

Case Report

Six cases of daptomycin-non-susceptible *Staphylococcus aureus* bacteraemia in Singapore

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We report what we believe to be the first six cases of daptomycin-non-susceptible *Staphylococcus aureus* infections from Singapore. These strains were rapidly isolated after bacteraemic patients were switched to daptomycin following initial prolonged unsuccessful therapy with vancomycin, despite confirmation of daptomycin susceptibility just prior to initiating daptomycin therapy. The majority of post-vancomycin therapy strains exhibited marked thickening of their cell walls on electron microscopic examination. In patients with persistent *S. aureus* bacteraemia, therapeutic failure with daptomycin may occur if used as salvage therapy following vancomycin failure, notwithstanding initial susceptibility testing results.

Introduction

Daptomycin is a cyclic lipopeptide that has received approval by the US Food and Drug Administration for the treatment of complicated skin and soft tissue infections and *Staphylococcus aureus* bacteraemia (Cubist Pharmaceuticals, 2008). Prior vancomycin exposure potentiates the development of daptomycin non-susceptibility in *S. aureus in vitro* (Camargo *et al.*, 2008; Cui *et al.*, 2006; Sakoulas *et al.*, 2006). In particular, Camargo and coworkers demonstrated overlapping phenotypic and gene expression profiles between daptomycin-non-susceptible and vancomycin-intermediate *S. aureus* (Camargo *et al.*, 2008). However, clinical outcomes have generally been more favourable, with relatively few publications of therapeutic failure to date regardless of whether daptomycin was used as first- or second-line therapy (Moise *et al.*, 2009; Sakoulas *et al.*, 2009).

Daptomycin was approved for use in Singapore in 2008 and has seen gradually increasing prescription primarily as a second-line agent for severe meticillin-resistant *S. aureus* (MRSA) infections, largely because of its considerable cost difference compared to vancomycin. We report what we believe to be the first six cases of daptomycin-non-susceptible *S. aureus* infection from three public hospitals in Singapore, and describe the phenotypic and molecular characteristics of the isolates. These cases were identified by clinician self-reporting and review of microbiology labo-

ratory records from local public hospitals. Clinical data for the subjects were obtained via retrospective chart review, while available bacterial isolates were further evaluated at a central laboratory.

Case reports

The clinical characteristics of the cases and bacterial profiles of the isolates are described below. All subjects had multiple comorbidities, and had received vancomycin for at least a week, with trough levels exceeding 10 µg ml⁻¹. Because daptomycin susceptibility testing is not routinely performed locally, all subjects had received appropriately dosed daptomycin [at 6 mg (kg body weight)⁻¹ per day or every other day depending on creatinine clearance] as second-line therapy for an extended period despite rapid development of daptomycin non-susceptibility.

Case 1

A 78-year-old woman with type 2 diabetes mellitus, haemodialysis-dependent renal failure, aortic stenosis and vasculopathy with multiple intra-arterial stents presented with fever secondary to MRSA bacteraemia to Hospital A. Her blood cultures were repeatedly positive for MRSA despite appropriately dosed vancomycin for 19 days followed by 15 days of daptomycin. She was finally switched to oral linezolid but died shortly thereafter. Transoesophageal echocardiography did not reveal any vegetations. She had persistent MRSA bacteraemia for 36 days.

Abbreviations: BORSA, borderline oxacillin-resistant *S. aureus*; hVISA, vancomycin-heteroresistant *S. aureus*; MRSA, meticillin-resistant *S. aureus*.

Case 2

A 61-year-old man with type 2 diabetes mellitus and vasculopathy presented to Hospital A with fever and an infected necrotic left foot ulcer. Blood and wound cultures grew MRSA, and he underwent below-knee amputation in addition to i.v. vancomycin. Because of persistent bacteraemia, he was switched to i.v. daptomycin after 7 days of vancomycin. Transthoracic echocardiography revealed moderate mitral valve regurgitation with a 1.0 cm vegetation. He died abruptly after having received 11 days of daptomycin therapy. The bacteraemia never cleared.

Case 3

A 28-year-old woman with type 1 diabetes mellitus, haemodialysis-dependent renal failure and a prosthetic mitral valve presented with prosthetic valve endocarditis secondary to borderline oxacillin-resistant *S. aureus* (BORSA) to Hospital A. She received 29 days of i.v. vancomycin with initial clearance of bacteraemia. However, BORSA bacteraemia recurred on day 28 of therapy and she was switched to i.v. daptomycin for 2 weeks without resolution of bacteraemia. Finally, the bacteraemia cleared with a combination of i.v. meropenem and oral moxifloxacin, and she was discharged well with long-term oral antibiotic suppression of her infection. Any attempt to replace her prosthetic valve had been deemed too hazardous because of anatomical considerations.

Case 4

A 75-year-old woman with metastatic ovarian cancer developed fever and new-onset lower back pain after receiving intravenous chemotherapy as an inpatient at Hospital B. Blood cultures grew MRSA while an MRI scan of the lumbar spine revealed osteomyelitis and discitis at L2/L3 vertebra. Her bacteraemia failed to clear despite 7 days of i.v. vancomycin followed by 18 days of i.v. daptomycin, and it resolved only after i.v. linezolid was initiated. No attempt at vertebral curettage was made in view of the advanced nature of her underlying cancer, but she was discharged well on oral linezolid.

Case 5

A 75-year-old woman with type 2 diabetes mellitus, Wegener's granulomatosis and Child's B liver cirrhosis developed catheter-related bloodstream infection with MRSA during her hospitalization at Hospital C for hypoglycaemia. Transthoracic echocardiography revealed a small 0.7 cm vegetation on her mitral valve. She continued to deteriorate and remained persistently bacteraemic despite 11 and 20 days of vancomycin and daptomycin therapy, respectively, and finally died a day after switching to i.v. linezolid.

Case 6

A 63-year-old man with a history of cervical myelopathy presented to Hospital C with generalized exfoliative

dermatitis secondary to an allergic reaction. He developed persistent MRSA bacteraemia during his hospitalization complicated by T3/T4 spinal discitis and mild mitral regurgitation secondary to endocarditis. The MRSA bacteraemia persisted through 17 and 29 days of i.v. vancomycin and daptomycin therapy, respectively, and cleared only after a further 9 days of i.v. linezolid. He was discharged well on long-term oral linezolid.

Microbiological investigations and results

Isolates were reconfirmed as *S. aureus* via coagulation of citrated rabbit plasma with EDTA (BBL Becton Dickinson) and by production of clumping factor and protein A (BactiStaph; Remel). Vancomycin and daptomycin MICs were obtained via duplicate testing using Etest (AB Biodisk) following the manufacturer's guidelines, using *S. aureus* ATCC 29213 as a reference strain. Screening for vancomycin-heteroresistant *S. aureus* (hVISA) was performed on isolates that had vancomycin MIC $\leq 2 \mu\text{g ml}^{-1}$ using Etest GRD strips (AB Biodisk) following the manufacturer's guidelines. All isolates were typed for isogenicity using PFGE (Maslow *et al.*, 1993), multilocus sequence typing (Enright *et al.*, 2000) and SCC*mec* typing where applicable (Ito *et al.*, 2004). Cell wall thickness was measured using transmission electron microscopy for morphometric evaluation of 30 cells of every isolate, with the difference in thickness between each subject's isolates pre- and post-daptomycin exposure tested for significance via paired *t*-test using Intercooled Stata 11.0 (StataCorp).

The bacteriological profiles of the isolates are shown in Table 1. There were five MRSA strains and one BORSA strain with an oxacillin MIC of $8 \mu\text{g ml}^{-1}$ that was *mecA*-negative (it was initially misidentified as an MRSA). All strains were initially sensitive to vancomycin, but two had vancomycin MICs exceeding $2 \mu\text{g ml}^{-1}$ just prior to switching to daptomycin. Although all isolates had very low daptomycin MICs at the start, the breakpoint of $1 \mu\text{g ml}^{-1}$ was exceeded within 2 weeks of daptomycin prescription in all but one case (where interim isolates were not available for testing). Vancomycin MICs continued to increase in two strains while on daptomycin.

The presence of hVISA was not detected among the three initial *S. aureus* isolates available for testing. However, interim isolates for cases 1 and 2 tested positive for hVISA, with teicoplanin inhibitory concentrations at $32 \mu\text{g ml}^{-1}$ and $16 \mu\text{g ml}^{-1}$ at 48 h, respectively.

PFGE typing of available isolates demonstrated isogenicity for each subject's *S. aureus* strains (Fig. 1). The predominant clone was ST239-MRSA-III, which is the major healthcare-associated MRSA clonal type locally (Hsu *et al.*, 2007). Electron microscopy images of the initial and final vancomycin-intermediate daptomycin-non-susceptible *S. aureus* isolates for subject 1 are shown in Fig. 2. The initial isolates of three subjects were not available for testing but all others showed a significant increase in cell wall thickness

Table 1. Microbiological characteristics of the *S. aureus* strains

Case	Organism	Isolates tested	Vancomycin MIC ($\mu\text{g ml}^{-1}$)	Daptomycin MIC ($\mu\text{g ml}^{-1}$)	Cell wall thickness (nm)	hVISA screening	Sequence type	SCCmec type
1	MRSA	(1) Initial pre-therapy	1.00	0.75	$32.97 \pm 6.41^*$	Negative	5	II
		(2) Day 19 vancomycin, pre-daptomycin	1.50	0.75	$39.07 \pm 7.14^\dagger$	Negative		
		(3) Day 9 daptomycin	2.00	3.00	NT	Positive		
		(4) Day 15 daptomycin	3.00	4.00	$55.47 \pm 7.97^{*\dagger}$	—		
2	MRSA	(1) Initial pre-therapy	2.00	0.50	$47.87 \pm 7.56^*$	Negative	239	III
		(2) Day 7 vancomycin, pre-daptomycin	2.00	0.50	61.10 ± 9.98	Positive		
		(3) Day 7 daptomycin	2.00	3.00	$63.50 \pm 9.42^*$	—		
3	MRSA	(1) Initial pre-therapy	NT	NT	NT	—	239	III
		(2) Day 6 vancomycin, pre-daptomycin	2.0	0.19	46.40 ± 7.01	Negative		
		(3) Day 13 daptomycin	4.0	4.0	46.80 ± 8.27	—		
4	MRSA	(1) Initial pre-therapy	NT	NT	NT	—	239	III
		(2) Day 10 vancomycin, pre-daptomycin	2.00	0.50	45.97 ± 8.26	Negative		
		(3) Day 14 daptomycin	3.00	2.00	49.43 ± 8.15	—		
5	MRSA	(1) Initial pre-therapy	NT	NT	NT	—	239	III
		(2) Day 15 vancomycin, pre-daptomycin	3.00	0.50	$46.30 \pm 5.52^\dagger$	—		
		(3) Day 28 daptomycin	3.00	6.00	$51.97 \pm 8.15^\dagger$	—		
6	BORSA	(1) Initial pre-therapy	0.50	0.25	$41.37 \pm 6.46^*$	Negative	361	No SCCmec
		(2) Day 28 vancomycin, pre-daptomycin	1.50	0.25	59.40 ± 12.10	Negative		
		(3) Day 14 daptomycin	3.00	2.00	$61.40 \pm 12.80^*$	—		

NT, Isolates not available for further testing.

*Significant difference in cell wall thickness ($P < 0.05$) between initial and final isolates.

†Significant difference in cell wall thickness ($P < 0.05$) between immediate pre-daptomycin and final isolates.

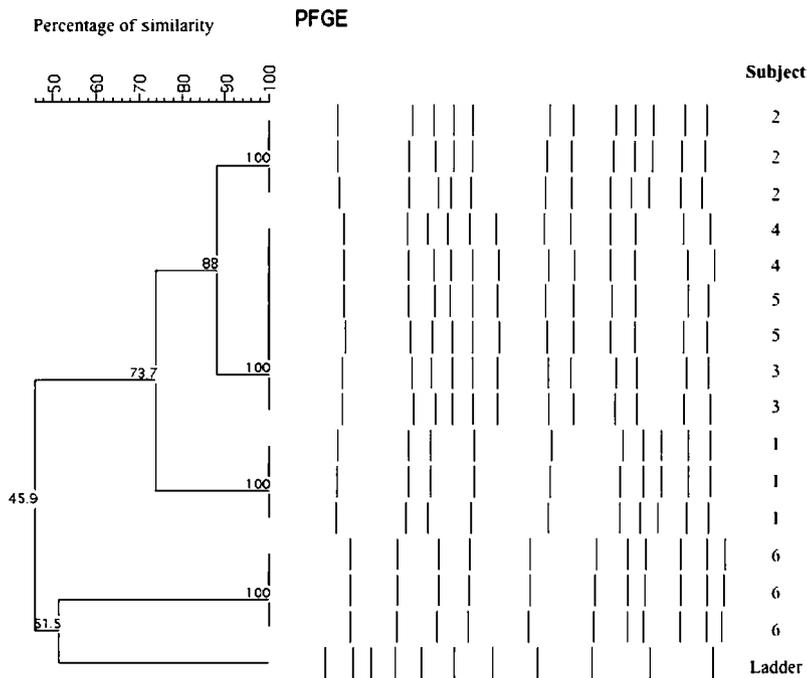


Fig. 1. Digitized PFGE patterns of *S. aureus* isolated from the subjects. Analysis was performed using Bionumerics 5.4 to calculate Dice coefficients of correlation, with a dendrogram generated by the unweighted pair group method using arithmetic averages clustering.

when initial isolates were compared to the daptomycin-non-susceptible counterparts.

Discussion

These cases accentuate an important axiom on the management of *S. aureus* bacteraemia: without achieving adequate source control, failure of medical therapy is not unexpected despite appropriate antibiotic prescription. Virtually all the cases reported here had infected sites that were not amenable to surgical excision because of various factors.

These are also, to our knowledge, the first daptomycin-non-susceptible *S. aureus* cases identified in local hospitals within a year of the launch of the antibiotic in Singapore. They provide *in vivo* corroboration of *in vitro* results linking the development of daptomycin non-susceptibility with vancomycin exposure (Cui *et al.*, 2006; Sakoulas *et al.*, 2006). It is striking that all post-vancomycin-, pre-daptomycin-exposed isolates remained susceptible to daptomycin with little or no increase in the daptomycin MICs compared to initial isolates (where available for testing). This suggests that testing *S. aureus* isolates at this point may not be a useful predictor for the efficacy of daptomycin in such clinical settings.

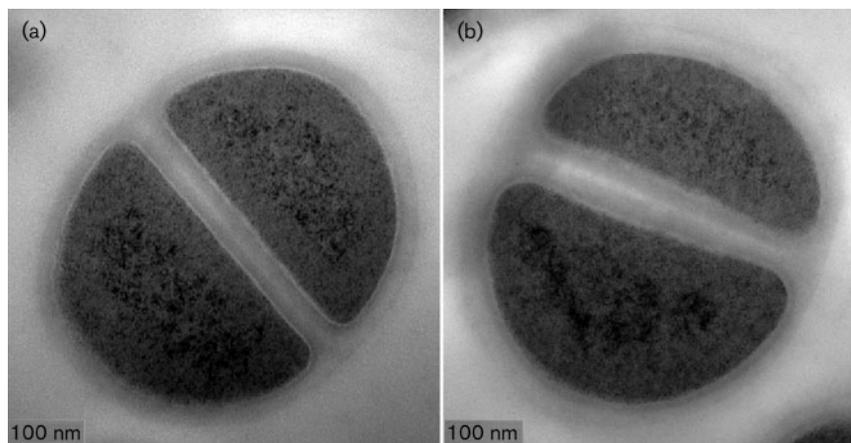


Fig. 2. Electron microscopy images of MRSA isolates from subject 1. (a) Initial (pre-therapy) MRSA isolate. (b) Final cultured MRSA isolate after 15 days of daptomycin therapy.

The exact mechanism for development of daptomycin resistance remains unclear and it is plausible that several possible pathways exist for different MRSA clones (Camargo *et al.*, 2008). Nonetheless, these appear to commonly result in a single phenotype where the organism develops an excessively thickened cell wall, conferring concomitant reduced susceptibility to vancomycin (Cui *et al.*, 2006). Despite non-exposure to vancomycin, the MIC of vancomycin for the terminal MRSA isolates of two subjects continued to increase while on daptomycin therapy, a phenomenon that has rarely been reported in the literature (Mariani *et al.*, 2006).

The precise correlation between cell wall thickness and increased vancomycin or daptomycin MIC is not known. Isolates from cases 3 and 4 did not develop a thicker cell wall despite a rise in both vancomycin and daptomycin MIC, whereas isolates from case 2 saw a marked increase in cell wall thickness despite no obvious change in vancomycin and daptomycin MIC. The latter (case 2) may possibly be attributed to the development of hVISA in the interim isolates, contributing to a phenotypically thickened cell wall but no change in susceptibility. Other mechanisms for reduced daptomycin susceptibility have been described, including alterations in cell wall surface charges and drug binding, as well as enhanced membrane fluidity resulting in diminished daptomycin-induced depolarization and cell membrane permeability (Jones *et al.*, 2008). It is plausible that these may have accounted for daptomycin non-susceptibility in isolates from cases 3 and 4.

Some experts have recommended higher doses of daptomycin [>6 mg (kg body weight)⁻¹] and/or combination therapy in order to prevent resistance development in situations where there is a high inoculum of bacteria or poorer drug penetration (Moise *et al.*, 2009). However, even dosing daptomycin as high as 12 mg (kg body weight)⁻¹ after vancomycin failure is not proof against the development of non-susceptibility (Lee *et al.*, 2010). Switching to daptomycin in a clinical situation where vancomycin therapy has failed appears to incur a heightened risk of treatment failure, and vice versa.

It is unsurprising that these isolates were obtained from subjects who had received prior treatment with vancomycin – the far higher cost of daptomycin relative to vancomycin has meant that patients in Singapore and many parts of the world are more likely to be prescribed the former only after therapeutic failure with the latter. This behaviour may have a negative impact on the long-term viability of daptomycin as an antistaphylococcal agent.

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