to the 0.5-McFarland turbidity standard was plated onto Mueller—Hinton agar, onto which Etest strips (AB BIODISK, Solna, Sweden) were applied. The plates were incubated at 35°C for 24 hours. The MIC was interpreted as the zone of inhibition that corresponded to a concentration gradient on the Etest strips, according to the manufacturer's guidelines. Quality control was done using the Clinical Laboratory Standards Institute-recommended reference strain (ATCC 29213).

Genotyping

PFGE was performed using the CHEF Mapper XA system (Bio-Rad Laboratories, Hercules, CA, USA) according to a published protocol.[10] We analyzed all findings according to standard criteria[15] and designated the pulsotypes in alphabetical order. PFGE patterns with fewer than four band differences from one existing pulsotype were categorized as subtypes and tagged with Arabic numbered suffixes.[15] In order to recognize PFGE polymorphisms, band patterns were analyzed by Molecular Analyst Fingerprinting, Fingerprinting Plus, and Fingerprinting DST software (Bio-Rad Laboratories). The grouping method was done to establish a dendrogram from the matrix using the unweighted pair group method with the arithmetic averages setting technique after estimation of similarities by using the Pearson correlation coefficient between each pair of organisms; the PFGE patterns were discriminated at the 80% similarity level. The chromosomal patterns were inspected visually and the similarities of the PFGE patterns were distributed using previously published criteria.[15] SCC*mec* typing was performed using a multiplex polymerase chain reaction strategy with sets of region-specific primers as previously portrayed. Screening for SCC*mec* was done with the primer and thermo cycler conditions described by Boyle-Vavra et al.[16]

Statistical analysis

Data were entered into Microsoft Access XP software and analysis performed using SPSS statistical software, version 10.0 (SPSS Inc., Chicago, IL, USA). The categorical parameters were compared using the Chi-square test, Mantel—Haenszel test, or Fisher exact test. The data from 2008 to 2012 were combined to provide more reliable estimates of the microbiological characteristics by strengthening the power of the investigation. All parameters were initially compared using univariate analysis; those with $p < 0.05$ and those that were biologically meaningful were included in the multivariate analysis. Nevertheless, parameters with colinearity tested by correlation matrices were not included in the final model at the same time. We assessed risk factors using SAS 9.1.3 (SAS Institute, Inc., Cary, NC, USA). All tests were two-tailed; $p < 0.05$ was considered statistically significant.

Results

A total of 195 MRSA strains were included in this study, of which 87 were isolated from patients with clinical MRSA infection. The sources of the clinical MRSA isolates are shown as Table 1. The major source was wound culture, and throat culture was secondary. Minor sources were stool culture and urine culture. Other 108 strains were isolated from nasally colonized healthy children. Among the 87 clinical isolates, 72 isolates belonged to CA-MRSA, while in the 108 colonizing strains, 106 isolates were CA-MRSA. We detected SCC*mec* gene in all CA-MRSA strains; the results revealed that in the 87 clinical isolate group, 52 (59.8%) strains contained type VT SCC*mec*, 20 (23%) strains were SCC*mec* type IV. In the colonizing group, 91 (85.8%) strains contained SCC*mec* type IV, and the other 15 (14.2%) strains were SCC*mec* type VT.

The susceptibility test to vancomycin and daptomycin revealed that all MRSA strains were sensitive to vancomycin and daptomycin. The result of the MRSA-vancomycin MICs for clinical and colonizing strains is shown in Table 2. Among the strains with vancomycin MIC $\geq$ 1.5 μg/mL, more were clinical isolates (60/87, 69.0%) than colonizing isolates (32/108, 29.6%), $p < 0.001$. The results of the MRSA-daptomycin MICs for the clinical and colonizing strains are shown in Table 3. There was no significant difference between the MICs of the two groups. The distribution of vancomycin MIC of clinical isolates is shown in Table 4.

Pulsed-field gel electrophoresis (PFGE) was performed on 60 clinical MRSA strains and 32 colonizing MRSA isolates with a vancomycin MIC $\geq$ 1.5 μg/mL. We defined similar PFGE types by using cutoffs of 80%. A great diversity of pulsotypes was noted among the 92 MRSA isolates subjected to PFGE typing. Figure 1 shows the distribution of the 40 PFGE types detected among the 60 clinical MRSA strains.

Table 1 The sources of the clinical methicillin-resistant *Staphylococcus aureus* (MRSA) isolates with vancomycin minimum inhibitory concentration $\geq$ 1.5 μg/mL

Sources	No. of clinical MRSA isolates	%
Urine	2	3.3
Wound	41	68.4
Throat	9	15
Blood	2	3.3
Sputum	4	6.7
Stool	2	3.3
Total	60	100

Table 2 The results of vancomycin minimum inhibitory concentration (MICs) for clinical and colonized methicillin-resistant *Staphylococcus aureus* strains*

Strains	No. of strains	MIC50 (μg/mL)	MIC90 (μg/mL)	MIC < 1.5 μg/mL	MIC ≥ 1.5 μg/mL	Geometric mean
				n (%)	*n* (%)	(μg/mL)
Clinical	87	1.5	1.5	27 (31.0)	60 (69.0)	1.39 ± 0.41
Colonized	108	1	1.5	76 (70.4)	32 (29.6)	1.15 ± 0.30

*$p < 0.001$.
MIC50 = concentration required to inhibit the growth of 50% of pathogens; MIC90 = concentration required to inhibit the growth of 90% of pathogens.

Table 3 The results of daptomycin minimum inhibitory concentration (MICs) for clinical and colonized methicillin-resistant *Staphylococcus aureus* for clinical and colonized strains

Strains	No. of strains	MIC50 (μg/mL)	MIC90 (μg/mL)	Geometric mean (μg/mL)
Clinical	87	0.19	0.38	0.2 ± 0.1
Colonized	108	0.19	0.38	0.2 ± 0.1

MIC50 = concentration required to inhibit the growth of 50% of pathogens; MIC90 = concentration required to inhibit the growth of 90% of pathogens.

Table 4 Vancomycin minimum inhibitory concentration (MICs) of clinical isolates in 2008–2012

Year	Vancomycin MIC (μg/mL) of clinical isolates						Total	MIC ≥ 1.5 (%)
	0.5	0.75	1.00	1.5	2.00	3.00		
2008	0	5	8	11	3	0	27	51.9
2009	1	1	0	12	4	0	18	88.9
2010	0	3	5	7	2	1	18	55.6
2011	0	0	5	15	4	0	24	79.2
2012	0	1	3	8	1	0	13	69.2

The majority belonged to PFGE type 1 or its four subtypes (6.7%), PFGE type 2–5 (each had 3 subtypes respectively; total 20%), and PFGE 6–14 (each had 2 subtypes respectively; total 30%). The other PFGE types occurred sporadically. In addition, we found that the distribution of clinical SCC*mec* genotypes were as follows: type II, seven isolates (11.7%); type III, seven isolates (11.7%); type IV, 16 isolates (26.7%); and type VT, 30 isolate (50%). Figure 2 shows the distribution of the 21 PFGE types among the 32 colonizing MRSA isolates. The diversity was also great. The majority belonged to PFGE type A or its five subtypes (15.6%), PFGE type B or its four subtypes (12.5%), and PFGE type C and D (each has 3 subtypes; total 18.8%). The other PFGE types were found sporadically. Besides, we noted that the distribution of colonizing SCC*mec* genotypes were: type II, one isolate (3.1%); type III, one isolate (3.1%); type IV, 28 isolates (87.5%); and type VT, 2 isolates (6.3%). No evidence of clonal dissemination was noted in these populations. The distribution and trend of vancomycin MICs over time is shown in Figure 3. The percentage of MRSA isolates with vancomycin MIC ≥ 1.5 μg/mL was always > 50% during the study period of 2008–2012.

Discussion

CA-MRSA was first found in Australia during the early 1990s and rapidly spread worldwide. CA-MRSA infections in pediatric patients have been increasing since 2002, and somewhere these SCC*mec* types are classically associated with CA-MRSA. The majority of the MRSA strains isolated from both pediatric patients and colonized healthy children in Taiwan are CA-MRSA.[9,10,17] In Taiwan, Tsao et al[18] also found that the prevalence rate of CA-MRSA increased obviously during recent years (from 25.6% in 2006 to 46% in 2010). Tsao et al[18] investigated the trend in vancomycin susceptibility and correlation with molecular characteristics of MRSA causing severe infections in Taiwan; there was no vancomycin creep among MRSA isolates, and the declining trend of vancomycin MIC against MRSA was ascribed to the increasing prevalence of CA-MRSA over time. Chen et al[19] published their study about susceptibilities of MRSA *in vitro* to nine antimicrobial agents in Taiwan; a total of 1725 isolates were acquired from 20 hospitals throughout Taiwan from 2006 to 2010. MIC creep was found only for daptomycin during this period, but not for vancomycin, teicoplanin, linezolid, or tigecycline.

In America and Canada, some similar results have been revealed already. Goldman et al[20] found that in the adult population, the trend during 2000–2005 toward increasing vancomycin MIC values for *S. aureus*, which is called *MIC creep*, had resulted in the concern that vancomycin may no longer be suitable to treat invasive staphylococcal infection in adults where the MIC is ≥ 2 μg/mL; however, a significant vancomycin MIC creep in pediatric populations was not manifested from 2006 to 2009. Adam et al[21] also noted that there had been no evidence of vancomycin MIC creep in 475 Canadian strains of MRSA isolates obtained from 1995 to 2006 in the adult population.

In our institution, the phenomenon of vancomycin MIC creep in MRSA exists not only in adults, but also children. Yeh et al[22] reported that the geometric mean of the vancomycin MIC for MRSA isolates obtained in 2009 was 1.39 ± 0.30 mg/mL, which was significantly higher than the mean vancomycin MIC obtained in 2001 (1.19 ± 0.34 mg/mL, $p < 0.01$) and 2005 (1.99 ± 0.25 mg/mL, $p < 0.001$). In our study, the percentile of vancomycin MIC ≥ 1.5 μg/mL is 62.5% of the clinical CA-MRSA strains and 28.3% of the colonizing CA-MRSA isolates. The clinical strains contribute

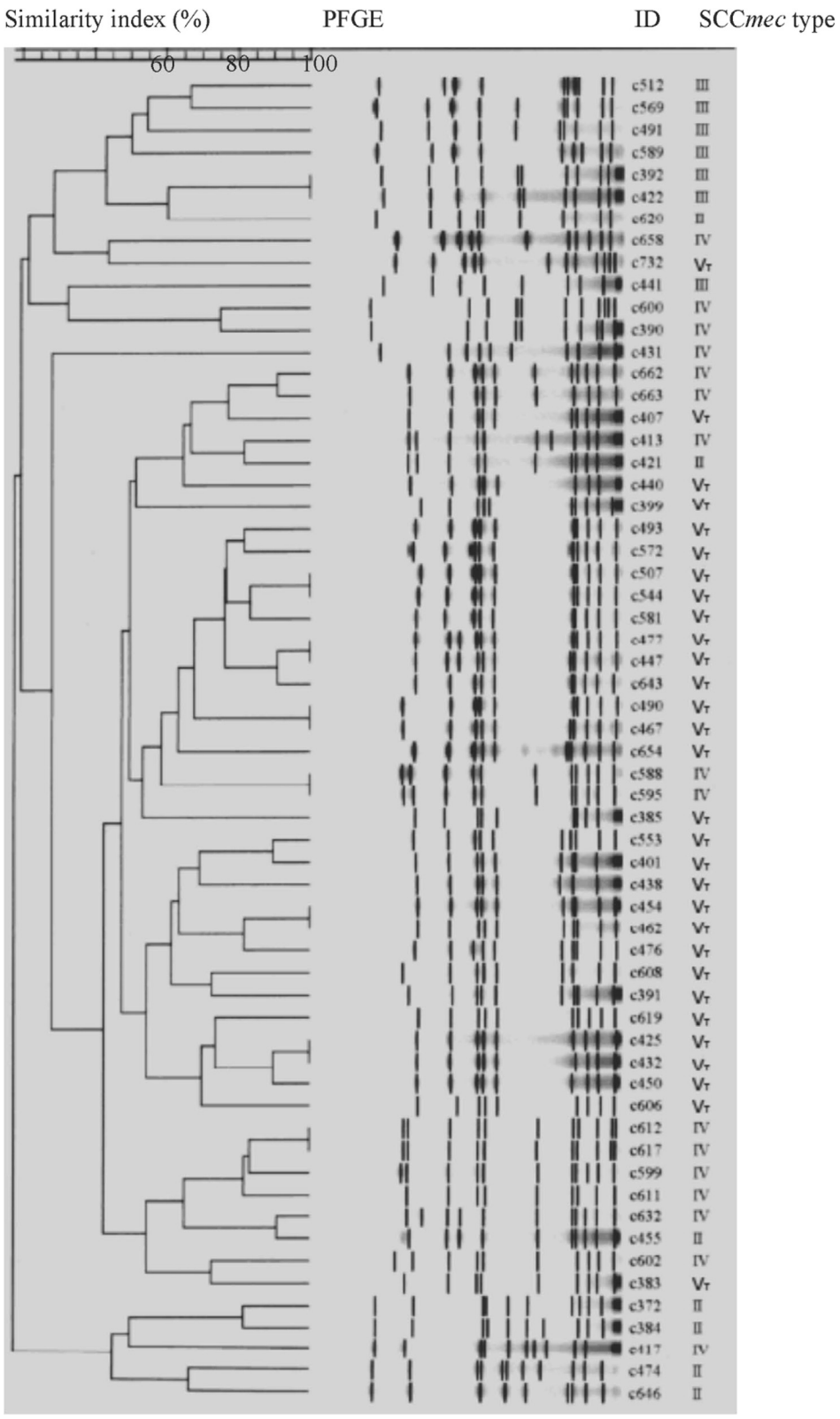

Figure 1. Genetic relatedness among clinical methicillin-resistant *Staphylococcus aureus* isolates with vancomycin minimum inhibitory concentration $\geq$ 1.5 μg/mL. Unweighted pair group method with arithmetic mean dendrogram revealing genetic relatedness among clinical methicillin-resistant *Staphylococcus aureus* isolates as determined by pulse-field gel electrophoresis with *smaI*. The broken line corresponds to the cutoff level (80%) used to define related pulse-field gel electrophoresis clones.

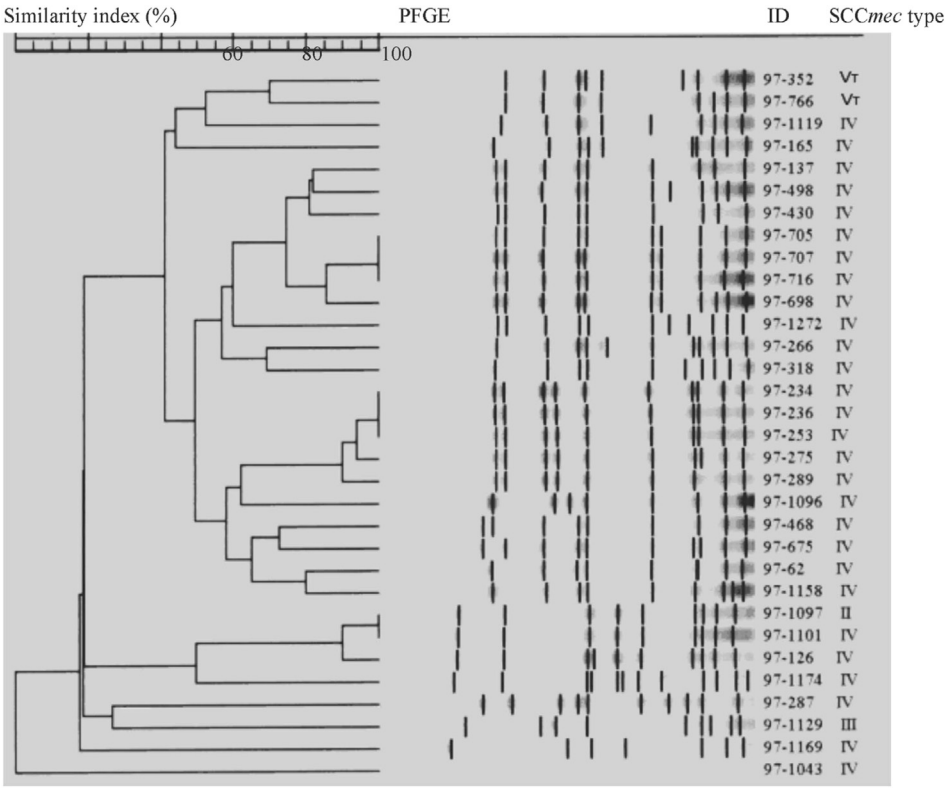

Figure 2. Genetic relatedness among colonizing methicillin-resistant *Staphylococcus aureus* isolates with vancomycin minimum inhibitory concentration $\geq$ 1.5 μg/mL. Unweighted pair group method with arithmetic mean dendrogram revealing genetic relatedness among colonizing methicillin-resistant *Staphylococcus aureus* isolates as determined by pulse-field gel electrophoresis with *smaI*. The broken line corresponds to the cutoff level (80%) used to define related pulse-field gel electrophoresis clones.

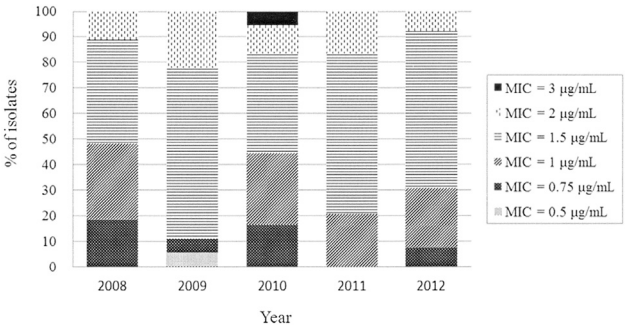

Figure 3. The distribution and trend of vancomycin MICs of methicillin-resistant *Staphylococcus aureus* clinical isolates between 2008—2012.

the majority of these strains possibly due to the frequent prescription of vancomycin in patients with MRSA infection over the past few decades. As a result, the decreased vancomycin susceptibility among clinical MRSA isolates has become increasingly common. Although the vancomycin MIC creep percentile of the colonizing CA-MRSA is lower than that of the clinical one, the possibility of MRSA infection due to colonization still exists.

Yeh et al[22] suggested that vancomycin should be prescribed to treat MRSA infections if the vancomycin MIC is $<$ 2 mg/mL, while alternative treatments should be considered if a clinical or microbiologic response to vancomycin fails. In the present study, we found that 69% of clinical MRSA isolates had vancomycin creep; a high dose of vancomycin (60 mg/kg/d) is recommended for patients infected MRSA with vancomycin MIC creep.[8] All of the MRSA strains were susceptible to daptomycin; thus daptomycin should be considered when vancomycin treatment fails or a poor response occurs or when a reasonable trough level could not be achieved.

In the present study we found that among the CA-MRSA strains, the clinical isolates contained 59.8% SCCmec type VT and 23% SCCmec type IV; the colonizing isolates included 85.8% SCCmec type IV and 14.2% SCCmec type VT. The phenomenon of vancomycin MIC creep actually exists in the colonized strains, which means that healthy children with colonized MRSA isolates have higher possibility to develop invasive disease caused by MRSA. Furthermore, the percentage of MRSA isolates with vancomycin MIC $\geq$ 1.5 μg/mL was always $>$ 50% during the study period of 2008—2012; the fluctuated curve revealed no declining or rising trend of vancomycin MIC creep but the creeping phenomenon really subsisted. Nevertheless, there were dissimilar molecular characteristics among the colonizing MRSA isolates and clinical strains. The results were similar to previous reports from Taiwan.[2,11,16] The risk of these strains spreading and leading to infection requires further evaluation.

Rodríguez et al[23] found that colonizing MRSA with similar molecular characteristics of those causing infection

revealed the dissemination capacity of *S. aureus* and the risk of infection among the child population. The participants of the study were children from a university hospital and day care centers. Javidnia et al[24] confirmed the presence of at least one clonal group of MRSA strains in each of the three hospitals investigated; through exact typing, the strains were shown to be found in the surroundings of the same hospital and had highly similar antibiotic resistance patterns to those isolated from patients. Colonizing MRSA with similar molecular characteristics to infectious strains displays the importance for public health of monitoring these populations owing to the risks of the strains disseminating and causing infection.

There was a large variety in PFGE types of MRSA with vancomycin MIC ≥ 1.5 μg/mL. No evidence of clonal dissemination of MRSA strains in this tertiary hospital was found. Great diversity of PFGE types existed in both clinical and colonizing MRSA isolates; there was no evidence or association between the clinical and colonizing MRSA isolates with vancomycin MIC creep. This phenomenon is meaningful for infection control both in the community and in the hospital.

The study has several limitations. First, this is a retrospective study for the pediatric population at a single medical center, perhaps providing a selection bias and the number of isolates is limited locally in Northern Taiwan. Second, MICs were only analyzed over a 5-year period; longer periods are needed to an expansive investigation of MIC change. Third, we obtained vancomycin MICs using the Etest method rather than the broth microdilution method; the two systems have been reported to yield MIC values differing by up to two-fold.[25]

In conclusion, we detected the existence of vancomycin MIC creep in our hospital and the community during the study period. The increase in vancomycin MICs has been associated with worse outcomes in children with invasive MRSA disease. Furthermore, using molecular analysis by PFGE and SCC*mec* typing MRSA in two different pediatric populations from the hospital and the community, we found great diversity in both strain populations. There was no association between the clinical and colonizing MRSA isolates with vancomycin MIC creep, which means that there was no mutual relationship between the MRSA isolates obtained from the hospital and the community. The dissimilarity in epidemiological characteristics between the two populations provides the basis for the design of control and prevention strategies for colonizing and infectious MRSA isolates.[23]

Conflicts of interest

All authors: No potential conflicts of interest.

Acknowledgments

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