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ORIGINAL ARTICLE

The association of molecular typing, vancomycin MIC, and clinical outcome for patients with methicillin-resistant *Staphylococcus aureus* infections

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Abstract *Background/Purpose:* There are reports of an increase in vancomycin minimum inhibitory concentration (MIC) against methicillin-resistant *Staphylococcus aureus* (MRSA) over time, a phenomenon referred to as "MIC creep", but some studies have conflicting results. The aim of this study is to evaluate the association of molecular typing, vancomycin MIC, and clinical outcome for patients with MRSA infections.
Methods: Thirty-two MRSA isolates from Taichung Veterans General Hospital (TCVGH), Taichung, Taiwan during the period of 2003 to 2008 were analyzed for the association of sequence typing, vancomycin MIC, and the correlated clinical outcome for patients with MRSA infections. The vancomycin MICs of 28 additional isolates from 2014 were used for the detection of MIC creep.
Results: Among the genotypes of 32 isolates, there were 17 (53.1%) isolates with ST239-SCC*mec*III, seven (21.9%) isolates with ST5-SCC*mec*II, six (18.8%) isolates with ST59-SCC*mec*IV, and two (6.2%) isolates with ST59-SCC*mec*V_T. Two isolates had an MIC of 2 μg/mL and were identified as ST239-SCC*mec*III. No statistically significant change in the distribution of MICs of all

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isolates was observed between 2003 and 2014 ($p = 0.263$). There was no significant difference in the mortality rates between two groups of patients with vancomycin MICs < 2 μg/mL and ≥ 2 μg/mL ($p = > 0.99$).
Conclusion: There was no vancomycin MIC creep in the period from 2003 to 2014 in this study. Appropriate prognostic models for assessment of the association among sequence types, vancomycin MICs, and clinical outcome warrant further investigation.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is highly prevalent in hospitals worldwide, with the highest rates (> 50%) reported in North America, South America, and Asia.[1] The rate of MRSA is about 60% in Taiwan, according to the data of the Taiwan Surveillance of Antimicrobial Resistance.[2] Clonal complex 5 (CC5) and CC8 are the most prevalent health care-associated MRSA (HA-MRSA) CCs worldwide.[1,3–5] CC8-ST239-SCC*mec*III (63.0%), CC8-ST241-SCC*mec*III (7.6%), CC5-ST59-SCC*mec*IV/V_T (17.2%), and CC5-ST5-SCC*mec*II (5.7%), are the most common sequence types in Taiwan.[6–8]

The mortality rate associated with invasive MRSA infections is estimated at 20%.[9] The risk factors of mortality of *S. aureus* bacteremia include age and comorbidities. The presence of shock and the source of *S. aureus* bacteremia are strong predictors of outcome.[10] Progressive increases in vancomycin minimum inhibitory concentrations (MICs) within a susceptible range (MIC creep) have been reported in several studies.[11,12] Treatment success is higher for MRSA bacteremia with vancomycin MIC ≤ 0.5 μg/mL (55.6%) than those with vancomycin MICs of 1–2 μg/mL (9.5%).[13] Some studies have shown that the increase in vancomycin MIC is associated with a substantial risk of vancomycin treatment failure for MRSA infections and a higher mortality rate,[14–16] but others have suggested no significant association between higher MICs and poor outcomes.[17,18]

To assess the above predictors of the clinical outcome of patients with MRSA infections, molecular typing, trend of vancomycin MIC, and the available clinical data were analyzed.

Methods

Bacterial strains

Thirty-two non-duplicate MRSA isolates were collected randomly from Taichung Veterans General Hospital (TCVGH), Taichung, Taiwan during the period from October 2003 to December 2008. About six isolates each year were randomly selected for the study. The isolates were collected from multiple sources, including blood (37.4%), bone (31.2%), sputum (12.5%), and skin and soft tissue (6.2%), etc. Furthermore, 28 isolates which were randomly collected from TCVGH during 2014 were used to detect vancomycin MIC creep.

Susceptibility test

These 32 strains were tested for susceptibility using the Phoenix automated microbiology system (BD Diagnostic Systems, Sparks, MD, USA). The panel of the Phoenix automated microbiology system contained nitrofurantoin, oxacillin, penicillin G, quinupristin-dalfopristin, rifampicin, streptomycin, teicoplanin, tetracycline, trimethoprim-sulfamethoxazole (TMP/SMX), and vancomycin for MIC analysis. The MIC breakpoints were interpreted according to the guidelines established by the Clinical and Laboratory Standards Institute (CLSI) 2015.[19]

Polymerase chain reaction techniques

Multiplex polymerase chain reaction (PCR) analysis was performed as described previously[20] to distinguish the four genetic elements for SCC*mec*. SCC*mec* typing for type V_T was determined by using a particular primer described previously.[21] The presence of Panton-Valentine leukocidin (PVL) genes was determined by a PCR strategy described previously.[22]

Multi-locus sequence typing

Multi-locus sequence typing (MLST) is well established as a valuable method for genotyping bacteria based on the sequence variation of housekeeping genes.[23] MLST techniques applied to diverse species of bacteria generally use at least seven loci. The DNA sequences of seven housekeeping genes were performed as described previously: carbamate kinase (*arcC*), shikimate dehydrogenase (*aroE*), glycerol kinase (*glp*), guanylate kinase (*gmk*), phosphate acetyltransferase (*pta*), triosephosphate isomerase (*tpi*), and acetyl coenzyme A acetyltransferase (*yqiL*). The sequence types (ST) were determined by comparing the sequences of each gene to those of the known alleles deposited in the *S. aureus* MLST database (http://saureus.mlst.net/).

Clinical and bacteriological assessments

The clinical characteristics and outcome were reviewed according to medical records. Community-associated MRSA (CA-MRSA) was defined as any MRSA infection diagnosed in an outpatient or within 48 hours of admission to hospital, of which the patient has none of the following risk factors for

HA-MRSA: hemodialysis, surgery, residence in a long-term care facility or treatment in hospital, presence of a permanent catheter or percutaneous device at the time of culture, or previous isolation of MRSA within the previous 12 months.[24] All other MRSA infections were considered to be HA-MRSA.[25]

Sepsis-induced hypotension is defined as a systolic blood pressure < 90 mmHg or mean arterial pressure < 70 mmHg, or a systolic blood pressure decrease > 40 mmHg. Septic shock is defined as sepsis-induced hypotension persisting despite adequate fluid resuscitation.[26]

Clinical conditions (such as age, sex, underlying diseases, source of infection, length of stay, clinical response, and death) associated with the MRSA infection were reviewed. The efficacy was assessed by the clinical and bacteriological response. The clinical response was evaluated at the end of antimicrobial treatment and defined as cure (disappearance of acute signs and symptoms related to the infection or sufficient improvement such that additional or alternative antimicrobial treatment was not required), failure (insufficient improvement of the signs and symptoms of infection and additional or alternative antimicrobial treatment was required), or indeterminate (a clinical assessment was not possible for any reason). The bacteriological response was evaluated at the 7th day after the discontinuation of antimicrobial treatment and defined as eradication (no more positive cultures yielded), presumed eradication (absence of evaluable culture in a patient with clinical cure), persistence (presence of baseline pathogen in a patient with clinical failure of treatment), presumed persistence (absence of evaluable culture in a patient with clinical failure of treatment), or indeterminate (if bacteriological response was not evaluable for any reason). Bacteriological success was defined if eradication or presumed eradication were present. Bacteriological failure was defined as persistence or presumed persistence.[27] The 30 day mortality rates after MRSA infection were analyzed.

Check the sampling bias of clinical data

To evaluate the sampling bias due to the relatively small number of patients, a comparison of the clinical data in this study with the previous report by Pan et al[8] was performed by the χ^2 test.

Statistical analysis

Comparisons of the categorical data of each study group were carried out using the χ^2 test or the Fisher's exact test, while comparisons of continuous data were performed using one-way analysis of variation or the Student t test. Variables are considered as significantly different if a two-tailed $p < 0.05$. Univariate analysis was first conducted to identify the risk factors associated with mortality. Multivariate analysis was then used for the analysis of variables with a $p < 0.05$. Isolates were classified into two groups, i.e., those with vancomycin MIC ≤ 1 μg/mL and those with vancomycin MIC ≥ 2 μg/mL. MIC trends were assessed using nonparametric methods. All analyses were performed using IBM SPSS Statistics for Windows, version 22.0.0.0 (IBM Corp., Armonk, NY, USA).

Results

There were no significant differences in the underlying diseases between this study and the report by Pan et al[8] ($p > 0.05$). These underlying diseases included diabetes mellitus, renal insufficiency, cardiovascular diseases, gastrointestinal diseases, malignancies, neutropenia, respiratory diseases, hepatobiliary diseases, autoimmune diseases, previous hospitalization, and previous antibiotic use (Table 1). There were no significant differences in the percentages of sources of infection, except for the percentage of osteomyelitis being higher in this study than those reported by Pan et al[8] (31.2% vs. 0%, $p < 0.001$). There were no significant differences in the 30 day all-cause mortality and MRSA related 30 day mortality (6.2% vs. 12.7%, $p = 0.382$; 6.2% vs. 4.2%, $p = 0.64$, respectively).

Among the genotypes of 32 isolates, there were 17 (53.1%) isolates with ST239-SCC*mec*III, seven (21.9%) isolates with ST5-SCC*mec*II, six (18.8%) isolates with ST59-SCC*mec*IV, and two (6.2%) isolates with ST59-SCC*mec*V$_T$ (Table 2). The isolates of ST5-SCC*mec*II and ST239-SCC*mec*III were all health care-associated. For ST59-SCC*mec*IV, two isolates were health care-associated and four isolates were community-associated. Two isolates of ST59-SCC*mec*V$_T$ were community-associated. The PVL gene was found in one isolate of ST59-SCC*mec*IV and two isolates of ST59-SCC*mec*V$_T$.

Table 1 Clinical characteristics and outcome for patients with methicillin-resistant *Staphylococcus aureus* (MRSA) infections.

	No. of patient (%)
Age (y), mean	56.1 ± 27.1
Sex	
Female	7 (21.9)
Male	25 (78.1)
Underlying diseases	
Diabetes mellitus	6 (18.8)
Renal insufficiency	1 (3.1)
Cardiovascular diseases	7 (21.9)
Gastrointestinal diseases	4 (12.5)
Malignancies	4 (12.5)
Neutropenia	1 (3.1)
Respiratory diseases	3 (9.3)
Hepatobiliary diseases	4 (12.5)
Autoimmune diseases	2 (6.2)
Previous hospitalization	20 (62.5)
Previous antibiotic use	23 (71.9)
Source of infection	
Primary blood stream infection	4 (12.5)
Osteomyelitis	10 (31.2)
Respiratory tract infection	4 (12.5)
Skin and soft tissue infection	10 (31.2)
Surgical site infection	2 (6.2)
Others	2 (6.2)
30 d all-cause mortality	2 (6.2)
MRSA-related 30 d mortality	2 (6.2)

Table 2 Comparison of molecular characteristics and clinical outcomes among different sequence types.

Characteristic	*n* (%)			
	ST5	ST239	ST59	ST59
	(*n* = 7)	(*n* = 17)	(*n* = 6)	(*n* = 2)
SCC*mec*	II	III	IV	V_T
Origin				
Health care-associated	7 (100)	17 (100)	2 (33)	0
Community-acquired	0	0	4 (67)	2 (100)
PVL genes	0	0	1 (17)	2 (100)
Susceptibility				
Ciprofloxacin	0	0	6 (100)	2 (100)
Clindamycin	0	2 (12)	1 (17)	0
Rifampicin	0	15 (88)	5 (83)	2 (100)
TMP/SMX	7 (100)	1 (5.8)	6 (100)	2 (100)
Vancomycin	7 (100)	17 (100)	6 (100)	2 (100)
30 d mortality	0	2 (12)	0	0
Diagnosis				
Primary BSI	0	4 (24)	0	0
Osteomyelitis	3 (43)	4 (24)	3 (50)	0
Pneumonia	2 (29)	2 (12)	0	0
SSTI	1 (14)	5 (29)	3 (50)	1 (50)
Others	1 (14)	2 (12)	0	1 (50)

BSI = blood stream infection; PVL = Panton-Valentine leukocidin; SCC*mec* = staphylococcal chromosomal cassette *mec*; SSTI = skin and soft-tissue infection; TMP/SMX = trimethoprim/sulfamethoxazole.

Ciprofloxacin was active against all the isolates of ST59-SCC*mec*IV and ST59-SCC*mec*V_T (Table 2). Rifampicin was active against 88% of 17 isolates with ST239-SCC*mec*III and 83% of six isolates with ST59-SCC*mec*IV. TMP/SMX was active against all the isolates of ST5-SCC*mec*II and ST59, but only active against 5.8% of 17 isolates with ST239-SCC*mec*III. Vancomycin was active against all the 32 isolates. Among the 17 patients with ST239 MRSA infections, skin and soft tissue infection (29%), bacteremia (24%), and osteomyelitis (24%) were the most common diseases.

Among the 32 isolates, 11 (34%) isolates had an MIC of 0.5 μg/mL, 19 (60%) isolates had an MIC of 1 μg/mL, and two (6%) isolates had an MIC of 2 μg/mL (Table 3). Only two (6%) of 32 isolates had an MIC of 2 μg/mL, and were identified as ST239-SCC*mec*III. The 32 isolates were classified into two groups by two time periods, i.e., 2003–2005 and 2006–2008 (Table 3). The geometric means of MICs of isolates in the periods of 2003–2008 and 2014 were 0.82 μg/mL and 0.90 μg/mL, respectively. There was no significant difference in the percentages of isolates with a vancomycin MIC $\geq$ 2 μg/mL between the periods of 2003–2008 and 2014 ($p > 0.99$). No statistically significant change in the distribution of MICs of all isolates was observed during the period from 2003 to 2014 ($p = 0.263$). There was also no significant difference in the percentages of ST239 isolates with a vancomycin MIC $\geq$ 2 μg/mL between the periods of 2003–2005 and 2006–2008 ($p = 0.471$). Therefore, there was no vancomycin MIC creep from the periods of 2003 to 2014 in this study.

Treatment with vancomycin achieved clinical cure and bacteriological eradication in 30 (94%) of 32 patients. Central line catheter was a risk factor for 30 day mortality, according to univariate analysis (Table 4).

Discussion

The 30 day all-cause and MRSA-related mortality rate was 6.2% in this study. The mortality rate associated with invasive MRSA infection varies from 10% to 34%[28–30] in previous studies in different settings. Although two patients expired with the conditions of septic shock, mechanic ventilation, and stay in ICU, the number of deaths was too small to demonstrate these three risk factors as statistically significant. Septic shock was found to be the only independent factor associated with MRSA bacteremia in a study.[31] Score systems for severity of illness and organ dysfunction have been validated and applied as useful tools to predict the risk factors of death in patients admitted to the intensive care unit (ICU), such as the acute physiology and chronic health evaluation (APACHE),[32,33] and sepsis-related organ failure assessment (SOFA).[34] However, the previous literature did not provide enough information to assess the accuracy of the prognostic models in patients with suspected infection in the emergency department and hospital ward.[35] In a recent study, sepsis severity score was evaluated by assessing the Surviving Sepsis Campaign database from 2005 to 2009, and the mortality rates were higher in patients with mechanical ventilation, hypotension, and stay in ICU.[36] The significance of these three risk factors could be evaluated by further studies.

Ciprofloxacin was active against CA-MRSA (ST59-SCC*mec*IV and ST59-SCC*mec*V_T), and TMP/SMX was also active against ST59-SCC*mec*IV and ST59-SCC*mec*V_T, and health care-associated isolates with ST5-SCC*mec*II, in this study. Vancomycin was active against all of the isolates. These susceptibility data are similar to those of other studies in Taiwan.[7,37] In these studies, ciprofloxacin was active against isolates of ST59 (> 85%). TMP/SMX was active against isolates with ST5-SCC*mec*II (> 85%), ST59-SCC*mec*IV (> 95%), and ST59-SCC*mec*V_T (> 95%).[7,37]

Only two isolates with the genotype of ST239-SCC*mec*III were revealed to have a high vancomycin MIC of 2 μg/mL in this study. The high vancomycin MIC of 2 μg/mL in MRSA isolates is attributable to the spread of the predominant ST239 strain.[38,39] The susceptibility tests for 470 MRSA isolates were performed by agar dilution in a hospital in northern Taiwan.[40] Among the 250 MRSA isolates of ST239, there were 29% of isolates with a vancomycin MIC of 2 μg/mL, 70% of isolates with a vancomycin MIC of 1 μg/mL, and 1% of isolates with a vancomycin MIC of 0.5 μg/mL (Table 3).[40] MRSA of ST239 is multiply antibiotic-resistant and accounts for the most predominant strain of HA-MRSA throughout Asia, South America, and Eastern Europe.[41–47] Seven (22%) isolates were identified as ST5-SCC*mec*II in this study, although a high vancomycin MIC $\geq$ 2 μg/mL against this clone was not detected. However, six (8%) of 74 isolates with ST5 have a vancomycin MIC of 2 μg/mL in that Taiwanese hospital.[40] ST5 was first identified as a nosocomial clone in 2000 in another Taiwanese hospital and became the major clone colonizing adult patients in ICUs in

Table 3 Trends of vancomycin minimum inhibitory concentrations (MICs) among different sequence types of methicillin-resistant *Staphylococcus aureus* (MRSA).

	No.	Range of vancomycin MICs, *n* (%)				*p*	
		Phoenix automated microbiology system					
		0.5 μg/mL	1 μg/mL	2 μg/mL	GM (μg/mL)		
2003–2005							
ST5	6	3 (50)	3 (50)	0	0.70		
ST59	3	0	3 (100)	0	1		
ST239	9	1 (11)	6 (67)	2 (22)	1.08		
2006–2008							
ST5	1	1 (100)	0	0	0.5		
ST59	5	4 (80)	1 (20)	0	0.57		
ST239	8	2 (25)	6 (75)	0	0.84	0.471[a]	
Total (2003–2008)	32	11 (34)	19 (60)	2 (6)	0.82		
2014	28	5 (18)	22 (79)	1 (3)	0.90	> 0.99[b]	0.263[c]
Kao et al[40]		Agar dilution method					
2006–2008		0.5 μg/mL	1 μg/mL	2 μg/mL			
ST5	74	3 (4.1)	65 (87.8)	6 (8.1)			
ST59	109	19 (17.4)	90 (82.6)	0			
ST239	250	2 (0.8)	175 (70)	73 (29.2)			
Yeh et al[31]		E test					
		< 1.5 μg/mL	≥ 1.5 μg/mL	GM (μg/mL)			
2001	45	26 (57.8)	19 (42.2)	1.19			
2005	46	38 (82.6)	8 (17.4)	0.99			
2009	49	14 (28.6)	35 (71.4)	1.39			

GM = geometric mean.
[a] χ^2 test, indicates the probability of significant difference in the percentages of ST239 isolates with a vancomycin MIC ≥ 2 μg/mL between the periods of 2003–2005 and 2006–2008.
[b] χ^2 test, indicates the probability of significant difference in the percentages of isolates with a vancomycin MIC ≥ 2 μg/mL between the periods of 2003–2008 and 2014.
[c] Mann–Whitney *U* test, indicates the probability of significant change in the distribution of MICs of all isolates between the periods of 2003–2014 ($p = 0.263$).

2008.[48–50] Continuous monitoring of the prevalence of sequence types and antimicrobial susceptibility of MRSA is needed to evaluate the impact of development of MRSA clones with high vancomycin MIC on the clinical outcome.

The phenomenon, vancomycin MIC creep, was not present and MRSA with a high vancomycin MIC of 2 μg/mL did not result in a higher mortality rate in this study. Vancomycin MIC creep has been reported in some studies.[11,12,51] An increase of vancomycin MIC in a northern Taiwanese hospital was also reported.[31] According to the report, 42% of 45 MRSA isolates had a vancomycin MIC ≥ 1.5 μg/mL in 2001, and 71.4% of 49 MRSA isolates had a vancomycin MIC ≥ 1.5 μg/mL in 2009 (Table 3). However, there was no significant difference in the in-hospital mortality rates between patients with MRSA isolates with MICs < 1.5 μg/mL or ≥ 1.5 μg/mL.[31] By contrast, there were several discordant reports for vancomycin MIC creep.[52,53] Reports from large multicenter surveillance studies have not reported changes in vancomycin susceptibilities over time[54,55] Combining data from multiple centers can obscure the MIC trends that may exist within a given institution or a geographic region as a result of differences in patient populations and antimicrobial usage patterns.[12,53] The use of less sensitive traditional susceptibility parameters (e.g., percentages, MIC50, and MIC90) in their analyses will limit the ability to detect MIC creep and their magnitude in MIC populations.[12,52] Vancomycin MICs produced by Etest have a tendency to be 0.5–1.5 $\log_2$ dilutions higher than those by

Table 4 Factors independently associated with mortality for patients with methicillin-resistant *Staphylococcus aureus* (MRSA) infection.

Factor	OR (95% CI)	*p*
Diabetes mellitus	5.0 (0.266–93.958)	0.345
Gastrointestinal diseases	9.0 (0.440–183.973)	0.238
Malignancies	9.0 (0.440–183.973)	0.238
Central line catheter	1.667 (0.815–3.409)	0.020
Shock	1.333 (0.894–1.989)	0.056
Mechanical ventilation	1.286 (0.907–1.823)	0.073
ICU stay	1.25 (0.917–1.704)	0.091

CI = confidence interval; ICU = intensive care unit; OR = odds ratio.

the broth dilution method.[56–58] Further evidence is warranted to determine if such small differences in the vancomycin MIC are indeed significant, and therapeutic recommendations should specify the MIC method on which they are based.[57]

There were some limitations in this study. First, the number of isolates is small. However, there were some major MRSA clones spreading in Taiwan, and each clone has its microbiological characteristics. It is expected that these isolates could have the microbiological characteristics of the major clones, although this is not a large scale study. Second, the number of deaths was not large enough to demonstrate statistical significance in the risk factors of mortality, although the odds ratios were high. Third, the MICs determined by the Phoenix automated microbiology system could have a tendency to be lower than those performed by the Etest. The clinical significance of these small differences in the vancomycin MIC should be clarified by further studies.

In conclusion, there was no vancomycin MIC creep from the periods of 2003 to 2014 in this study. There was no significant difference in the mortality rates between two groups of patients with vancomycin MICs < 2 μg/mL and ≥ 2 μg/mL ($p > 0.99$). Appropriate prognostic models for assessment of the association among sequence types, vancomycin MICs, and clinical outcome warrant further investigation.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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References

1. Stefani S, Chung DR, Lindsay JA, Friedrich AW, Kearns AM, Westh H, et al. Meticillin-resistant *Staphylococcus aureus* (MRSA): global epidemiology and harmonisation of typing methods. *Int J Antimicrob Agents* 2012;**39**:273–82.
2. McDonald LC, Lauderdale TL, Shiau YR, Chen PC, Lai JF, Wang HY, et al. The status of antimicrobial resistance in Taiwan among Gram-positive pathogens: the Taiwan Surveillance of Antimicrobial Resistance (TSAR) programme, 2000. *Int J Antimicrob Agents* 2004;**23**:362–70.
3. Feil EJ, Cooper JE, Grundmann H, Robinson DA, Enright MC, Berendt T, et al. How clonal is *Staphylococcus aureus*? *J Bacteriol* 2003;**185**:3307–16.
4. Campanile F, Bongiorno D, Borbone S, Stefani S. Methicillin-resistant *Staphylococcus aureus* evolution: the multiple facets of an old pathogen. *Eur Infect Dis* 2010;**4**:70–6.
5. Chambers HF, Deleo FR. Waves of resistance: *Staphylococcus aureus* in the antibiotic era. *Nat Rev Microbiol* 2009;**7**:629–41.
6. Wang WY, Chiueh TS, Sun JR, Tsao SM, Lu JJ. Molecular typing and phenotype characterization of methicillin-resistant *Staphylococcus aureus* isolates from blood in Taiwan. *PloS One* 2012;**7**:e30394.
7. Huang YC, Chen CJ. Community-associated meticillin-resistant *Staphylococcus aureus* in children in Taiwan. *Int J Antimicrob Agents* 2000s;**2011**(38):2–8.
8. Pan SC, Wang JT, Lauderdale TL, Ko WC, Chen YS, Liu JW, et al. Epidemiology and staphylococcal cassette chromosome mec typing of methicillin-resistant *Staphylococcus aureus* isolates in Taiwan: A multicenter study. *J Formos Med Assoc* 2014;**113**:409–16.
9. Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, Ray S, et al. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. *JAMA* 2007;**298**:1763–71.
10. van Hal SJ, Jensen SO, Vaska VL, Espedido BA, Paterson DL, Gosbell IB. Predictors of mortality in *Staphylococcus aureus* bacteremia. *Clin Microbiol Rev* 2012;**25**:362–86.
11. Robert J, Bismuth R, Jarlier V. Decreased susceptibility to glycopeptides in methicillin-resistant *Staphylococcus aureus*: a 20 year study in a large French teaching hospital, 1983-2002. *J Antimicrob Chemother* 2006;**57**:506–10.
12. Steinkraus G, White R, Friedrich L. Vancomycin MIC creep in non-vancomycin-intermediate *Staphylococcus aureus* (VISA), vancomycin-susceptible clinical methicillin-resistant *S. aureus* (MRSA) blood isolates from 2001-05. *J Antimicrob Chemother* 2007;**60**:788–94.
13. Sakoulas G, Moise-Broder PA, Schentag J, Forrest A, Moellering Jr RC, Eliopoulos GM. Relationship of MIC and bactericidal activity to efficacy of vancomycin for treatment of methicillin-resistant *Staphylococcus aureus* bacteremia. *J Clin Microbiol* 2004;**42**:2398–402.
14. Soriano A, Marco F, Martinez JA, Pisos E, Almela M, Dimova VP, et al. Influence of vancomycin minimum inhibitory concentration on the treatment of methicillin-resistant *Staphylococcus aureus* bacteremia. *Clin Infect Dis* 2008;**46**:193–200.
15. Howden BP, Ward PB, Charles PG, Korman TM, Fuller A, du Cros P, et al. Treatment outcomes for serious infections caused by methicillin-resistant *Staphylococcus aureus* with reduced vancomycin susceptibility. *Clinical Infect Dis* 2004;**38**:521–8.
16. Wang JL, Wang JT, Sheng WH, Chen YC, Chang SC. Nosocomial methicillin-resistant *Staphylococcus aureus* (MRSA) bacteremia in Taiwan: mortality analyses and the impact of vancomycin, MIC = 2 mg/L, by the broth microdilution method. *BMC Infect Dis* 2010;**10**:159.
17. Liao CH, Chen SY, Huang YT, Hsueh PR. Outcome of patients with meticillin-resistant *Staphylococcus aureus* bacteraemia at an emergency department of a medical centre in Taiwan. *Int J Antimicrob Agents* 2008;**32**:326–32.
18. Crompton JA, North DS, Yoon M, Steenbergen JN, Lamp KC, Forrest GN. Outcomes with daptomycin in the treatment of *Staphylococcus aureus* infections with a range of vancomycin MICs. *J Antimicrob Chemother* 2010;**65**:1784–91.
19. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement. CLSI document M100–S25*. Wayne, PA: Clinical and Laboratory Standards Institute; 2015.
20. Oliveira DC, de Lencastre H. Multiplex PCR strategy for rapid identification of structural types and variants of the mec element in methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2002;**46**:2155–61.
21. Boyle-Vavra S, Ereshefsky B, Wang CC, Daum RS. Successful multiresistant community-associated methicillin-resistant *Staphylococcus aureus* lineage from Taipei, Taiwan, that carries either the novel *Staphylococcal* chromosome cassette mec (SCCmec) type VT or SCCmec type IV. *J Clin Microbiol* 2005;**43**:4719–30.
22. Lina G, Piemont Y, Godail-Gamot F, Bes M, Peter MO, Gauduchon V, et al. Involvement of Panton-Valentine leukocidin-

producing *Staphylococcus aureus* in primary skin infections and pneumonia. *Clinical Infect Dis* 1999;**29**:1128—32.
23. Spratt BG. Multilocus sequence typing: molecular typing of bacterial pathogens in an era of rapid DNA sequencing and the internet. *Curr Opin Microbiol* 1999;**2**:312—6.
24. Lin JC, Yeh KM, Peng MY, Chang FY. Community-acquired methicillin-resistant Staphylococcus aureus bacteremia in Taiwan: risk factors for acquisition, clinical features and outcome. *J Microbiol Immunol Infect* 2004;**37**:24—8.
25. Morrison MA, Hageman JC, Klevens RM. Case definition for community-associated methicillin-resistant *Staphylococcus aureus*. *J Hosp Infect* 2006;**62**:241.
26. Dellinger RP, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, et al. Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2012. *Crit Care Med* 2013;**41**:580—637.
27. Chuang CH, Liu MF, Lin CF, Shi ZY. Impact of revised susceptibility breakpoints on bacteremia of *Klebsiella pneumoniae*: Minimum inhibitory concentration of cefazolin and clinical outcomes. *J Microbiol Immunol Infect* 2016;**49**(5):679—84.
28. Wang JL, Chen SY, Wang JT, Wu GH, Chiang WC, Hsueh PR, et al. Comparison of both clinical features and mortality risk associated with bacteremia due to community-acquired methicillin-resistant *Staphylococcus aureus* and methicillin-susceptible S. *aureus*. *Clin Infect Dis* 2008;**46**:799—806.
29. Cosgrove SE, Qi Y, Kaye KS, Harbarth S, Karchmer AW, Carmeli Y. The impact of methicillin resistance in *Staphylococcus aureus* bacteremia on patient outcomes: mortality, length of stay, and hospital charges. *Infect Control Hosp Epidemiol* 2005;**26**:166—74.
30. Shurland S, Zhan M, Bradham DD, Roghmann MC. Comparison of mortality risk associated with bacteremia due to methicillin-resistant and methicillin-susceptible *Staphylococcus aureus*. *Infect Control Hosp Epidemiol* 2007;**28**:273—9.
31. Yeh YC, Yeh KM, Lin TY, Chiu SK, Yang YS, Wang YC, et al. Impact of vancomycin MIC creep on patients with methicillin-resistant *Staphylococcus aureus* bacteremia. *J Microbiol Immunol Infect* 2012;**45**:214—20.
32. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. *Crit Care Med* 1985;**13**:818—29.
33. Carneiro AV, Leitão MP, Lopes MG, DP F. Risk stratification and prognosis in critical surgical patients using the Acute Physiology, Age and Chronic Health III System (APACHE III). *Acta Med Port* 1997;**10**:751—60 [Article in Portuguese].
34. Vincent JL, Moreno R, Takala J, Willatts S, De Mendonca A, Bruining H, et al. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. *Intensive Care Med* 1996;**22**:707—10.
35. Calle P, Cerro L, Valencia J, Jaimes F. Usefulness of severity scores in patients with suspected infection in the emergency department: a systematic review. *J Emerg Med* 2012;**42**:379—91.
36. Osborn TM, Phillips G, Lemeshow S, Townsend S, Schorr CA, Levy MM, et al. Sepsis severity score: an internationally derived scoring system from the surviving sepsis campaign database*. *Crit Care Med* 2014;**42**:1969—76.
37. Chen CJ, Huang YC, Su LH, Wu TL, Huang SH, Chien CC, et al. Molecular epidemiology and antimicrobial resistance of methicillin-resistant *Staphylococcus aureus* bloodstream isolates in Taiwan, 2010. *PloS one* 2014;**9**:e101184.
38. Horne KC, Howden BP, Grabsch EA, Graham M, Ward PB, Xie S, et al. Prospective comparison of the clinical impacts of heterogeneous vancomycin-intermediate methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-susceptible MRSA. *Antimicrob Agents Chemother* 2009;**53**:3447—52.
39. Holmes NE, Turnidge JD, Munckhof WJ, Robinson JO, Korman TM, O'Sullivan MV, et al. Genetic and molecular predictors of high vancomycin MIC in *Staphylococcus aureus* bacteremia isolates. *J Clin Microbiol* 2014;**52**:3384—93.
40. Kao TM, Wang JT, Weng CM, Chen YC, Chang SC. In vitro activity of linezolid, tigecycline, and daptomycin on methicillin-resistant *Staphylococcus aureus* blood isolates from adult patients, 2006-2008: stratified analysis by vancomycin MIC. *J Microbiol Immunol Infect* 2011;**44**:346—51.
41. Xu BL, Zhang G, Ye HF, Feil EJ, Chen GR, Zhou XM, et al. Predominance of the Hungarian clone (ST 239-III) among hospital-acquired meticillin-resistant *Staphylococcus aureus* isolates recovered throughout mainland China. *J Hosp Infect* 2009;**71**:245—55.
42. Alp E, Klaassen CH, Doganay M, Altoparlak U, Aydin K, Engin A, et al. MRSA genotypes in Turkey: persistence over 10 years of a single clone of ST239. *J Infect* 2009;**58**:433—8.
43. Oliveira DC, Tomasz A, de Lencastre H. The evolution of pandemic clones of methicillin-resistant *Staphylococcus aureus*: identification of two ancestral genetic backgrounds and the associated mec elements. *Microb Drug Resist* 2001;**7**:349—61.
44. Vivoni AM, Diep BA, de Gouveia Magalhaes AC, Santos KR, Riley LW, Sensabaugh GF, et al. Clonal composition of *Staphylococcus aureus* isolates at a Brazilian university hospital: identification of international circulating lineages. *J Clin Microbiol* 2006;**44**:1686—91.
45. Bartels MD, Nanuashvili A, Boye K, Rohde SM, Jashiashvili N, Faria NA, et al. Methicillin-resistant *Staphylococcus aureus* in hospitals in Tbilisi, the Republic of Georgia, are variants of the Brazilian clone. *Eur J Clin Microbiol Infect Dis* 2008;**27**:757—60.
46. Melter O, Aires de Sousa M, Urbaskova P, Jakubu V, Zemlickova H, de Lencastre H. Update on the major clonal types of methicillin-resistant *Staphylococcus aureus* in the Czech Republic. *J Clin Microbiol* 2003;**41**:4998—5005.
47. Uhlemann AC, Otto M, Lowy FD, DeLeo FR. Evolution of community- and healthcare-associated methicillin-resistant *Staphylococcus aureus*. *Infect Genet Evol* 2014;**21**:563—74.
48. Chen CB, Chang HC, Huang YC. Nasal meticillin-resistant *Staphylococcus aureus* carriage among intensive care unit hospitalised adult patients in a Taiwanese medical centre: one time-point prevalence, molecular characteristics and risk factors for carriage. *J Hosp Infect* 2010;**74**:238—44.
49. Chen CJ, Hsueh PR, Su LH, Chiu CH, Lin TY, Huang YC. Change in the molecular epidemiology of methicillin-resistant *Staphylococcus aureus* bloodstream infections in Taiwan. *Diagn Microbiol Infect Dis* 2009;**65**:199—201.
50. Huang YC, Su LH, Wu TL, Lin TY. Changing molecular epidemiology of methicillin-resistant *Staphylococcus aureus* bloodstream isolates from a teaching hospital in Northern Taiwan. *J Clin Microbiol* 2006;**44**:2268—70.
51. Wang G, Hindler JF, Ward KW, Bruckner DA. Increased vancomycin MICs for Staphylococcus aureus clinical isolates from a university hospital during a 5-year period. *J Clin Microbiol* 2006;**44**:3883—6.
52. Alos JI, Garcia-Canas A, Garcia-Hierro P, Rodriguez-Salvanes F. Vancomycin MICs did not creep in *Staphylococcus aureus* isolates from 2002 to 2006 in a setting with low vancomycin usage. *J Antimicrob Chemother* 2008;**62**:773—5.
53. Kehrmann J, Kaase M, Szabados F, Gatermann SG, Buer J, Rath PM, et al. Vancomycin MIC creep in MRSA blood culture isolates from Germany: a regional problem? *Eur J Clin Microbiol Infect Dis* 2011;**30**:677—83.

54. Joana S, Pedro P, Elsa G, Filomena M. Is vancomycin MIC creep a worldwide phenomenon? Assessment of *S. aureus* vancomycin MIC in a tertiary university hospital. *BMC Res Notes* 2013;**6**:65.
55. Jones RN. Microbiological features of vancomycin in the 21st century: minimum inhibitory concentration creep, bactericidal/static activity, and applied breakpoints to predict clinical outcomes or detect resistant strains. *Clin Infect Dis* 2006; **42**(Suppl. 1):S13—24.
56. Sader HS, Rhomberg PR, Jones RN. Nine-hospital study comparing broth microdilution and Etest method results for vancomycin and daptomycin against methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2009; **53**:3162—5.
57. Prakash V, Lewis 2nd JS, Jorgensen JH. Vancomycin MICs for methicillin-resistant Staphylococcus aureus isolates differ based upon the susceptibility test method used. *Antimicrob Agents Chemother* 2008;**52**:4528.
58. Leonard SN, Rossi KL, Newton KL, Rybak MJ. Evaluation of the Etest GRD for the detection of *Staphylococcus aureus* with reduced susceptibility to glycopeptides. *J Antimicrob Chemother* 2009;**63**:489—92.