

Daptomycin-nonsusceptible, vancomycin-intermediate, methicillin-resistant *Staphylococcus aureus* endocarditis

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Due to the emergence of *Staphylococcus aureus* with reduced vancomycin susceptibility, newer antibiotics, including daptomycin, have been used to treat methicillin-resistant *S aureus* infections. Daptomycin is a cyclic lipopeptide that is approved to treat *S aureus* bacteremia and right-sided endocarditis, and reports of *S aureus* with reduced susceptibility to daptomycin are infrequent. To our knowledge, the present report describes the first Canadian case of daptomycin-nonsusceptible, vancomycin-intermediate *S aureus* infection.

Key Words: Daptomycin nonsusceptible; Endocarditis; Methicillin-resistant *Staphylococcus aureus*; Vancomycin intermediate resistance

L'endocardite à *Staphylococcus aureus* résistant à la méthicilline, ayant une résistance intermédiaire à la vancomycine et sans sensibilité à la daptomycine

Étant donné l'émergence d'infections à *Staphylococcus aureus* peu sensibles à la vancomycine, de nouveaux antibiotiques, y compris la daptomycine, sont utilisés pour traiter les infections à *S aureus* résistant à la méthicilline (SARM). La daptomycine est un lipopeptide cyclique approuvé pour le traitement de la bactériémie à *S aureus* et de l'endocardite du cœur droit, et les déclarations de *S aureus* peu sensibles à la daptomycine sont peu fréquentes. En autant que nous le sachions, le présent rapport est le premier cas d'infection à *S aureus* ayant une résistance intermédiaire à la vancomycine et sans sensibilité à la daptomycine au Canada.

CASE PRESENTATION

A 75-year-old woman was admitted to a tertiary care hospital with chest pain, myalgia, arthralgia and acute-on-chronic renal failure requiring hemodialysis (HD). Her medical history included type II diabetes, obesity, hypertension, dyslipidemia, pulmonary hypertension and a bioprosthetic aortic valve, which was replaced four years before presentation. The patient's admission surveillance cultures of the nose and groin were positive for methicillin-resistant *Staphylococcus aureus* (MRSA). During the second month of her hospital stay, blood cultures grew MRSA for which she was treated with a nine-day course of intravenous vancomycin; a loading dose of 1 g and 300 mg following each HD session. Her vancomycin trough level five days after starting therapy was 4.8 mg/L. The vancomycin dose was increased to 500 mg following each HD; repeat trough levels were 13.5 mg/L, 7.7 mg/L and 15.5 mg/L on the fifth, eighth and 10th day, respectively, following the first trough level measurement. Despite vancomycin therapy, the patient remained febrile and multiple blood cultures grew MRSA. Due to the persistence of MRSA bacteremia, the antibiotic was changed to daptomycin (6 mg/kg every 48 h) on day 8 after the repeat positive blood culture results. A repeat blood culture performed at 72 h after initiating daptomycin was negative. A transesophageal echocardiogram demonstrated vegetative masses on the bioprosthetic aortic and native mitral valves. Two days after discontinuing the 37-day course of daptomycin, blood cultures grew MRSA. Due to the underlying multiple comorbidities and associated high surgical risks, the patient was deemed not to be a candidate for valve replacement. In the laboratory, the MRSA isolate from this episode showed a vancomycin minimum inhibitory concentration (MIC) of 4 mg/L and a daptomycin MIC >4 mg/L by Etest (bioMérieux Inc, USA) and Sensititre (TREK Diagnostics Inc, USA). Linezolid (600 mg orally, twice daily) was initiated but the patient expired shortly after starting therapy.

LABORATORY METHODS

Susceptibility testing was performed using the VITEK2 GP67 card (bioMérieux Inc, USA), Etest and Sensititre at the local laboratory. To confirm the increase in vancomycin MIC, the isolates were tested by the glycopeptides resistance determinant Etest and broth microdilution for vancomycin at the National Microbiology Laboratory (Winnipeg, Manitoba) and the Central Public Health Laboratory (Toronto, Ontario), respectively. Susceptibility testing results are summarized in Table 1.

Retrospectively, additional specialized susceptibility testing was performed to determine whether the heterogeneous vancomycin-intermediate *S aureus* (hVISA) phenotype developed during the course of antibiotic therapy. Phenotypically, only the isolate obtained after daptomycin administration showed reduced hemolytic activity on blood agar and the individual colonies significantly varied in size. When the modified population analysis profiling technique by Wooten et al (1) was used, the hVISA, daptomycin-nonsusceptible isolate had an area under the curve (AUC) ratio of 0.93 compared with a well characterized vancomycin-intermediate *S aureus* strain (VISA), *mu3* (American Type Culture Collection 700698). In contrast, the surveillance isolate had an AUC ratio of 0.09 compared with *mu3*, indicating that vancomycin heteroresistance was not an intrinsic property of these isolates. Genotypically, all isolates were characterized as *spa* type t032, ST-22, CMRSA-8 (EMRSA-15) and harboured *sccMec* Type IVc (2-4).

DISCUSSION

We described a case of persistent MRSA bacteremia with fatal endocarditis. Isolates were found to be both vancomycin- and daptomycin-susceptible initially by VITEK2, and subsequently by broth microdilution and/or Etest, albeit with each methodology yielding

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TABLE 1
Results of susceptibility testing using various methodologies to determine the presence of the heterogeneous vancomycin-intermediate *Staphylococcus aureus* phenotype

Isolate	Vancomycin MIC, mg/L					Daptomycin MIC, mg/L	
	VITEK2 ^{*,†}	Standard Etest ^{*,†}	Macro Etest ^{*,‡}	GRD Etest ^{*,‡}	BMD [§]	Etest ^{*,†}	BMD [¶]
Surveillance	0.5	1	2	1.5	2	1.0	Not done
Following nine days of vancomycin therapy	1	1	3	1.5	2	1.0	1.0
Following 37 days of daptomycin therapy	4	3	8	6	4	≥4.0	> 4

^{*}bioMérieux Inc, USA; [†]VITEK2 and standard Etest for vancomycin and daptomycin were performed at the Hamilton Regional Laboratory Medicine Program, Hamilton, Ontario; [‡]Etest using 2.0 McFarland inoculum (Macro Etest) and the glycopeptide resistance determinant (GRD) Etest was performed at the National Microbiology Laboratory, Winnipeg, Manitoba; [§]Broth microdilution (BMD) testing performed at the Ontario Agency for Health Protection and Promotion, Central Public Health Laboratory, Toronto, Ontario; [¶]BMD testing performed at the Hamilton Regional Laboratory Medicine Program using Sensititre (TREK Diagnostics Inc, USA). MIC Minimum inhibitory concentration

different MIC values. The variation in MIC values obtained for *S aureus* tested for vancomycin susceptibility by different methodologies has been well described and may mask the presence of hVISA subpopulations (5-7). The macro Etest for vancomycin is a more sensitive screening test for the detection of hVISA; however, false positives can occur and ideally should be confirmed by a gold standard method, such as population analysis profiling (1,7). The MIC for the macro Etest should not be reported because it may not reflect a true MIC (7).

In our patient, before initiating daptomycin, and while on vancomycin therapy, the MIC for vancomycin increased from 1 mg/L to 2 mg/L (data not shown). Moreover, the vancomycin trough level was lower than the recommended level of 15 mg/L to 20 mg/L during the majority of the duration of vancomycin therapy, reflecting the difficulty in dosing patients on HD (8). Adjustment of the vancomycin dose to obtain a vancomycin trough level of 15 mg/L to 20 mg/L is recommended for serious MRSA infections, especially in patients who are morbidly obese or have renal dysfunction (8,9). A low vancomycin trough level in the present case may also have contributed to the development of heteroresistance, which has been described (10). Following the five-week course of daptomycin, the isolates were vancomycin-intermediate (MIC=4 mg/L, by VITEK2 and broth microdilution) and daptomycin-nonsusceptible (MIC ≥4 mg/L) as defined by the Clinical and Laboratory Standards Institute standards (11). Similar cases of daptomycin-nonsusceptible, vancomycin-intermediate MRSA have been reported in the literature; however, to date none have been reported in Canada (12-18).

The Infectious Diseases Society of America recommends consideration of the use of high-dose daptomycin (10 mg/kg/day) in combination with an additional agent for management of persistent MRSA bacteremia in a setting of vancomycin treatment failure (9).

Prolonged monotherapy with vancomycin may select *S aureus* with reduced susceptibility to both vancomycin and daptomycin (19). Vancomycin is a large glycopeptide antibiotic that inhibits the formation of nascent peptidoglycan in most Gram-positive bacteria by binding to the D-alanyl-D-alanine residues of murein monomers in the cytoplasmic membrane, thereby preventing their use as substrates in polymerization reactions by glycosyltransferase (18,20). The genetic basis for the development of hVISA/VISA is not well understood; however, these isolates develop thickened cell walls that may trap greater quantities of vancomycin compared with susceptible *S aureus* strains (21). Trapped vancomycin molecules, in turn, may limit further diffusion of vancomycin into the inner layers of the cell wall by 'clogging' the mesh network of the outer layers of the cell wall (8,22). Other cell wall-associated changes, including a reduction in peptidoglycan cross-linking and reduced muramic acid O-acetylation have also been observed (10). Alterations in cell membrane fluidity and surface charge may also contribute to the hVISA/VISA phenotype (23). Taken together, these cell wall and membrane changes may hinder the diffusion of daptomycin to the cytoplasmic membrane, preventing the calcium-dependent membrane depolarization and resultant cell lysis. Cross resistance between vancomycin and daptomycin has been previously observed (19).

The emergence of hVISA/VISA during treatment with vancomycin is difficult for many clinical laboratories to detect because specialized testing methods are required (5-7). Given that the phenotypic basis for the development of hVISA/VISA is a thickened cell wall with cell membrane alterations, careful monitoring of susceptibility to other antibiotics, such as daptomycin, is warranted. Clinicians should be vigilant for the development of resistance to daptomycin in a setting of MRSA bacteremia that persists despite vancomycin therapy (24). Standard susceptibility testing by Etest or macro Etest is a useful screening test for hVISA but should be confirmed by population analysis profiling.

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