Daptomycin non-susceptibility in vancomycin-intermediate Staphylococcus aureus (VISA) and heterogeneous-VISA (hVISA): implications for therapy after vancomycin treatment failure

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Received 8 December 2010; returned 23 January 2011; revised 26 January 2011; accepted 8 February 2011

Objectives: The aim of this study was to establish the relationship between reduced vancomycin and daptomycin susceptibility among Australasian vancomycin-intermediate Staphylococcus aureus (VISA) and heterogeneous-VISA (hVISA) isolates from patients never exposed to daptomycin.

Methods: Forty-seven stored clinical isolates of hVISA/VISA collected before November 2008 from around Australia and New Zealand were selected. Daptomycin and vancomycin MIC testing was performed using broth microdilution (BMD) and Etest methods. Daptomycin population analysis was performed on a subset of isolates.

Results: The percentage of daptomycin non-susceptible isolates was 0% for vancomycin-susceptible S. aureus (VSSA) (Etest and BMD), for hVISA it was 26% by Etest and 15% by BMD, and for VISA 62% by Etest and 38% by BMD. Population analysis profile testing demonstrated daptomycin heteroresistance among the hVISA and VISA strains tested.

Conclusions: This is the highest rate of daptomycin non-susceptibility reported among hVISA isolates to date. Clinicians should exhibit caution when using daptomycin in situations where serious hVISA or VISA infection is a possibility.

Keywords: staphylococci, vancomycin resistance, daptomycin resistance, antibiotic resistance

Introduction

Vancomycin is commonly used to treat serious methicillin-resistant Staphylococcus aureus (MRSA) infections although treatment failures have been well described, requiring a switch to alternative antibiotics such as daptomycin.1,2 An association between reduced susceptibility to vancomycin, which often occurs after vancomycin treatment failure, and daptomycin non-susceptibility has been reported by several investigators.3–5

To date, daptomycin non-susceptibility has been described primarily among vancomycin-intermediate S. aureus (VISA, vancomycin MICs 4–8 mg/L) with low rates reported among heterogeneous-VISA (hVISA, vancomycin MICs in the susceptible range), which are much more common.2,4 Daptomycin was not released in Australia until November 2008. The aim of this study was therefore to establish the relationship between reduced vancomycin and daptomycin susceptibility among Australasian hVISA and VISA isolates from patients never exposed to daptomycin.

Materials and methods

Bacterial isolates

Forty-seven stored clinical isolates of hVISA/VISA collected before November 2008 from around Australia and New Zealand were selected. These isolates were previously defined as hVISA/VISA based on vancomycin broth MIC and vancomycin population analysis profile (PAP).2 Control MRSA strains (n = 20) were selected from the Australia–New Zealand co-operative on outcomes in staphylococcal sepsis (ANZCOSS) S. aureus bacteraemia study.5

Susceptibility testing

Daptomycin and vancomycin CLSI broth microdilution (BMD) MICs were determined in 96-well plates (100 μL/well) with BBL Mueller–Hinton II broth (Becton Dickinson), adjusted to contain calcium (50 mg/L) when testing daptomycin. Daptomycin susceptibility (MIC ≤ 1 mg/L) and non-susceptibility (MIC > 1 mg/L) were defined according to the CLSI breakpoints.5 Daptomycin and vancomycin MICs were also determined by
Etest (AB bioMérieux) on BBL Mueller–Hinton II agar (MHA; Becton Dickinson) plates according to the manufacturer’s instructions. Control strains Enterococcus faecalis ATCC 29212 (1–4 mg/L) and S. aureus ATCC 29213 (0.25–1 mg/L) were included with every analysis. Isolates were screened using glycopeptide resistance detection (GRD) Etests (AB bioMérieux) and classified into three phenotypes including: (i) vancomycin-susceptible S. aureus (VSSA) if they had a vancomycin BMD MIC $\leq 2$ mg/L with a GRD MIC $\leq 8$ mg/L of vancomycin and teicoplanin; (ii) hVISA if they had a vancomycin BMD MIC $\leq 2$ mg/L and a GRD MIC $\geq 8$ mg/L of either vancomycin and/or teicoplanin; and (iii) VISA if the vancomycin MIC was between 4 and 8 mg/L as determined by BMD.$^2$

**PAPs**

PAPs were performed for daptomycin as previously described$^{2,8}$ using MHA plates containing 50 mg/L calcium with daptomycin concentrations from 0 to 12.0 mg/L. Two vancomycin-susceptible MRSA strains (from ANZCOSS isolates) and S. aureus ATCC 29213 were included as controls.

**Results**

The 47 S. aureus isolates, which had previously been confirmed as hVISA or VISA, were assigned to three phenotypes after retesting (see Table S1, available as Supplementary data at JAC Online). In total, 15% [7/47, 6 MRSA and 1 methicillin-susceptible S. aureus (MSSA)] of the isolates were categorized as VSSA, 57% (27/47, including 3 MSSA) as hVISA, and 28% (13/47) as VISA. Vancomycin MICs ranged from 1.0 to 4.0 mg/L when tested by BMD and 1.5 to 6.0 mg/L by Etest. Daptomycin MICs ranged from 0.25 to 4 mg/L by BMD and 0.19 to 6.0 mg/L by Etest.

Table 1 shows the in vitro activity of daptomycin against the three S. aureus phenotypes. Increasing daptomycin MICs were observed as the vancomycin susceptibility was reduced, with the VISA isolates showing the highest daptomycin MIC results. The percentage of daptomycin non-susceptible isolates was 0% for VSSA (Etest and BMD), for hVISA it was 26% by Etest and 15% by BMD, and for VISA 62% by Etest and 38% by BMD. A total of 15 isolates were considered daptomycin non-susceptible according to Etest results; however, only nine had an MIC of $>1$ mg/L when tested by the reference BMD method, demonstrating a positive predictive value of only 60%.

Figure 1(a) shows the daptomycin Etest MICs for the three groups of isolates including the geometric mean. An additional group of vancomycin-susceptible MRSA isolates from the ANZCOSS study was included to provide a more representative
sample of daptomycin MICs. Using one-way analysis of variance (ANOVA) a significant difference in daptomycin geometric mean MICs was found when the hVISA and VISA isolates were compared with either the seven VSSA isolates (P=0.025) or with the ANZCOSS MRSA isolates (P<0.0001).

Daptomycin population analysis was performed on a subset of isolates, and confirmed daptomycin heteroresistance among the hVISA and VISA isolates (Figure 1b).

Discussion
Vancomycin remains the first-line agent for the treatment of serious MRSA infections, with clinical failure often prompting a change to alternative antibiotics such as daptomycin. In this study, the rate of daptomycin non-susceptibility among Austral-ian hVISA isolates never exposed to daptomycin is much higher than the rate of 0%–1.8% previously reported. While Patel et al. found that 3% of MRSA isolates with vancomycin MICs ≤2 mg/L were daptomycin non-susceptible, they did not comment on whether these isolates were hVISA. Among our VISA isolates the rate of daptomycin non-susceptibility was comparable to rates of 13.3%–80% reported by others. Similar to other studies there was no daptomycin non-susceptibility observed among the seven VSSA isolates.

Our results show a clear association between increasing vancomycin MICs and increasing daptomycin non-susceptibility. Other investigators have noted an in vitro relationship between vancomycin and daptomycin susceptibility in S. aureus, with isolates showing increased vancomycin MICs also demonstrating higher daptomycin MICs. Sakoulas et al. suggested that vancomycin exposure may induce physiological changes that influence daptomycin susceptibility. The clinical significance of this in vitro microbiological observation is unknown, and data from a recent retrospective observational study have shown no difference in daptomycin success rates against MRSA strains for which vancomycin MICs were <2 or ≥2 mg/L. Daptomycin’s activity is concentration dependent and so it is possible that higher doses may be required to treat hVISA and VISA strains for which daptomycin MICs are elevated although this requires further confirmation.

PAP testing demonstrated daptomycin heteroresistance among the hVISA and VISA strains. Daptomycin heteroresistance among S. aureus isolates has been previously reported although the clinical relevance remains unclear. The emergence of daptomycin non-susceptible subpopulations among high-inoculum infections and those in which there is poor antibiotic penetration is likely to be the first step in selecting S. aureus strains with higher-level daptomycin resistance.

There are a number of potential limitations to this study. Firstly, we intentionally selected S. aureus isolates that had previously been defined as hVISA or VISA by PAP testing; thus we investigated a heavily biased strain population. Nonetheless, a clear association between hVISA/VISA and reduced daptomycin susceptibility was demonstrated. The inclusion of randomly selected VSSA control strains from the ANZCOSS dataset provided a good comparator group representative of daptomycin MICs in Australian MRSA isolates. Secondly, a number of previously defined hVISA/VISA strains were found to be VSSA when retested using the GRD Tetest method from frozen storage. This method was selected as several studies have shown it has high sensitivity for detecting hVISA and VISA isolates when read after 48 h of incubation. The hVISA phenotype can be unstable and may be lost during storage, which may explain why these strains were found to be phenotypically VSSA on repeat testing. Interestingly, daptomycin MICs were higher for these strains than for control MRSA strains suggesting that some effect on daptomycin activity was retained. Finally, while the link between daptomycin non-susceptibility and hVISA/VISA is clearly demonstrated, the impact of these in vitro observations on daptomycin treatment outcome has not been defined.

In summary, using the reference BMD method we found that 15% of hVISA and 38% of VISA strains were daptomycin non-susceptible among an Australasian collection of hVISA/VISA isolates never exposed to daptomycin. This is the highest rate of daptomycin non-susceptibility reported among hVISA isolates to date. Vancomycin exposure may induce low-level daptomycin resistance or daptomycin heteroresistance although the precise mechanisms involved are not yet fully understood. The clinical significance of this in vitro microbiological observation is not known and it is unclear whether daptomycin should be avoided in patients who have failed prior vancomycin therapy. Thus, while uncertainty remains regarding the clinical impact of reduced daptomycin susceptibility we encourage caution when using daptomycin in situations where serious hVISA or VISA infection is a possibility. This includes high-bacterial-load infections or where prolonged glycopeptide therapy has failed to eradicate infection. In addition, it is important for diagnostic laboratories to have the capabilities to detect reduced vancomycin and daptomycin susceptibility in isolates from such patients.

Acknowledgements
We would like to thank Dr Natasha Holmes for supplying the 20 ANZCOSS isolates.

Funding
This work was supported by the Microbiology and Infectious Diseases Departments at Austin Health.

Transparency declarations
None to declare.

Supplementary data
Table S1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References


