Screening and detection of heterogenous vancomycin intermediate *Staphylococcus aureus* in Hospital Kuala Lumpur Malaysia, using the glycopeptide resistance detection Etest and population analysis profiling

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Abstract

In a 3-month study done in Hospital Kuala Lumpur (HKL), 7 out of 320 methicillin resistant *Staphylococcus aureus* isolates were confirmed as heterogeneous vancomycin intermediate *S. aureus* (hVISA) using the glycopeptide resistance detection e-test and population analysis, giving a prevalence rate of 2.19%. This is the first report of hVISA in Malaysia.

Introduction

Heterogenous vancomycin intermediate *Staphylococcus aureus* (hVISA) strains have been reported as indicators for reduced vancomycin susceptibility in *S. aureus*,[1] and various studies associated its presence with vancomycin treatment failure.[2] It has been shown that methicillin resistant *S. aureus* (MRSA) has the propensity to evolve into hVISA phenotype during *in vitro* exposure to subinhibitory concentrations of vancomycin.[3] During the last decade, hVISAs had been isolated in many countries including those in South East Asia;[4] nevertheless, in our knowledge, its emergence has not been reported in Malaysia. As a pilot study, we investigated the prevalence of hVISA among MRSA strains isolated at Hospital Kuala Lumpur (HKL) in a 3-month period and determined factors associated with its infections.

Case Report

Hospital Kuala Lumpur is the largest hospital in Malaysia with the highest MRSA burden in the country.[5] In this hospital, vancomycin is used as the standard first line treatment for MRSA infection; however, recently, its efficacy has been a subject of discussion due to several anecdotal vancomycin treatment failure cases in Hospital Kuala Lumpur. We also wondered if some of the MRSAs isolated in the hospital were actually hVISAs with reduced susceptibilities to vancomycin that could not be detected by routine microbiological tests used in our hospital diagnostic laboratory. To investigate this, from 25th February to 25th May 2009, we collected a total of 320 index MRSA isolates (first MRSA isolated from the corresponding patients) and established them as strains for vancomycin resistance testing. As it is cost, time and labor consuming to perform vancomycin population analysis[6] on all 320 strains to test for heterogenous vancomycin resistance, strains were first screened for the phenotype using Glycopeptide Resistance Detection (GRD) Etest antibiotic strips (AB BIODISK, Sweden).[7] After GRD screening, a total of 8 strains were defined as presumptive hVISA, no VISA strain was detected. Following that, to confirm the results of the GRD screening, the 8 presumptive hVISAs were subjected to vancomycin population analysis. Interestingly, area under the curve (AUC) analyses[8] of the strains' population analysis profiles confirmed that 7 out of the 8 tested strains were hVISA (Table 1), giving a prevalence rate of 2.19%.

Discussion

All hVISA strains isolated in this study were hospital acquired as they were isolated from their corresponding patients after 48 hours of hospital admission. To determine factors associated with the 7 hVISA infections, demographic data of all corresponding patients of each index MRSA isolate were retrieved from medical records. Medical history of each patient such as diabetes mellitus, renal failure, malignancy, together with prescription history of vancomycin and beta-lactam antibiotics (as these were the only classes of antibiotics prescribed to the corresponding patients of the study isolates during this investigation), length of hospitalization and intensive care unit (ICU) admission were recorded. Continuous variables were then assessed by independent samples t-test, while categorical variables were analyzed using Pearson's Chi-square. Calculations were performed using Statistical Package for Social Science (SPSS) 12.0 (SPSS Inc., Chicago, USA) where a P-value of <0.05 was considered as significant. After performing multivariate linear regression, we found that ICU admission (P<0.004), hospitalization of more than 14 days (P<0.014) and vancomycin administration of more than 7 days (P<0.016) were independent factors associated with hVISA infections in our group of patients. Our findings were in line with those of Charles *et al.* in 2004, where hVISA/VISA infections were found to be associated with longer antibiotic treatment periods and longer hospitalization.[9] In a separate report, ICU admission was also stated as a significant associated factor for hVISA infections.[10] It seems that patients who are severely ill, hospitalized for long durations with ICU admissions might have a higher chance of developing hVISA infections. As many patients in HKL fulfill some or all of the above criteria, taking it together, we suspect that the prevalence of hVISA in HKL might be high; however, these strains are not being actively detected by

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Table 1. Heterogenous vancomycin intermediate *Staphylococcus aureus* strains and their corresponding patients in this study.

Specimen No	Age	Gender	Primary diagnosis	Specimen	Diabetes mellitus	Renal failure	Malignancy	Admission to ICU	Days of hospital stay	Days of IV VCM	On Beta-lactam	Area under curve Ratio[8]
682	14	Male	Right hip osteomyelitis	Nasal swab	No	No	No	Yes	44 days	0	Yes	0.90
582	53	Female	Left diabetic foot ulcer	Pus swab	Yes	Yes	No	Yes	52 days	14	Yes	0.93
182	58	Male	Acute ventriculitis	CSF	Yes	No	No	Yes	98 days	14	Yes	1.01
252	20	Male	Gluteal sarcoma with HAP	Sputum	No	No	Yes	Yes	24 days	10	Yes	0.90
978	71	Male	Pemphigus folliaceous	Pus swab	Yes	No	No	No	34 days	7	Yes	1.01
215	4	Female	Acute encephalitis with HAP	Tracheal aspirate	No	No	No	Yes	32 days	14	Yes	0.96
460	29	Female	Meningo-encephalitis with HAP	Sputum	No	No	No	Yes	38 days	10	Yes	0.98

ICU, intensive care unit; IV, intravenous; VCM, vancomycin; CSF, cerebrospinal fluid; HAP, hospital acquired pneumonia.

the hospital diagnostics laboratory. As hVISA and MRSA with reduced vancomycin susceptibility has been reported to cause treatment failure,[11] given the hVISA prevalence rate detected in this study, it is not surprising that vancomycin treatment failure cases among MRSA infected patients are increasing in HKL. In our study, we employed the GRD test as a screening tool for hVISA before confirming the resistance with population analysis, and found that the GRD Etest was fairly specific with only one false positive result. In a review, Howden and colleagues have reported the test's sensitivity as 93-94% with a 82-95% specificity for hVISA detection.[2] Therefore, the GRD might be considered a good screening tool for hVISA in hospitals where most hospitalized patients are severely ill with long hospitalization durations. Once identified as hVISA infected, optimal treatment could be prescribed to the corresponding patient to prevent vancomycin treatment failure, thereby increasing the chance of a good clinical outcome for the patient.

Conclusions

This is the first report of hVISA in Malaysia. As the strains used in this study were collected in a short span of 3 months, and that vancomycin treatment failure is on the rise in HKL, we suspect that the actual prevalence of hVISA in this hospital might be even higher. We found the GRD test useful for hVISA screening, nevertheless PAP-AUC analysis still remains the gold standard for hVISA confirmation. A more comprehensive, case control study involving major hospitals in the country would be important to better understand the significance and distribution of hVISA in Malaysian hospitals.

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