# The Presence of Heterogeneous Vancomycin-Intermediate *Staphylococcus aureus* (heteroVISA) in a Major Malaysian Hospital

**Norazah Ahmad, MD, MSc, PhD*, Law Ngiik Ling, BSc**, Mohamed Kamel Abd Ghani, MD**, Salbiah Nawi, MPath*****

*Institute for Medical Research, Bacteriology Unit, Infectious Diseases Research Centre, Jalan Pahang, Kuala Lumpur, Wilayah Persekutuan 50588 Malaysia, **Faculty of Biomedicine, National University of Malaysia, Kuala Lumpur, Malaysia, ***Selayang Hospital, Selangor, Malaysia

**SUMMARY**

**This study was conducted to detect the presence of heterogenous vancomycin-intermediate *Staphylococcus aureus* (heteroVISA) among MRSA isolates in a major hospital. Forty-three MRSA isolates with vancomycin MIC 2 µg/ml collected in 2009 was screened for heteroVISA using Etest Glycopeptide Resistance Detection (GRD) and confirmed by population analysis profile-area under curve method. The genetic relatedness of heteroVISA strains with other MRSA was examined by pulsed-field gel electrophoresis (PFGE) method. Two isolates were shown to be heteroVISA and derived from the same clone. This showed that heteroVISA strains were already present among our local strains since 2009 and were genetically related to other susceptible strains.**

**KEY WORDS:**
*MRSA, HeteroVISA, Etest GRD, population analysis, pulsed-field gel electrophoresis*

## INTRODUCTION

*Staphylococcus aureus* is one of the major pathogens causing various infections ranging from skin and soft tissue infections to life-threatening infections. Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the major causes of nosocomial infections around the world. In Malaysia, the overall prevalence of MRSA in hospitals was 22% in 2009 and the MRSA rates in 16 major hospitals ranged from 3.5% to 28.5% [1].

*S. aureus* with reduced susceptibility towards vancomycin was first reported in Japan by Hiramatsu *et al*[2]. Since then, heteroVISA had been reported in many countries around the world including Spain, Korea, Italy, Singapore, Thailand and Hong Kong[3,4,5,6,7,8].

The efficacy of vancomycin was shown to decline in cases in which vancomycin MICs for MRSA were 1 to 2 µg/ml compared to isolates with MICs < 0.5 µg/ml[9]. The prevalence rate of heteroVISA among MRSA with vancomycin MIC 2 µg/ml is also increasing[10]. HeteroVISA infections were associated with higher mortality rate compared to vancomycin-sensitive *Staphylococcus aureus* (VSSA) infections[11]. Although heteroVISA has not been reported in Malaysia, treatment failure with vancomycin had been documented in a clinical MRSA isolate with vancomycin MIC 2 µg/ml from Hospital Selayang[12].

HeteroVISA strains are difficult to detect by standard MIC methods because of the low frequency of resistant subpopulations. Population analysis profiles combined with area under the curve analysis is the gold standard for heteroVISA identification. However, it is labour-intensive and not practical for routine use. E-test GRD had been evaluated as an alternative test for detection of heteroVISA and had shown promising results[13]. This study was carried on clinical MRSA isolates with vancomycin MIC 2 µg/ml to determine the presence of heteroVISA among clinical isolates of MRSA from a major referral hospital.

## MATERIALS AND METHODS

*Bacterial isolates*

Forty-three clinical MRSA isolates used in this study were acquired from the bacterial storage in vials at -70°C in Bacteriology Unit, Institute for Medical Research (IMR). The vancomycin MICs of these strains were 2µg/mL, as determined by E-test method and collected between January to December 2009 from Hospital Selayang. Hospital Selayang is a tertiary referral hospital with 960 in-patient beds and 20 clinical disciplines.

*GRD Etest*

HeteroVISA screening with Etest GRD was carried out following the manufacturer's instructions (bioMérieux). An overnight culture was inoculated into Mueller-Hinton broth to achieve a bacterial suspension corresponding to a 0.5 McFarland standard. The suspension was then spread onto a Mueller-Hinton agar + 5% blood plate (MHB; Becton Dickinson, MD USA) and a GRD Etest strip consisting of a double-sided gradient with vancomycin and teicoplanin was then applied onto the plate. The zone of the GRD Etest strip was read at 24h and 48h incubation and MIC showing the complete inhibition of growth recorded. The test isolate was considered positive for heteroVISA if the GRD Etest strip was ≥ 8 µg/ml for either vancomycin or teicoplanin. ATCC 29213 (VSSA strain) and ATCC 700698 (heteroVISA strain also known as Mu3) were used as controls.

*This article was accepted: 22 February 2012*
*Corresponding Author: Norazah Ahmad, Institute for Medical Research, Bacteriology Unit, Infectious Diseases Research Centre, Jalan Pahang, Kuala Lumpur, Wilayah Persekutuan 50588 Malaysia Email: norazah@imr.gov.my*

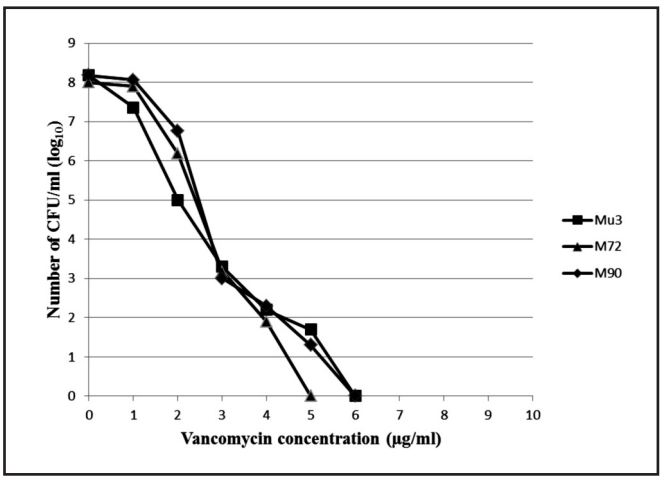


**Fig. 1:** Population analysis of two heteroVISA strains (M72 and M90) and Mu3 (reference heteroVISA strain).

*Population analysis profile-Area under curve (PAP-AUC )*
Any heteroVISA-positive isolates screened by Etest GRD were subjected to PAP-AUC method as described by Wootton *et al*[14]. A 100 µl aliquot of the overnight isolates and control strain (Mu3) in tryptic soy broth (TSB) were adjusted to optical density of 0.03 at 578 nm ($10^8$ CFUs/ml) and serial 10-fold dilutions were spread over Brain Heart Infusion (BHI; Becton Dickinson, MD USA) agar plates containing vancomycin at concentrations ranging from 1 to 10 µg/ml. After incubation at 37°C for 48 hours, the number of viable colonies was counted. The number of resistant cells contained in 1ml of the starting cell suspension was calculated and plotted on a semi-logarithmic scale. The AUC for each test isolate and Mu3 was measured from the graph by the construction of trapezoids. A ratio was then calculated by dividing the AUC of the test isolate by the AUC of Mu3. Interpretation of PAP-AUC was as follows: ratio of the AUC of the test isolate to Mu3 < 0.9 was considered VSSA, ratio of the AUC of the test isolate to Mu3 ≥ 0.9 and < 1.3 was considered heteroVISA and ratio of the AUC of the test isolate to Mu3 ≥ 1.3 was considered VISA[15] . Mu3 (heteroVISA strain) was used as positive control in PAP-AUC.

*Pulsed-field gel electrophoresis (PFGE)*
All of the 43 clinical MRSA isolates were subjected to PFGE analysis. PFGE profiles were determined as described previously by McDougal *et al*[16]. DNA electrophoresis was carried out using a countour-clamped homogenous electric field system (CHEF-MAPPER, Bio-Rad) with pulse time 5-15 s for 8 hours, followed by 15-25 s for 10 hours. Gel was stained with ethidium bromide (0.5 mg/ml), destained in distilled water and photographed under UV illumination. DNA fragment patterns were analyzed using *Fingerprinting FP* Quest software (Bio-Rad) by unweighted pair group method with averages (UPGMA) based on Dice Coefficient to construct a dendogram. Tolerance and optimization were set at 1%. Isolates which showed similarity values of ≥ 80% were defined as sharing a common PFGE pattern and assigned with a capital letter. Isolates with similarity values ≥ 80% were closely related with each other. Each PFGE pattern was further classified into subtypes based on similarity values of 80-100% [16]. *S. aureus* NCTC 8325 was used as standard molecular marker for gel normalization in PFGE.

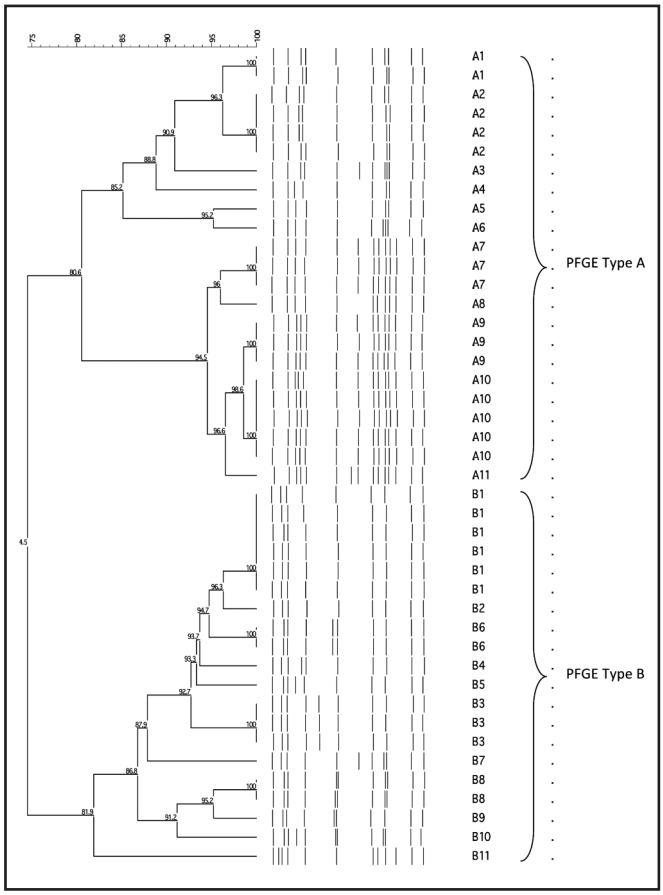


**Fig. 2:** Dendogram and PFGE type for the MRSA isolates.

## RESULTS

Most of the MRSA strains were isolated from blood (46.5%), followed by pus (30.2%), intravenous catheters (4.7%), respiratory secretions (4.7%), abdominal fluid (4.7%), wound swab (2.3%), urine (2.3%), tissue (2.3%) and bronchoalveolar lavage (2.3%).

These strains were isolated from patients from various wards namely medical (30.2%), nephrology (20.9%), intensive care unit (13.9%), orthopaedic (9.3%), hepatology (7.0%), cardiology (4.7%), surgery (4.7%), hepatobilliary (4.7%), accident and emergency (2.3%) and urology (2.3%).

Using Etest GRD method, all of the isolates had vancomycin MIC values ranging from 1 µg/mL to 2 µg/ml and teicoplanin MIC values ranging from 1.5 µg/ml to 8 µg/mL. All isolates showed higher teicoplanin MIC values than vancomycin MIC. Of the 43 isolates, 2 heteroVISA strains M72 and M90 were identified by Etest GRD method, with MIC teicoplanin 8 µg/m. Both of these heteroVISA strains were then confirmed by PAP-AUC method, showing AUC ratio of 0.98 and 1.08 respectively. Population analysis of these 2 heteroVISA strains is shown in Figure 1. Both of these heteroVISA strains were isolated from intravenous catheters from two different patients.

Restriction enzyme *Sma1* resolved the genomic DNA of 43 MRSA isolates into two main distinct PFGE patterns A and B (Figure 2). The isolates were classified almost equally into

both PFGE pattern type A (53.5%; 23 isolates) and B (46.5%; 20 isolates). PFGE pattern type A was further classified into 11 subtypes (A1 to A11) in which subtype A10 represented the majority of the isolate (21.7%), followed by subtype A2 (17.4%), while other subtypes were exhibited in one to three isolates only. PFGE pattern type B was also classified into 11 subtypes (B1 to B11) with subtype B1 representing the majority subtype (30%), while other subtypes which was shown in one to three isolates only.

MRSA with PFGE pattern type A were isolated from all the wards mentioned above while MRSA with PFGE pattern type B were only isolated from five wards namely medical ward, nephrology ward, ICU, orthopedic ward and hepatology ward. This may imply that strains with PFGE type A are widely disseminated than PFGE type B strains in this hospital. Most of the isolates with PFGE pattern type A were isolated from nephrology ward while isolates with PFGE pattern type B were from medical ward.

Both heteroVISA strains were classified in PFGE pattern type B and further subtyped as B6. PFGE showed that both heteroVISA strains belong to the same clone. These strains were isolated from different wards in which one strain was from nephrology ward while the other was from medical ward. The heteroVISA strains shared the same PFGE type B pattern with the other vancomycin-sensitive strains.

## DISCUSSION

Vancomycin is the empirical agent in treating serious MRSA infections. However, vancomycin treatment failures have been increasingly reported from all around the world[17]. Some of the treatment failures were caused by resistance of heteroVISA strains to vancomycin[18]. The minority intermediate-resistant cells are difficult to be detected by traditional laboratory testing therefore heteroVISA infections are always underestimated[19]. HeteroVISA is associated with prolonged bacteremia, high burden bacterial infection, prolonged antibiotic therapy, prolonged hospitalization, treatment failures and increase in death potential[20]. Patients with heteroVISA infections have higher complication rates and more persistent infections compared to patients with VSSA infections[21, 22].

Vancomycin treatment failures have been associated with MRSA strains with vancomycin MIC ≤ 2 µg/mL[9,23]. There is a possibility that these strains were heteroVISA but had not been identified as the conventional standard MIC methods are not sufficient in detecting the minority intermediate-resistant subpopulations[24].

In this study, most of the MRSA isolates with vancomycin MIC 2 µg/ml were cultured from blood. Previous studies had shown that the prevalence rates of MRSA isolates with reduced susceptibility to vancomycin (MIC 2 µg/ml) were very high among MRSA strains which caused invasive infections like bloodstream infections[25]. A study by Mohr and Murray has reported that as much as 30% of MRSA isolated from blood samples had a vancomycin MIC of 2 µg/mL[26]. A high percentage of MRSA strains from local Malaysian hospitals were also observed to have increased vancomycin MIC[27]. The significant relationship between clinical outcomes and the percentage of MRSA with vancomycin MIC 2 µg/mL isolated from blood samples would be more relevant if prior exposure to vancomycin is known. Clinicians will be guided to consider the alternative treatment in vancomycin treatment failure cases if the history of previous vancomycin therapy is available.

In this study, all clinical MRSA isolates showed higher teicoplanin MIC values compared to vancomycin MIC values using Etest GRD. Previous studies had shown that *S. aureus* acquired resistance to teicoplanin before it acquired resistance to vancomycin[28,29]. An experimental study had shown that increase of glycopeptides MIC in *S. aureus* was caused by overproduction of penicillin binding protein 2 (PBP2) which caused cell wall thickening. Overproduction of PBP2 in VSSA strain caused vancomycin MIC to increase 1 µg/ml (from 1 µg/ml to 2 µg/ml) while teicoplanin MIC increases significantly from 2 µg/ml to 8 µg/ml[30].

Two heteroVISA strains had been detected and confirmed among MRSA isolates with vancomycin MIC 2 µg/ml in this study. M72 was isolated from a 54 year-old end stage renal failure patient with history of multiple readmissions into the hospital. On this admission he had cardiac arrhythmia and was transferred to the intensive care unit (ICU). He developed ventilator-acquired pneumonia and was treated with intravenous imipenem for 13 days. No history of vancomycin treatment was obtained during this admission. M90 was isolated from a 20 year old leptospirosis patient complicated with acute kidney insufficiency. He developed pulmonary bleeding and Acinetobacter pneumonia and was transferred to the ICU. The patient received intravenous vancomycin in bolus doses once a day for 3 days, plus a battery of other antibiotics such as cefoperazone-sulbactam, azithromycin, ceftazidime, sulfamethoxazole-trimethoprim, imipenem and piperacillin-tazobactam.

HeteroVISA strains had been reported globally including neighbouring countries like Singapore and Thailand[6,7]. Therefore it is a matter of time that heteroVISA strains will finally be detected in Malaysia because of the increasing usage of vancomycin. HeteroVISA strains may have been present in hospitals in Malaysia for quite a time but may be misidentified as susceptible strains because the minority resistant cells are difficult to detect. Furthermore, this study also supported the previous report on a vancomycin treatment failure case caused by MRSA strain with MIC 2 µg/ml in Hospital Selayang[31]. The MRSA strain was most probably a heteroVISA strain.

Both the heteroVISA strains were isolated from intravenous catheters. A study by Horne et al had shown that MRSA isolates with reduced susceptibility to vancomycin would cause clinical problems if the infection occurred at low vancomycin penetration sites like cardiac vegetation, bones or around the prosthetic devices[32]. However, the clinical importance of these strains was unknown as information regarding treatment outcome was not available and there was no history of prior vancomycin treatment in these patients. According to a study conducted in a France hospital, an outbreak involving heteroVISA strain had been reported

among patients who were not receiving any vancomycin treatment and the primary risk for the patients was the exposure to hospital environment[33]. Besides, a study by Howden *et al*. showed that heteroVISA infections were prevalent in the presence of foreign bodies, for example catheter[34].

Two major types of PFGE patterns were identified among the 43 MRSA with vancomycin MIC 2 µg/ml in this study. In a previous study, a total of 31 PFGE major patterns had been identified among MRSA isolates in Malaysia hospitals[12]. Inter-hospital spread and transmission of MRSA occurs frequently and regularly in Malaysia most probably due to increased frequency of transfers of patients between hospitals. There is a possibility that MRSA with vancomycin MIC 2 µg/ml from Hospital Selayang had been transmitted to or from another hospital, since this hospital is the major referral hospital for hepatobiliary diseases. It would be interesting to know the major PFGE patterns of MRSA endemic in Hospital Selayang so that the genetic relatedness of MRSA with vancomycin MIC 2 µg/ml with other endemic MRSA strains can be determined.

Both heteroVISA strains identified in this study belonged to PFGE type B and were further classified into subtype B6. This showed that the heteroVISA strains originated from the same clone by having closely related genetic profile. However, the strains were isolated from patients in different wards. This suggested that heteroVISA clone was disseminated between nephrology and medical wad. Dissemination of heteroVISA clone between wards might occur through direct contact of healthcare personnel or via hospital environment. The nephrology and medical wards also have the most varieties of MRSA subtypes compared to other wards.

HeteroVISA strains with PFGE subtype B6 was closely related to other MRSA strains with PFGE subtype B by showing only one to three fragment differences. These could result from genetic events causing point mutations in DNA of MRSA[35]. However, these heteroVISA showed different phenotype compared to other strains with PFGE type B which were VSSA, therefore phenotypic method was unhelpful in confirming the clonal relationship of these strains. DNA typing technique using PFGE had shown clearly the genetic relatedness of these strains.

In conclusion, heteroVISA strains had been identified among clinical MRSA isolates with vancomycin MIC 2 µg/ml isolated in 2009 from Hospital Selayang. These heteroVISA strains belonged to the same MRSA clone as shown by the same PFGE type B and was well disseminated in Hospital Selayang. There is also the possibility of heteroVISA strains present in other healthcare institutions in Malaysia. Further studies on MRSA isolates from other healthcare institutions need to be carried out in order to identify the epidemiology of heteroVISA strains in Malaysia.

**ACKNOWLEDGEMENT**

The authors thank the Director General of Health, Ministry of Health Malaysia for permission to publish this article.

**REFERENCES**

1. Ministry of Health, Malaysia. National Surveillance of Antibiotic Resistance Report 2009. Available at: www.imr.gov.my/report/nsar2009.htm.
2. Hiramatsu K, Aritaka N, Hanaki H *et al*. Dissemination in Japanese hospitals of strains of *Staphylococcus aureus* heterogeneously resistant to vancomycin. Lancet 1997; 350: 1670-3.
3. Ariza J, Pujol M, Cabo J *et al*. Vancomycin in surgical infections due to methicillin-resistant *Staphylococcus aureus* with heterogeneous resistance to vancomycin. Lancet; 1999: 353: 1587-8.
4. Kim MN, Pai CH, Woo JH, Ryu JS, Hiramatsu K. Vancomycin-intermediate Staphylococcus aureus in Korea. J Clin Microbiol 2000; 38: 3879-81.
5. Marchese A, Balistreri G, Tonoli E, Bebbia EA, Schito GC. Heterogenous vancomycin-resistance in methicillin–resistant *Staphylococus aureus* strains in a large Italian hospital. J Clin Microbiol 2000; 38: 866-9.
6. Sng LH, Koh TH, Wang GC, Hsu LY, Kapi M, Hiramatsu K. Heterogeneous vancomycin-resistant *Staphylococcus aureus* (hetero-VISA) in Singapore. Int J Antimicrob Agents 2005; 25: 177-9.
7. Trakulsomboon S, Danchaivijitr S, Rongrungruang Y *et al*. First report of methicillin-resistant *Staphylococcus aureus* with reduced susceptibility to vancomycin in Thailand. J Clin Microbiol 2001; 39: 591-5.
8. Wong SS, Ng TK, Yam WC. Bacteremia due to *Staphylococcus aureus* with reduced susceptibility to vancomycin. Diagn Microbiol Infect Dis 2000; 36: 261-8.
9. Sakoulas G, Moise-Broder PA, Schentag J, Forrest A, Moellering RC Jr, Eliopoulos GM. Relationship of MIC and bactericidal activity to efficacy of vancomycin for treatment of methicillin-resistant *Staphylococcus aureus* bacteremia. J Clinical Microbiol 2004; 42: 2398-402.
10. Sader HS, Becke, HK, Moe GJ, Jones RN. Antimicrobial activity of daptomycin tested against *Staphylococcus aureus* with vancomycin MIC of 2 microg/mL isolated in the United States and European hospitals (2006-2008). Diagn Microbiol and Infect Dis 2010; 66: 329-31.
11. Wong SS, Ho PL, Woo PC, Yuen KY. Bacteremia caused by staphylococci with inducible vancomycin heteroresistance. Clin Infect Dis 1999; 29: 760-7.
12. Norazah A, Lim VK, Rohani MY, Alfizah H, Koh YT, Kamel AG. A major methicillin-resistant Staphylococcus aureus clone predominates in Malaysian hospitals. Epidemiol Infect 2003; 130: 407-11.
13. Tenover FC, Sinner SW, Segal RE *et al*. Characterisation of a Staphylococcus aureus strain with progressive loss of susceptibility to vancomycin and daptomycin during therapy. Int J Antimicrob Agents 2009; 33: 564–8.
14. Wootton M, Howe RA, Hillman R, Walsh TR, Bennett PM, MacGowan AP. A modified population analysis profile (PAP) method to detect hetero-resistance to vancomycin in *Staphylococcus aureus* in a UK hospital. J Antimicrob Chemother 2001; 47: 399-403.
15. 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 Antimicrobial Chemother 2009; 63: 489–92.
16. McDougal LK, Steward CD, Killgore GE, Chaitram JM, McAllister SK, Tenover FC. Pulsed-field gel electrophoresis typing of oxacillin-resistant Staphylococcus aureus isolates from the United States: establishing a national database. J Clin Microbiol 2003; 41: 5113-20.
17. Liu C, Chambers HF. *Staphylococcus aureus* with heterogeneous resistance to vancomycin: epidemiology, clinical significance, and critical assessment of diagnostic methods. Antimicrob Agents Chemother 2003; 47: 3040-5.
18. Maor Y, Hagin M, Belausov N, Keller N, Ben-David D, Rahav G. Clinical features of heteroresistant vancomycin-intermediate *Staphylococcus aureus* bacteremia versus those of methicillin-resistant S. aureus bacteremia. J Infect Dis 2009; 199: 619-24.
19. Howden BP, Johnson PD, Ward PB, Stinear TP, Davies JK. Isolates with low-level vancomycin resistance associated with persistent methicillin-resistant *Staphylococcus aureus* bacteremia. Antimicrob Agents Chemother 2006; 50: 3039-47.
20. Rong SL, Leonard SN. Heterogeneous vancomycin resistance in *Staphylococcus aureus*: a review of epidemiology, diagnosis, and clinical significance. Ann Pharmacother 2010; 44: 844-50.
21. Charles PG, Ward PB, Johnson PD, Howden BP, Grayson ML. Clinical features associated with bacteraemia due to heterogenous vancomycin-intermediate *Staphylococcus aureus* Clin Infect Dis 2004; 38:448-51.
22. Neoh HM, Hori S, Komatsu M *et al*. Impact of reduced vancomycin susceptibility on the therapeutic outcome of MRSA bloodstream infections. Annals of Clinical Microbiology and Antimicrobials 2007; 6:13. Available at: www.ann-clinmicrob.com/content/6/1/13.
23. Moise PA, Schentag JJ. Vancomycin treatment failures in *Staphylococcus aureus* lower respiratory tract infections. Int J of Antimicrob Agents 2000; 16 (Suppl 1): S31-4.
24. Hiramatsu K. Vancomycin resistance in staphylococci. Drug Resist Updates 1998; 1: 135-50.

25. Hidayat LK, Hsu DI, Quist R, Shriner KA, Wong-Beringer A. High-dose vancomycin therapy for methicillin-resistant *Staphylococcus aureus* infections: efficacy and toxicity. Arch Int Med 2006; 166: 2138-44.
26. Mohr JF, Murray BE. Point: Vancomycin is not obsolete for the treatment of infection caused by methicillin-resistant Staphylococcus aureus. Clin Infect Dis 2007; 44: 1536-42.
27. Ahmad N, Nawi S, Rajasekaran G *et al*. Increased vancomycin minimum inhibitory concentration among Staphylococcus aureus isolates in Malaysia. J Med Microbiol 2010; 59: 1530-2.
28. Brunet, F., Vedel, G., Dreyfus F *et al*. Failure of teicoplanin therapy in two neutropenic patients with staphylococcal septicemia who recovered after administration of vancomycin. Eur J of Clin Microbiol Infect Dis 1990; 9: 145-7.
29. Kaatz, GW, Seo SM, Dorman NJ, Lerner SA. Emergence of teicoplanin resistance during therapy of *Staphylococcus aureus* endocarditis. Journal of Infectious Diseases 1990; 162: 103-8.
30. Hanaki H, Kuwahara-Arai K, Boyle-Vavra S, Daum RS, Labischinski H, Hiramatsu K. Activated cell-wall synthesis is associated with vancomycin resistance in methicillin-resistant *Staphylococcus aureus* clinical strains Mu3 and Mu50. J Antimicrob Chemother 1998; 42: 199-209.
31. Norazah A, Salbiah N, Nurizzat M, Santhana, R. Vancomycin treatment failure in a vancomycin susceptible methicillin-resistant Staphylococcus aureus (MRSA) infected patient. Med J Mal 2009; 64: 166-7.
32. Horne KC, Howden BP, Grabsch EA *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.
33. Pina P, Marliere C, Vandenesch F, Bedos JP, Etienne J, Allouch PY. An outbreak of *Staphylococcus aureus* strains with reduced susceptibility to glycopeptides in a French general hospital. Clin Infect Dis 2000; 31: 1306-8.
34. Howden BP, Ward PB, Charles PG *et al*. Treatment outcomes for serious infections caused by methicillin-resistant *Staphylococcus aureus* with reduced vancomycin susceptibility. Clin Infect Dis 2004; 38: 521-8.
35. Tenover FC, Arbeit RD, Goering RV *x0* Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 1995; 33: 2233-9.