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Review Article

Methicillin-resistant *Staphylococcus aureus* with reduced vancomycin susceptibility in Taiwan

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Abstract

Staphylococcus aureus is a versatile pathogen which can cause various mild to life-threatening infectious diseases. The evolution of *S. aureus* resistance is notorious, from penicillin and oxacillin to vancomycin. Vancomycin, introduced in 1956, was once considered a most reliable antibiotic for methicillin-resistant *S. aureus* (MRSA); unfortunately, the first strain of *S. aureus* with decreased susceptibility to vancomycin emerged in 1996. Vancomycin has been approved in Taiwan since 1983, and the prevalence rates of heteroresistant vancomycin-intermediate *S. aureus* (hVISA) and vancomycin-intermediate *S. aureus* (VISA) in 2003 were 0.7% and 0.2%, respectively. However, a ten-fold increase of hVISA and VISA to 10% and 2.7%, respectively, in 2012–2013 could indicate a challenging clinical situation in Taiwan. The most commonly reported staphylococcal cassette chromosome *mec* (SCC*mec*) types of hVISA and VISA are usually SCC*mec* type III or II, typical nosocomial MRSA strains. Preventing the spread of resistant pathogens through infection control interventions and judicious antibiotic stewardship is a serious medical issue.

Keywords: *Heteroresistant vancomycin-intermediate Staphylococcus aureus, Methicillin-resistant Staphylococcus aureus, Vancomycin, Vancomycin-intermediate Staphylococcus aureus, Vancomycin-resistant Staphylococcus aureus*

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Introduction

Staphylococcus aureus is a versatile facultative anaerobic, non-spore-forming, high environmental-resistant, Gram-positive coccus, with the ability to grow on mannitol salt agar which inhibits many organisms because of its high 7.5% salt concentration. The differentiation between *S. aureus* and other staphylococci species depends on positive coagulase and clumping factors [1]. Before the 1880s, Ogston described clinical diseases such as sepsis and abscesses resulting from *S. aureus*. Nowadays, *S. aureus* remains one of the most important clinical pathogens. It causes many diseases, including superficial skin and soft-tissue infections, food poisoning, and various invasive diseases, such as endocarditis, osteomyelitis, pneumonia, meningitis, and even septic shock and death [1]. According to the Taiwan Nosocomial Infections Surveillance System, *S. aureus* was always one of the top ten causative pathogens of nosocomial infections in intensive care units in medical centers between 2006 and 2015. The percentage of methicillin-resistant *Staphylococcus aureus* (MRSA) among all *S. aureus* strains ranges from 66.9% to 84.5% [2]. In addition to being one of the most important clinical pathogens, the resistance or decreased susceptibility to various antimicrobial agents (from penicillin to methicillin and even vancomycin or antiseptics) of *S. aureus* presents troublesome clinical problems [3,4]. Due to increasing antibiotic resistance, it is essential to prevent the spread of these resistant pathogens or genetic determinants using infection control interventions and antimicrobial stewardship.

Development of antimicrobial resistance in *Staphylococcus aureus*, from penicillin to methicillin and vancomycin

Penicillin – discovered by Alexander Fleming in 1928 – radically changed the relationship between humans and microorganisms after its mass production and clinical prescription in the 1940s [3]. Thereafter, effective, inexpensive antimicrobial agents with limited side effects were available for various bacterial infectious diseases. However, resistance to various antimicrobial agents in clinical isolates developed

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under evolutionary selected pressure in the current antibiotics era. The development and spread of resistant *S. aureus* strains directly resulted from frequent clinical overprescription of antibiotics [5]. *S. aureus* rapidly developed penicillin resistance after the introduction of this drug into clinical use, with penicillin-resistant *S. aureus* strains emerging within 1–2 years, followed by 25% resistance of *S. aureus* strains in hospitals after 6 years and 25% resistance of *S. aureus* strains in communities after 15–20 years. Currently, <3% of clinical *S. aureus* isolates are susceptible to penicillin [5]. The penicillin resistance came from *blaZ*, a plasmid-carried gene, which has propagated rapidly among bacteria populations [6]. *S. aureus*-carrying *blaZ* is resistant to penicillin, ampicillin, amoxicillin, ticarcillin, and piperacillin, which are all labile to penicillinase. These *blaZ*-carrying penicillin-resistant *S. aureus* strains are still susceptible to penicillinase-stable penicillins such as oxacillin, methicillin, cloxacillin, dicloxacillin, and nafcillin; β-lactam/β-lactamase inhibitor combinations such as amoxicillin-clavulanate, ampicillin-sulbactam, and piperacillin-tazobactam; carbapenems, including doripenem, ertapenem, imipenem, and meropenem; and most cephalosporins [7].

Methicillin was first introduced into clinical usage in 1961, but the first MRSA strain emerged within 1 year. Twenty-five percent of intrahospital *S. aureus* strains were methicillin-resistant 25–30 years after the introduction of methicillin [5]. The resistance mechanisms of MRSA originate from the *mecA* gene in staphylococcal cassette chromosome *mec* (SCC*mec*), a mobile genetic element, which would lead to the production of penicillin-binding protein 2a which cannot bind with most β-lactam antibiotics [8]. That means with the exception of some new anti-MRSA cephalosporins such as ceftaroline and ceftobiprole, MRSA strains are resistant to all other β-lactam antimicrobial agents [7]. Eleven different SCC*mec* types have been reported, and all contain the *mecA* gene, except for SCC*mec* type XI strains which harbor the *mecC* gene, also known as $mecA_{LGA251}$ [9-11]. Before 1996, MRSA was always considered a typical nosocomial pathogen, harboring SCC*mec* type II or type III (around 34–67 kb) [12]. Even today, MRSA strains with SCC*mec* type II or III are still considered typical nosocomial pathogens worldwide, including in Taiwan [13-15]. After 1996, MRSA strains with the smaller SCC*mec* (type IV or V, around 20–27 kb) emerged in the community; these MRSA strains harbor fewer non-β-lactam-resistant genes and might be susceptible to macrolides, tetracyclines, fluoroquinolones, lincosamides, and folate pathway inhibitors [12,16,17]. Now, nosocomial infections can result from these MRSA strains that were once thought to be limited to the community [15,18].

Vancomycin was introduced in 1956 because of emerging penicillinase-producing *S. aureus* [5,19]. There was a temporary reduction of vancomycin in clinical use because of the introduction of methicillin, a penicillinase-stable penicillin, in 1961. As new MRSA strains emerged and the number of β-lactam-allergic patients increased, vancomycin use increased gradually after the 1970s [20]. Unlike the strains which rapidly developed resistance to penicillin and methicillin, the first *S. aureus* with decreased susceptibility to vancomycin was reported in 1996, 40 years after introduction of this drug [21]. This difference might result from different resistance mechanisms between vancomycin and β-lactams. The genetic determinants of β-lactam resistance are usually transmitted through plasmids or as a mobile genetic element and could propagate rapidly between bacteria.

Revision of vancomycin susceptibility interpretation criteria for *Staphylococcus aureus*

With growing prescription of vancomycin, the vancomycin minimal inhibitory concentration (MIC) of clinical *S. aureus* isolates has gradually elevated [22], which might result in more vancomycin treatment failures [23-25]. The Clinical and Laboratory Standards Institute (CLSI) lowered the clinical susceptible MIC breakpoint from ≤4 μg/mL to ≤2 μg/mL to increase clinical applications in 2006 [26]. According to the current CLSI suggestions, *S. aureus* with reduced susceptibility to vancomycin could be categorized into vancomycin-resistant *S. aureus* (VRSA) with a vancomycin MIC ≥16 μg/mL; vancomycin-intermediate *S. aureus* (VISA) with a vancomycin MIC of 4–8 μg/mL; and heteroresistant VISA (hVISA) with a vancomycin MIC of 1–2 μg/mL [Table 1] [27].

Despite lowering these interpretation breakpoints to ensure compatibility with clinical treatment responses, there were still troublesome issues because of the existence of hVISA and the inability of routine clinical antimicrobial susceptibility testing to detect VISA precisely. Since 2009, the vancomycin susceptibility of *S. aureus* cannot be determined by the disk diffusion method because it fails to differentiate vancomycin-susceptible *S. aureus* (VSSA, MIC ≤2 μg/mL) isolates from VISA (MIC 4–8 μg/mL) and VRSA (MIC ≥16 μg/mL) strains [26]. Because time-consuming and labor-intensive standard dilution methods (microdilution, macrodilution, and agar dilution) are not applicable for routine mass clinical use, automated platforms such as the BD Phoenix™ automated testing system, the VITEK® 2 automated instrument, and the MicroScan system are used in clinical laboratories in Taiwan. However, there are still inconsistencies between standard dilution methods and these FDA-approved testing systems [28,29]. Vancomycin screening agar, which contains vancomycin 6 μg/mL and is usually used to detect vancomycin-resistant enterococci (VRE), is one of the methods suggested by the CLSI to detect vancomycin

Table 1: Vancomycin susceptibility tests for *Staphylococcus aureus*

Vancomycin susceptibility classifications	Broth dilution (μg/ml)			Vancomycin BHI agar screen (6 μg/mL)
	CLSI (before 2005)	CLSI (after 2006)	EUCAST	
VSSA	≤4	≤2	≤2	Negative
hVISA	N/A	1-2	1-2	Negative
VISA	8-16	4-8	N/A	Variable
VRSA	≥32	≥16	≥4	Positive

VSSA: Vancomycin-susceptible *Staphylococcus aureus*, hVISA: Heteroresistant vancomycin-intermediate *Staphylococcus aureus*, VISA: Vancomycin-intermediate *Staphylococcus aureus*, VRSA: Vancomycin-resistant *Staphylococcus aureus*, CLSI: Clinical and Laboratory Standards Institute, EUCAST: European Committee on Antimicrobial Susceptibility Testing, BHI: Brain heart infusion, N/A: Not available

resistance in *S. aureus,* but it could miss VISA isolates with a vancomycin MIC of 4–6 μg/mL [30].

Vancomycin resistance mechanisms in *Staphylococcus aureus*

The resistance mechanisms of VRSA originated from *S. aureus* isolates gaining the vancomycin-resistant determinant *vanA* from VRE [31,32]. However, the genetic determinants of VISA and hVISA are still controversial but are usually related to mutations in cell wall building genes [28,33-35]. The only consistent feature of these low-level vancomycin-resistant isolates is cell wall thickening, and there is a positive correlation between cell wall thickness and vancomycin MIC levels [36,37]. This thickening locks many vancomycin molecules in the bacterial cell wall, which results in less vancomycin diffusion from outside into the division septum, and vancomycin tolerance develops [28]. Currently approved methods or automated systems are reliable in the detection of VRSA and VISA among clinical isolates but not for discovery of hVISA, which harbors a few vancomycin MIC >2 μg/mL subpopulations (in $< 1 \times 10^{-6}$/mL concentration), although the MIC of hVISA is still within the susceptible range [26]. This extremely low concentration of resistant subpopulations cannot be detected by regular standard dilution methods because the bacterial amounts or concentrations used in microdilution, macrodilution, and agar dilution are about 5×10^4 colony-forming units (CFU)/well or 5×10^5 CFU/mL, 5×10^5 CFU/mL, and 1×10^4 CFU/spot, respectively [30]. More than half of *S. aureus* isolates with a vancomycin MIC of 2–3 μg/mL and 10%–20% of *S. aureus* with a vancomycin MIC of 1.5 μg/mL are hVISA [26]. The reported epidemiology of hVISA varies significantly, from 0% to more than 30%, because of different time periods, different regions, and different screening methods [28,38]. No approved method has been developed to detect of hVISA, and most reported procedures rely on elevated test bacteria concentrations or prolonged incubation periods [28]. The population analysis profile is considered the most reliable detection method for hVISA, but it is time-consuming and labor-intensive and is not practical in clinical laboratories [39]. In patients receiving vancomycin treatment, higher failure rates, longer inhospital stays, and prolonged bacteremia periods have been reported in those with hVISA infections than those with VSSA [40,41]. Vancomycin was approved in Taiwan in 1983 [42]. In 2003, the prevalence rates of VISA and hVISA in Taiwan were 0.2% and 0.7%, respectively, based on MRSA isolates from ten medical centers [43]. A report from one hospital in southern Taiwan in 2009 showed that there were five cases of hVISA (8.1%) in 62 blood MRSA isolates [44]. Another study in 15 hospitals from 2012 to 2013 revealed that the prevalence rates of VISA and hVISA in Taiwan had increased to 2.7% and 10%, respectively, based on 622 MRSA isolates with a vancomycin MIC ≥1 μg/mL [45]. The most reported SCC*mec* types of hVISA and VISA were usually SCC*mec* type III or II, typical nosocomial MRSA strains [44,45]. The ten-fold rise in the prevalence of VISA and hVISA over 10 years indicates rigorous, inevitable clinical challenges for infection control and treatment.

Treatment and infection control of methicillin-resistant *Staphylococcus aureus* infections

According to the practice guidelines of the Infectious Diseases Society of America and Infectious Diseases Society of Taiwan [46,47], not all MRSA infections should be treated with antibiotics. Incision and drainage with adequate wound care are sufficient for a simple abscess; antimicrobial agents should be considered when extended local infection is suspected. Repeating blood cultures to rule out persistent bacteremia after 2–4 days of antibiotic treatment is suggested for all patients with MRSA bacteremia, and identifying and eradicating the possible infection focus are also warranted. The suggested dosage of vancomycin is 30–60 mg/kg/day in two or three divided doses for patients with normal renal function, and the target trough serum concentration for therapeutic drug monitoring is 15–20 μg/mL [48]. Close monitoring of the vancomycin treatment response is suggested. Alternative therapeutic drugs should be considered, even in VSSA-related infections, if the infection focus is eradicated, and an adequate dose is prescribed, but the clinical response is inadequate [49]. For isolates with a vancomycin MIC >2 μg/mL, antibiotics other than vancomycin, such as linezolid, daptomycin, tigecycline, and fusidate sodium, are suggested. For community-acquired MRSA infections, trimethoprim-sulfamethoxazole, tetracyclines, fluoroquinolones, and clindamycin are alternative treatment choices depending on susceptibility results.

To prevent the spread of MRSA, both standard precautions and contact precautions are suggested, including cohort nursing and frequent cleaning and disinfection of patient care equipment, instruments, devices, and the environment [50,51]. The suggested screening sites for MRSA colonization include the nares, wounds, tracheostomy, sputum, invasive catheter sites, axilla, perineum, groin, and throat. Patients with nasal carriage are prone to *S. aureus*-related infections [52]. Universal decolonization (intranasal mupirocin ointment 2% and 2% chlorhexidine-impregnated cloths) was more effective in decreasing MRSA-related infections than target decolonization in one study [53]. Adequate antimicrobial stewardship is required because inappropriate antibiotic consumption is one of the most important etiologies of emerging resistance [54], and there is potential collateral damage between different antibiotics, such as fluoroquinolones and MRSA, and third-generation cephalosporins and VRE [55,56]. Various molecular typing methods could help to elucidate the epidemiology of MRSA and its evolution [57-59]. Multilocus sequence typing (MLST) is suitable for determining macro-variations or long-term revolution on a large scale, but pulsed-field gel electrophoresis (PFGE) and even whole-genome sequencing are used for investigating micro-variations or short-term revolution on a smaller scale [3,60]. Another common method, *spa* typing, based on the variable number of tandem repeats in the gene of protein A (*spa*), has a discriminatory power between PFGE and MLST [61,62]. In Taiwan, the most common MLST-spa types of SCCmec types II, III, and IV MRSA isolates were ST5-t002 (USA100, New York/Japan clone), ST239-t037 (Brazilian/Hungarian clone), and ST59-t437, respectively. For SCCmec

type V MRSA isolates, the most common MLST-spa types were ST59-t437 and ST45-t1081 [14,15,63-65].

Conclusion

S. aureus is a versatile pathogen which could lead to various diseases and evolution of rapid resistance under the selection pressure of various antibiotics. With the emerging reduced susceptibility of *S. aureus* to vancomycin (hVISA, VISA, and VRSA), vancomycin is no longer the first choice or surefire antimicrobial agent for treatment of infectious diseases caused by MRSA [66]. Both careful antimicrobial agent selection and infection control interventions are all essential in treating patients with MRSA infections, especially for life-threatening cases. If a reduced vancomycin-susceptibility MRSA strain is suspected or there is a poor treatment response to vancomycin, alternative antibiotics should be considered. However, *S. aureus* strains nonsusceptible to daptomycin or resistant to linezolid and tigecycline have been reported [67-69]. The battle between human beings and microorganisms is never ending. Under selection pressure of with continuing antimicrobial agent consumption, medical personnel should anticipate the emergence of various resistant pathogens, such as "ESKAPE," [70] where the "S" in the abbreviation stands for *S. aureus*. In the face of these multidrug-resistant pathogens, preventing the spread of resistant microorganisms and their resistance determinant genes and adequate antibiotics stewardship to prevent unnecessary selection pressures are inevitable medical issues [71].

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Conflicts of interest

There are no conflicts of interest.

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