Methicillin-resistant *Staphylococcus aureus* with Reduced Susceptibility to Vancomycin in Sanprasitthiprasong Hospital

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Background: *Staphylococcus aureus is a species of bacteria that causes a number of diseases and more than 60% of it is presently resistant to methicillin. Vancomycin is the drug of choice for the eradication of methicillin-resistant S. aureus (MRSA).*
Objective: *This study aimed to investigate the susceptibility of heterogeneous vancomycin intermediate S. aureus (hVISA) and vancomycin intermediate S. aureus (VISA) to vancomycin by standard disk diffusion, microbroth dilution, a one-point population assay, and a population analysis profile.*
Material and Method: *Sixty-eight MRSA isolates from patients admitted to Sanprasitthiprasong Hospital between November 2010 and November 2011 were tested.*
Results: *Standard disk diffusion showed that all the MRSA isolates were susceptible to vancomycin. Vancomycin MICs for all isolates were 1-2 μg/mL. Only two MRSA isolates (2.9%) were able to grow on brain heart infusion agar supplemented with vancomycin 4 μg/mL and were confirmed by a population analysis as hVISA.*
Conclusion: *This study showed the effect of vancomycin on MRSA and the need for early detection and controlled planning.*

Keywords: *Methicillin-resistant Staphylococcus aureus (MRSA), Vancomycin, Heterogeneous vancomycin-intermediate S. aureus (hVISA), Vancomycin-intermediate S. aureus (VISA), High level vancomycin-resistant S. aureus (VRSA)*

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Staphylococcus aureus is a gram-positive coccus that can cause infections in several systems, such as the respiratory and urinary tracts, and in the blood and open wounds. Most *S. aureus* are methicillin-resistant (MRSA) and the preferred drugs for the treatment of these are glycopeptides, such as vancomycin and teicoplanin.

Several research studies found MRSA with reduced susceptibility to vancomycin, the first being the work in Japan by Hiramatsu et al. The isolates in this study were vancomycin-intermediate *S. aureus* (VISA)[1]. Currently, the mechanism of intermediate resistance to vancomycin in *S. aureus* is unknown. The transfer of the *vanA* gene-resistant determinants from vancomycin-resistant enterococci (VRE) to *S. aureus* by cell-to-cell mating was demonstrated in vitro[2]. However, none of the VISA strains was shown to have any *van* determinants that were present in VRE[3]. VISA strains had lower growth rates and thicker cell walls[4] and produced three to five times more penicillin-binding proteins 2 and 2' and three to eight times more cell wall precursors in comparison to susceptible strains[5].

There have been reports of the prevalence of heterogeneous vancomycin-intermediate *S. aureus* (hVISA) ranging from 0-74%[3,6]. hVISA, namely Mu3, was first reported in 1997 in Japan[7] and classified by the Criteria of Clinical Laboratory Standards Institute (CLSI) as having a vancomycin broth microdilution MIC in the susceptible range. The isolate contained sub-populations that were able to grow in media containing 5 to 9 μg/mL of vancomycin. VISA or Mu50 was recovered several months later in the same hospital in Japan[7] and both strains were suggested to be closely related. Hiramatsu et al initially discovered hVISA, then VISA, and finally high level vancomycin resistant *S. aureus* (VRSA)[8]. In Thailand, *S. aureus* with reduced susceptibility towards vancomycin was first reported

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by Trakulsomboon et al[9]. They found three isolates of VISA by a one-point population analysis and later reported VRSA. In addition, a study in Srinagarind Hospital found 7 hVISA by a population analysis profile curve[10].

The present study that is the subject of this paper took place in Sanprasitthiprasong Hospital, a medical school with a high rate of MRSA infection and a high use of vancomycin therapy.

Material and Method

Bacterial strains

S. aureus isolates were obtained from patients admitted to Sanprasitthiprasong Hostpital from November 2010 to November 2011. All isolates were tested by 1 μg oxacillin disk, oxacillin resistance screening agar (Oxoid, Basingstoke, Hants, UK), and oxacillin microbroth dilution (Trek diagnostic systems, Biosciences Inc., Magellan, USA). *S. aureus* isolates that were resistant to oxacillin by all methods were selected for further study. Sixty-eight MRSA isolates from blood (36 isolates), respiratory secretions and sputum (22 isolates), and pus (10 isolates) were included in the present study.

Standard disk diffusion for vancomycin

The disk diffusion test with 30 μg vancomycin was performed on Mueller Hinton agar (Hardy diagnostics, Santa Maria, USA) using the Kirby-Bauer method. *S. aureus* ATCC 25923 was used as a vancomycin-sensitivity control with an expected inhibition zone of between 18 and 23 mm. The results were interpreted according to CLSI[11]. The inhibition zone of a susceptible (S) organism was considered to be ≥15 mm.

Minimal inhibitory concentration (MIC) for vancomycin

MIC was tested by Sensititre gram-positive plate (Trek diagnostic systems, Biosciences Inc., Magellan, USA) and performed according to the manufacturer's instructions. The concentrations of vancomycin were 0.25-32 μg/mL. Isolates with vancomycin MIC of ≤2 μg/mL were interpreted as susceptible as recommended by CLSI[11].

One-point population assay

A one-point population assay was performed by the method of screening agar[7,12]. Briefly, 100 μL of bacterial suspension adjusted to McFarland No. 0.5 was spread onto brain heart infusion (BHI) agar (Hardy diagnostics) supplemented with 4 μg/ml of vancomycin (Merck KGaA, Darmstadt, Germany). Plates were incubated at 35°C for 24-48 hours. *S. aureus* strains Mu3 (hVISA) and Mu50 (VISA) were used as positive controls. *S. aureus* ATCC 29213 was used as the negative control strain.

Population analysis profile [7,13]

The bacterial suspension was adjusted to an optical density of 10^8 CFU/ml and serial 10-fold dilutions were spread over brain heart infusion (BHI) agar plates containing vancomycin at concentrations ranging from 1 to 64 μg/mL. After inoculation at 35°C for 48 hours, the number of viable colonies was counted. *S. aureus* strains Mu3, Mu50, and ATCC 29213 were used as controls. The number of resistant cells contained in 1 ml of the starting cells suspension was calculated and plotted on a semi-logarithmic scale.

Results

Standard disk diffusion and MIC

Through the use of standard disk diffusion, it was found that all isolates were susceptible to vancomycin with inhibition zones ranging from 16 mm to 24 mm. Vancomycin MIC for all isolates ranged from 1 μg/mL to 2 μg/mL, which were interpreted as susceptible[11].

One-point population assay

Of the 68 MRSA isolates, only two isolates (M005 and M102) grew on BHI supplemented with 4 μg/mL of vancomycin after 24-h incubation.

Population analysis profile

Isolates M005 and M102 had hVISA curves by a population analysis profile versus the reference strains (Fig. 1). Calculation of the areas under the curves (AUC) showed Mu3 was 19.7, M005 was 19.05, and M102 was 19.65. The ratio of the AUC of the test were 0.97 and 0.99, respectively. Mu50 was 51.90 and gave an AUC ration of 2.63. The vancomycin MIC for both hVISA isolates were 2 μg/mL.

Discussion

In the present study, two of the 68 MRSA isolates were hVISA that were susceptible to vancomycin by both standard disk diffusion and microbroth dilution. They were isolated from sputum of different hospitalized patients. These patients were admitted for pneumonia, had never been treated with vancomycin before, and were treated with ceftriaxone.

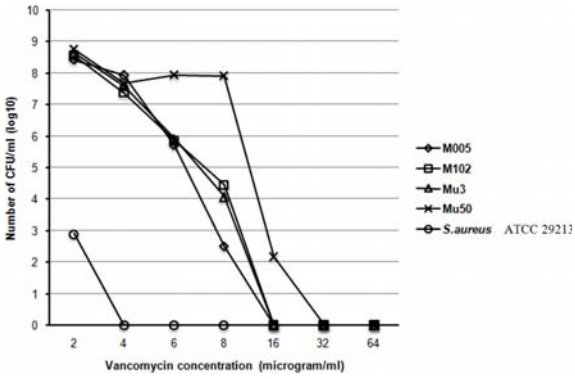

Fig. 1 Population analysis profiles of three controls (*S. aureus* strains Mu3, Mu50, and ATCC 29213) and two MRSA clinical strains (M005 and M102).

M005 was resistant to tetracycline, erythromycin, and ciprofloxacin by standard disk diffusion and microbroth dilution. By the time, M102 was resistant to erythromycin and ciprofloxacin but susceptible to tetracycline. However, both M005 and M102 were susceptible to tigecycline.

This was Sanprasitthiprasong Hospital's first warning about MRSA, an important hospital pathogen with reduced susceptibility to vancomycin. The concern over the extent of hVISA was that it was not detectable with standard disk diffusion and microbroth dilution. Therefore, these isolates were not known to have reduced susceptibility to vancomycin in clinical microbiology laboratories. Moreover, vancomycin treatment clinically failed regarding hVISA strains[4,7,14].

The Mu3 strain was serially passaged in increasing concentrations of vancomycin in vitro, giving rise to sub-populations with levels of resistance comparable to that of Mu50. This phenomenon suggests that colonization or infection with VISA or hVISA, with repeated vancomycin exposure, acts as a selection pressure on the development of the resistant population[15]. Thus, incorrect identification of hVISA or VISA may increase the incidence of resistant strains. This poses a problem for future anti-microbial therapy and patients infected with these organisms may fail to respond to treatment.

The identification of hVISA is important in a clinical microbiology laboratory. Multiple studies demonstrated high sensitivity and specificity in hVISA detection. However, variations between laboratories were observed by the Macro E-test[12,16-18]. A population analysis profile sets a high standard for hVISA detection but is labor intensive. In this study, hVISA was detected by a one- point population assay. The susceptible strain was not able to grow in media containing 4 μg/mL vancomycin. However, only two strains of hVISA were found. Further study is required for the validation of a one-point population assay in a large sample of hVISA.

Conclusion

MRSA from patients admitted at Sanprasitthiprasong Hospital were found to have two isolates of hVISA that were not detected by routine susceptibility testing. This was the first warning to Sanprasitthiprasong Hospital about MRSA with reduced susceptibility to vancomycin, an issue that may become a problem in anti-microbial therapy in the future.

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Potential conflicts of interest

None.

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Methicillin-resistant Staphylococcus aureus ที่มีความไวต่อยา vancomycin ลดลง ในโรงพยาบาล สรรพสิทธิประสงค์

ภาวนา พนมเขต, ศุทธินี ธิราช, สุรศักดิ์ แว่นรัมย์, รุ่งนภา ปานกล้า

ภูมิหลัง: *Staphylococcus aureus เป็นเชื้อแบคทีเรียที่ก่อให้เกิดโรคหลายชนิด ปัจจุบันมากกว่าร้อยละ 60 ของ S. aureus ดื้อต่อยา methicillin ยา vancomycin เป็นยาที่ใช้ในการรักษาเพื่อกำจัดเชื้อ Methicillin-resistant Staphylococcus aureus (MRSA)*

วัตถุประสงค์: *การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อตรวจหา heterogeneous vancomycin intermediate S. aureus (hVISA) และ vancomycin intermediate S. aureus (VISA) โดยทดสอบความไวของยา vancomycin ด้วยวิธี standard disk diffusion microbroth dilution one-point population assay และ population analysis profile*

วัสดุและวิธีการ: *เชื้อ MRSA ทั้งหมดจำนวน 68 สายพันธุ์ ที่แยกได้จากผู้ป่วยที่เข้ารับการรักษาที่โรงพยาบาลสรรพสิทธิประสงค์ ตั้งแต่เดือนพฤศจิกายน พ.ศ. 2553 ถึง เดือนพฤศจิกายน พ.ศ. 2554 ถูกนำมาทดสอบ*

ผลการศึกษา: *ด้วยวิธี standard disk diffusion เชื้อ MRSA ทุกสายพันธุ์ไวต่อยา vancomycin เชื้อทั้งหมดมี vancomycin MIC 1-2 μg/mL มีเพียง 2 สายพันธุ์ (ร้อยละ 2.9) สามารถเจริญได้บน Brain heart infusion agar ที่มียา vancomycin 4 μg/mL และยืนยันด้วย Population analysis profile เป็น hVISA*

สรุป: *การศึกษาครั้งนี้แสดงให้เห็นอุบัติการณ์ของเชื้อ MRSA กับยา vancomycin และจำเป็นต้องมีการตรวจวินิจฉัยในระยะเริ่มต้น เพื่อวางแผนควบคุม*