Case Report

Daptomycin non-susceptible meticillin-resistant *Staphylococcus aureus* USA 300 isolate

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Daptomycin is a novel bactericidal agent active against Gram-positive pathogens including meticillin-resistant *Staphylococcus aureus* (MRSA). Our case is unique in the description of an MRSA USA 300 isolate that developed decreased susceptibility to daptomycin during daptomycin and vancomycin therapy. Directed sequencing detected a previously reported mutation in *mprF*, resulting in a T345A substitution, associated with non-susceptibility to daptomycin.

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Case report

A 40-year-old white male with a previous history of intravenous drug abuse and hepatitis C was admitted to a local hospital for fever and shortness of breath. He was empirically treated with intravenous vancomycin (1 g i.v. q.12 h) starting 11/11/2006 (dates in American format throughout). The first set of blood cultures drawn on the day of admission turned positive after 12 h incubation. A subsequent transoesophageal echocardiogram showed native aortic valve endocarditis.

The first set of blood cultures grew meticillin-resistant *Staphylococcus aureus* (MRSA) with a vancomycin MIC of $\leq 2 \mu\text{g ml}^{-1}$ (Microscan; Dode-Behring). The patient had repeat blood cultures on 11/17/2006, which were persistently positive for MRSA (with blood cultures turning positive after 20 h incubation), after 5 days of vancomycin therapy. No serum vancomycin levels were available from the local hospital.

Vancomycin was discontinued and intravenous daptomycin 6 mg kg^{-1} per day was started on 11/17/2006 at the local hospital. No baseline serum creatine phosphokinase level results were available. A computed tomography scan of the head to evaluate agitation revealed right temporal lesions, which were thought to be embolic in nature. The third set of repeat blood cultures dated 11/21/2006 also turned positive for MRSA after 12 h incubation.

The patient was transferred to our facility on 11/24/2006 with persistent MRSA bacteraemia, aortic valve endocarditis and intracranial haemorrhagic septic emboli. On examination, he was found to be tachypnoeic and in moderate respiratory distress with a respiratory rate of 44 min^{-1} , pulse 131 beats min^{-1} , blood pressure 135/

83 mmHg, temperature 37 °C and weight of 176 lbs. Laboratory results showed a white blood cell count of 2200 cells μl^{-1} , with 86 % polymorphonuclear leukocytes; a platelet count of 73 000 cells μl^{-1} ; blood urea nitrogen level of 52 mg dl^{-1} ; creatinine level of 2.2 mg dl^{-1} (baseline creatinine 1.4); and bicarbonate level of 14 mg dl^{-1} . His estimated creatinine clearance was 47.5 ml min^{-1} at the time of transfer.

Chest X-ray showed a mild cardiomegaly with bilateral oedema and basilar opacities. The patient was later intubated for respiratory distress and airway protection. Review of the blood culture and susceptibility testing results (11/11, 11/17, 11/21) from the local hospital revealed that the MRSA isolates were susceptible to vancomycin with an MIC of $\leq 2 \mu\text{g ml}^{-1}$. Daptomycin therapy was discontinued on the day of transfer, and vancomycin (15 mg kg^{-1} i.v. q.12 h) and gentamicin (1 mg kg^{-1} i.v. q.24 h) were initiated.

Blood cultures drawn at our facility on 11/24/2006 turned positive after 9 h incubation with cultures finalized as MRSA. Review of the susceptibility results at our facility (Microscan; Dode-Behring) showed that the MRSA isolate (REF2145) was susceptible to vancomycin (MIC $\leq 2 \mu\text{g ml}^{-1}$), linezolid (MIC $\leq 2 \mu\text{g ml}^{-1}$), quinopristin/dalfopristin (MIC $\leq 1 \mu\text{g ml}^{-1}$), trimethoprim-sulfamethoxazole (MIC $\leq 2 \mu\text{g ml}^{-1}$) and gentamicin (MIC $\leq 4 \mu\text{g ml}^{-1}$), but non-susceptible to daptomycin (MIC 4 $\mu\text{g ml}^{-1}$). The susceptibility results were unchanged among all the isolates except for the daptomycin MIC in the REF2145 isolate. The E-test (AB Biodisk) confirmed the daptomycin MIC of 4 $\mu\text{g ml}^{-1}$. Vancomycin E-test results demonstrated that all four MRSA isolates (11/11, 11/17, 11/21 and REF2145) had vancomycin MICs of 2 $\mu\text{g ml}^{-1}$. Vancomycin and gentamicin trough levels were monitored

Abbreviation: MRSA, meticillin-resistant *Staphylococcus aureus*.

and dose adjustments were made to improve renal function (creatinine clearance $>50 \text{ ml min}^{-1}$). Vancomycin and gentamicin trough levels were in the range of $15\text{--}22 \mu\text{g ml}^{-1}$ and $1\text{--}2 \mu\text{g ml}^{-1}$, respectively.

Neurosurgical and cardiothoracic surgery evaluation were obtained. A magnetic resonance angiogram and a diagnostic cerebral angiogram were unremarkable for arteriovenous malformations or aneurysms. Rifampicin (600 mg per day) was added to the vancomycin and gentamicin regimen for persistent fevers and MRSA bacteraemia. All the intravascular devices were changed.

The patient underwent aortic valve replacement with an Edwards-Magna pericardial prosthesis (on 12/1/2006) following 7 days of antibiotic therapy (vancomycin, gentamicin and rifampicin) at our facility. His repeat blood cultures post-surgery remained negative from 12/3/2006 onwards. Rifampicin and gentamicin were discontinued after a total of 2 weeks of therapy and vancomycin was continued for a total of 6 weeks. He was successfully weaned off the ventilator and subsequently underwent physical therapy and recovered with no neurological deficits.

The three additional MRSA blood culture isolates (11/1, 11/17 and 11/21) from the patient were available from the local hospital for further evaluation. This investigation demonstrated that the MIC for daptomycin was also elevated in one of these three isolates. Using E-test methodology, the MRSA isolates dated 11/11 (MRSA 11/11) and 11/17 (MRSA 11/17) had MICs to daptomycin of $1.0 \mu\text{g ml}^{-1}$. However, the MRSA isolate dated 11/21 (MRSA 11/21) had an MIC of $3 \mu\text{g ml}^{-1}$ to daptomycin. Using previously published methodology (Bannerman *et al.*, 1995), all four isolates (MRSA 11/11, MRSA 11/17, MRSA 11/21 and REF2145) were indistinguishable by PFGE (data not shown). Subsequent comparative analysis (McDougal *et al.*, 2003; Bionumerics software, Applied Maths) demonstrated that the *Sma*I restriction pattern of the four MRSA isolates, including REF2145, was consistent with USA 300. In agreement with these data, using previously published primers and methods, REF2145 was also shown to contain the Pantone-Valentine leukocidin genes by PCR (Fey *et al.*, 2003).

Oligonucleotide primers were designed to detect known mutations associated with reduced susceptibility to daptomycin (Friedman *et al.*, 2006) in MRSA 11/11 (MIC=1 for daptomycin) and REF2145 (MIC=4 for daptomycin). These primers were designed to detect known single nucleotide changes in open reading frames of *mprF* (lysylphosphatidylglycerol synthetase), *yycG* (histidine kinase), *rpoB* (β -subunit of RNA polymerase) and *rpoC* (β' -subunit of RNA polymerase) (Friedman *et al.*, 2006). Only the mutation T345A in *mprF* was detected in REF2145 as compared to MRSA 11/11. This mutation has been previously detected in daptomycin non-susceptible isolates (Friedman *et al.*, 2006).

Discussion

We describe a patient with persistent MRSA bacteraemia while on daptomycin therapy. The MRSA isolates were noted to be community-associated as the patient had no exposure to health care in the preceding 1 year of the current hospitalization. Furthermore, PFGE analysis demonstrated that the genetic background of all four isolates was indistinguishable from USA 300, a common community-associated MRSA genetic background (Diep *et al.*, 2006). In addition, two of the isolates (REF2145 and MRSA 11/21) were noted to be non-susceptible to daptomycin with MICs of $4 \mu\text{g ml}^{-1}$ and $3 \mu\text{g ml}^{-1}$, respectively.

Daptomycin is a semi-synthetic cyclic lipopeptide derived from *Streptomyces roseosporus* with a susceptibility breakpoint of $\leq 1 \mu\text{g ml}^{-1}$ for methicillin-sensitive *S. aureus* and MRSA and $\leq 4 \mu\text{g ml}^{-1}$ for vancomycin-susceptible *Enterococcus faecalis* (CLSI, 2007; Eisenstein, 2004). The mechanism of bactericidal action involves calcium-dependent entry into the bacterial cytoplasmic membrane, causing depolarization and potassium ion leakage (Alborn *et al.*, 1991). This membrane disruption results in cessation of macromolecular synthesis and subsequent bacterial cell death (Silverman *et al.*, 2003).

Development of decreased susceptibility to daptomycin during long-term therapy has been previously reported in patients with MRSA bacteraemia (Mangili *et al.*, 2005). Similar reports of daptomycin resistance and treatment failure during daptomycin therapy for MRSA bacteraemia and osteomyelitis have also been described (Skiest, 2006; Marty *et al.*, 2006).

Several recent reports have focused on the elucidation of the mechanism of resistance to daptomycin within *S. aureus*. The loss of an 81 kDa membrane protein correlated with decreased susceptibility to daptomycin (Kaatz *et al.*, 2006). In addition, loss of this membrane protein was correlated with decreased binding of daptomycin to isolated cell membranes and resistance to daptomycin-mediated membrane potential dissipation. A recent report from Jones *et al.* (2008) has corroborated these data demonstrating significant changes in membrane structure/function in daptomycin non-susceptible isolates. In addition, vancomycin-intermediate *S. aureus* (VISA) isolates have reduced susceptibility to daptomycin; a potential explanation is that the thicker cell walls associated with VISA strains may provide a physical barrier for the daptomycin (Cui *et al.*, 2006). Also, previous exposure of *S. aureus* to vancomycin and/or daptomycin may also affect daptomycin susceptibility (Sakoulas *et al.*, 2006). Finally, using complete genome comparison, Friedman *et al.* (2006) demonstrated that mutations within four genes, *mprF*, *yycG*, *rpoB* and *rpoC*, correlate with decreased susceptibility to daptomycin. In agreement with this report, we identified one mutation (T345A) in REF2145 as compared to MRSA 11/11. The contribution of each one

of these genes to the mechanism of decreased susceptibility to daptomycin is not known at this time.

CA-MRSA USA 300 has been identified as the major cause of epidemic MRSA skin and soft-tissue infections in the United States in addition to more serious infections such as necrotizing pneumonia (Diep *et al.*, 2006). The USA 300 genotype is preliminarily distinguished from other MRSA backgrounds by the presence of Panton–Valentine leukocidin and the ACME (arginine catabolic mobile element) island containing the arginine deiminase gene cluster (Diep *et al.*, 2006; Naimi *et al.*, 2003). Fortunately, CA-MRSA USA 300 isolates generally remain susceptible to clindamycin, doxycycline, trimethoprim–sulfamethoxazole and also fluoroquinolones.

Development of daptomycin non-susceptible MRSA isolates in both hospital-associated and community-associated settings is a concern. Further studies are needed to provide a better insight into the development of daptomycin non-susceptibility in *S. aureus*.

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