CASE REPORT

Methicillin-resistant *Staphylococcus aureus* endocarditis and de novo development of daptomycin resistance during therapy

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Daptomycin resistance in *Staphylococcus aureus* has been previously reported, but the development of resistance while on therapy with subsequent clinical failure for endocarditis has been infrequently reported. A case of persistent methicillin-resistant *S aureus* (MRSA) bacteremia in the setting of right-sided endocarditis in a 38-year-old man with a history of intravenous drug use is presented. He developed de novo resistance to daptomycin during therapy after several courses of antibiotics, with subsequent clinical failure. Isolates were identified by molecular characterization to be community-acquired MRSA 10 (USA300). To the authors' knowledge, the present case was the first in Canada to involve the de novo development of daptomycin resistance with clinical failure due to MRSA during therapy for endocarditis. Clinicians and microbiologists must be aware of this phenomenon given the implications for treatment and transmission of the strain. It also raises questions regarding the use of daptomycin in settings of heavily pretreated patients with persistent MRSA bacteremia.

Key Words: *Daptomycin; Endocarditis; MRSA; Resistance*

L'endocardite à *Staphylococcus aureus* résistant à la méthicilline et l'apparition *de novo* d'une résistance à la daptomycine pendant le traitement

On a déjà rendu compte de résistance à la daptomycine en présence d'un *Staphylococcus aureus*, mais l'apparition d'une résistance en cours de traitement entraînant un échec clinique de l'endocardite l'a été plus rarement. Est présenté un cas de bactériémie à *Staphylococcus aureus* résistant à la méthicilline (SARM) persistante d'une endocardite droite chez un homme de 38 ans ayant des antécédents de consommation de drogues intraveineuses. Cet homme a développé une résistance *de novo* à la daptomycine après plusieurs séries de traitements antibiotiques, qui ont été suivies d'un échec clinique. La caractérisation moléculaire a déterminé que les isolats étaient un SARM 10 (USA300) d'origine non nosocomiale. En autant que les auteurs le sachent, le présent cas était le premier au Canada caractérisé par l'apparition *de novo* de résistance à la daptomycine accompagnée d'un échec clinique causé par le SARM pendant un traitement contre l'endocardite. Les cliniciens et les microbiologistes doivent être au courant de ce phénomène étant donné les répercussions sur le traitement et la transmission de la souche. Ce phénomène soulève également des questions au sujet de l'utilisation de daptomycine chez des patients ayant une bactériémie à SARM persistante soumis à un lourd traitement préalable.

Endocarditis and bacteremia are devastating infections associated with mortality rates of between 16% and 25% of affected individuals (1,2). *Staphylococcus aureus* represents the most common etiological agent in bacterial endocarditis in developed countries. In a recent observational cohort of 1779 patients with infectious endocarditis, *Staphylococcus aureus* was the most common pathogen and methicillin-resistant *S aureus* (MRSA) was identified in more than 25% of the cases (3). Treatment options for bacteremia and endocarditis caused by MRSA are more limited than methicillin-sensitive *S aureus*. Vancomycin, the standard therapy for bloodstream infections attributable to MRSA, has been associated with suboptimal outcomes (4,5); new agents for the treatment of *S aureus* bacteremia and endocarditis are needed. Daptomycin, a fermentation product of *Streptomyces roseosporus*, is a cyclic lipopeptide antibiotic with potent bactericidal activity against most Gram-positive organisms, including multiple antibiotic-resistant strains (6). It has a novel mechanism of action – insertion into and disruption of the functional integrity of the Gram-positive plasma membrane – resulting in rapid loss of membrane potential, cessation of macromolecular synthesis and cell death (7). The United States Food and Drug Administration approved daptomycin (4 mg/kg) in 2003 for the treatment of complicated skin and soft tissue infections caused by susceptible strains of *S aureus*, including MRSA strains and other Gram-positive bacteria (8) and, in May 2006 (6 mg/kg), for the treatment of *S aureus* bloodstream infections including right-sided infective endocarditis (9). In 2006, it was found that a dose of 12 mg/kg/day was well-tolerated and may be considered for the treatment of difficult-to-treat infections (10). Daptomycin resistance in *S aureus* has been rarely reported previously (11-13), but the development of resistance while on therapy with subsequent clinical failure during treatment for endocarditis has been infrequently documented (14,15).

A case of persistent MRSA bacteremia is presented in the setting of right-sided endocarditis, septic pulmonary emboli and empyema with de novo resistance development to daptomycin during therapy, with subsequent clinical failure.

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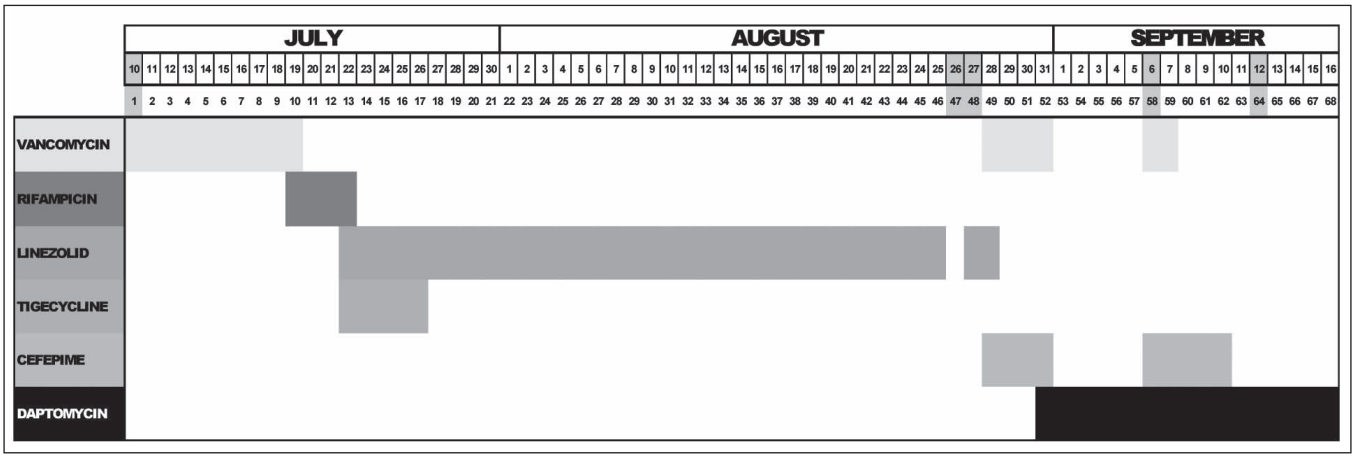

Figure 1) *Flow chart of antimicrobial use before implementation of the successful combination of gentamicin, ertapenem and linezolid*

CASE PRESENTATION

A 38-year-old man with a history of polysubstance abuse and hepatitis C presented to the emergency department with a 10-day history of pleuritic chest pain, intermittent fever and chills, cough as well as dyspnea. His chest x-ray and computed tomography scan revealed findings suggestive of a large right-sided empyema, bilateral pulmonary infiltrates compatible with pneumonia and septic emboli. Blood, sputum and pleural fluid cultures on day 1 were positive for MRSA resistant to erythromycin and sensitive to trimethoprim-sulfamethoxazole, clindamycin, vancomycin (minimum inhibitory concentration [MIC] of 1.0 mg/L) and daptomycin (MIC of 0.094 mg/L). During the patient's hospitalization, he required four chest tubes to drain bilateral loculated pleural empyemas. They were inserted on days 1, 3 and 5, and removed on days 12, 20, 60 and 82. On day 3, he was transferred to the intensive care unit for five days because of respiratory distress; the patient required mechanical ventilation for three days. Persistent bacteremia due to MRSA was documented from day 1 to day 14. During this period, the patient was treated with different combinations of antibiotics including vancomycin, azithromycin and ceftriaxone for two days; vancomycin alone for seven days; and vancomycin, rifampicin and trimethoprim-sulfamethoxazole for three days. The bacteremia was cleared for 33 days, during which time he was on linezolid and tigecycline for four days and linezolid alone for 29 days. Cefepime was used empirically in combination with vancomycin and daptomycin after a recurrence of the bacteremia was documented (Figure 1). His initial transesophageal echocardiograms at days 3 and 16 were normal; transesophageal echocardiograms at days 41, 43 and 51 showed vegetations on the atrial side of the anterior tricuspid valve leaflet, moderate tricuspid regurgitation and moderate pulmonary hypertension. From days 47 to 64, the patient was again bacteremic. After assessment, he was not considered to be a surgical candidate by the cardiovascular surgery unit. On day 51, daptomycin was started at a dose of 6 mg/kg/day and six days later, the dose was increased to 12 mg/kg/day, at which time the isolate was determined to be sensitive by E-testing (MIC of 0.125mg/L). Six days later, the patient was persistently bacteremic, and daptomycin was discontinued. The bacteremia cleared after two days of therapy with gentamicin, linezolid and ertapenem. A transesophageal echocardiogram at day 62 demonstrated a vegetation on the tricuspid valve that extended to the anterior chordae with a 2 cm annular abscess with severe tricuspid regurgitation. The patient was maintained on the triple combination of gentamicin, linezolid and ertapenem for the remainder of his hospitalization and gradually recovered with no further episodes of bacteremia. All pleural drains were removed after 82 days, and the patient was discharged home after 111 days of hospitalization. On follow-up over the ensuing 12 months, the patient remained stable without evidence of recurrent illness. A repeat echocardiogram revealed a tricuspid valve vegetation 2.4 cm in size and moderate to severe tricuspid regurgitation.

METHODS

Isolates were collected from days 1 (pretreatment), 47 and 48 (postvancomycin and predaptomycin), 58 and 64 (during daptomycin treatment) and were submitted for detailed phenotypic and molecular characterization to ensure that the isolates were identical. MRSA was identified using standard laboratory procedures and confirmed by polymerase chain reaction assay for *nuc*, *femA* and *mecA*. MICs were determined by E-testing, according to the Clinical and Laboratory Standards Institute guidelines, in duplicate and with higher inoculum, and correlated with a daptomycin growth inhibition assay (16). A MIC breakpoint of 1 mg/L or less to daptomycin was considered to be susceptible, and a breakpoint of greater than 1 mg/L was considered to be nonsusceptible. The isolates were further characterized by pulsed-field gel electrophoresis (17), *SCCmec* typing (18), staphylococcal protein A typing (19), accessory gene regulator typing (20), multilocus sequence typing (21), and polymerase chain reaction assays for the Panton-Valentine leukocidin genes (22), CMRSA 10 (USA300) strain molecular markers of the arginine catabolic mobile element and prophage ΦSa2usa/ΦSa2mw (23). No additional testing was performed to assess either further resistance inducibility or the mechanisms of resistance.

RESULTS

On day 1 before therapy, the isolates were sensitive to both vancomycin and daptomycin, with MICs of 1.0 mg/L and 0.094 mg/L, respectively. On days 47 and 48 (Figure 1) including 12 days of vancomycin, the isolates remained susceptible to both drugs (MICs of 1.0 mg/L and 0.125 mg/L for vancomycin and daptomycin,

TABLE 1
Susceptibility testing (E-test) of patient methicillin-resistant *Staphylococcus aureus* isolates before and during vancomycin and pre- and postdaptomycin

Sample date	Vancomycin, MIC, mg/L	Daptomycin, MIC, mg/L	Therapy
Day 1	1.0 (S)	0.094 (S)	No antibiotics
Day 47	1.0 (S)	0.125 (S)	12 days vancomycin
Day 48	1.0 (S)	0.125 (S)	12 days vancomycin
Day 58	2.0 (S)	1.5 (R)	15 days vancomycin + 6 days daptomycin
Day 64	2.0 (S)	1.5 (R)	15 days vancomycin + 12 days daptomycin

MIC Minimum inhibitory concentration; R Resistant; S Susceptible

respectively). However, on days 58 and 64, the MRSA isolates from the patient remained sensitive to vancomycin (MIC of 2.0 mg/L) in comparison with the original isolates, but became nonsusceptible to daptomycin, with a MIC of 1.5 mg/L (Table 1). A daptomycin growth inhibition assay demonstrated correspondingly greater growth for the isolates from the latter time points, with reduced susceptibility to daptomycin. Molecular testing and pulsed-field gel electrophoresis analysis revealed that all five isolates derived from different time points were identical and were CMRSA 10 (USA300) with *mecA*+, Panton-Valentine leukocidin positive, arginine catabolic mobile element positive, ΦSa2usa phage positive, and accessory gene regulator type I multilocus sequence type ST8, but staphylococcal protein A type t024, and carried SCC*mec* type IVa (Figure 2).

DISCUSSION

The daptomycin nonsusceptible mutation frequency in *S aureus* is believed to be low (24). It has been suggested that reduced susceptibility to daptomycin is associated with MRSA strains with reduced susceptibility to vancomycin (heterogeneous vancomycin-intermediate *S aureus* [VISA] and VISA strains) (25-29). Previous studies (30,31) have indicated that VISA strains produce a thickened cell wall due to the excess production of peptidoglycan, which prevents the penetration of vancomycin. The daptomycin susceptibilities of these strains were found to be reduced compared with those of nonheterogeneous VISA and non-VISA isolates. Of interest, several investigations (29,32,33) have also demonstrated that daptomycin retains its bactericidal activity against these strains. Several investigators have examined the phenomenon of previous vancomycin exposure and its role in daptomycin nonsusceptibility.

In a study by Sakoulas et al (28), isolates with reduced susceptibility to vancomycin or the potential for developing reduced susceptibility were evaluated for reduced cross-susceptibility. Population analysis profiles of clinical isolates recovered from patients exposed to vancomycin during therapy revealed that after vancomycin exposure, three of four isolates concomitantly displayed vancomycin and daptomycin heteroresistance in vivo. These findings suggested that in the clinical setting of exposure to vancomycin, changes occur within the MRSA strains that influence daptomycin susceptibility (28).

Rose et al (24) evaluated five clinical isolates of *S aureus* (four MRSA isolates and one methicillin-susceptible *S aureus* isolate) with reduced susceptibility to daptomycin following vancomycin therapeutic exposure in an in vitro pharmacodynamic

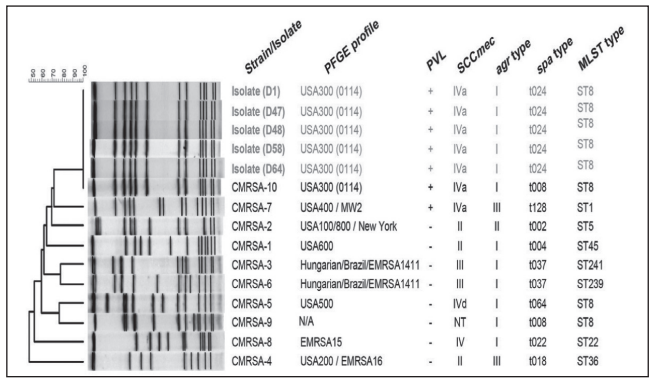

Figure 2) *Molecular characterization of methicillin-resistant* Staphylococcus aureus *(MRSA) isolates*. agr *Accessory gene regulator; CMRSA Community-associated MRSA; EMRSA Epidemic MRSA; MLST Multilocus sequence typing; N/A Not available; PFGE Pulsed-field gel electrophoresis; PVL Panton-Valentine leukocidin;* spa *Staphylococcal protein A*

model. Results showed that bactericidal activity was maintained across all daptomycin regimens used in the model regardless of previous vancomycin exposure. No change in MIC was detected for any MRSA isolate treated with daptomycin following vancomycin exposure. The authors suggested that some strains are more likely than others to lose susceptibility to daptomycin.

Sakoulas et al (34) examined sequential bloodstream isolates from a patient with community-onset native valve endocarditis due to methicillin-susceptible *S aureus*, in which rapid loss of susceptibility to daptomycin (MIC of greater than 1 g/L) occurred with accompanying treatment failure, following initial treatment with vancomycin. These isolates were further examined in an in vitro pharmacodynamic model, which demonstrated that reduced killing was observed before a rise in daptomycin MIC to the nonsusceptible range – this would not be detected by routine laboratory investigation. The authors hypothesized that vancomycin affects subpopulations of *S aureus* within settings in which a higher bacterial inoculum exists, thereby affecting daptomycin susceptibility (34).

The emergence of daptomycin nonsusceptibility has also been documented in the absence of vancomycin exposure. Fowler et al (15) reported an increase in the MICs of daptomycin to *S aureus* in seven isolates (five MRSA), with increases to 2 mg/L or greater in six patients who were randomly assigned to daptomycin only. The mechanism of daptomycin nonsusceptibility in *S aureus* is not completely understood, but has been recently described in association with alterations in surface charge, membrane phospholipid asymmetry and drug binding (35). Friedman et al (36) demonstrated a series of genetic perturbations induced by serial in vitro passage in *S aureus* isolates obtained from patients failing daptomycin therapy, including mutations in the *mprF* gene that contribute to membrane charge through lysinylation of peptidoglycan; the *yycG* histidine kinase gene, which has multiple functions including impact on membrane fatty acid biosynthesis; and *rpoB* and *rpoC*, which are subunits of RNA polymerase.

Kaatz et al (37) reported the emergence of daptomycin resistance in a patient with MRSA tricuspid valve endocarditis who failed daptomycin treatment, in which the daptomycin-resistant

strain exhibited reduced daptomycin binding to both whole cells and cytoplasmic membranes, and reduced membrane depolarization. The resistant isolates demonstrated the loss of an 81-kDa membrane protein postulated to represent a daptomycin membrane 'chaperone' (37). Silverman et al (7) demonstrated that some daptomycin-resistant bacterial strains selected in vitro have altered membrane potential – a feature shared by selected antimicrobial peptide-resistant *S aureus* strains.

Antimicrobial combination therapy consisting of linezolid, ertapenem and gentamicin was administered with the knowledge that linezolid combined with gentamicin and linezolid combined with ertapenem exhibits highly effective bactericidal activity against strains of MRSA in experimental in vivo endocarditis models (38,39).

To our knowledge, the present case is the first to be reported in Canada regarding the de novo development of daptomycin resistance with clinical failure to MRSA during endocarditis therapy. Clinicians and microbiologists must be aware of this phenomenon given the implications for treatment and transmission of the strain. It also raises questions regarding the use of daptomycin in settings of heavily pretreated patients or high bioburden and/or biofilm disease with MRSA, such as our patient who had bilateral empyemas and an annular abscess of the tricuspid valve.

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