

Correspondence

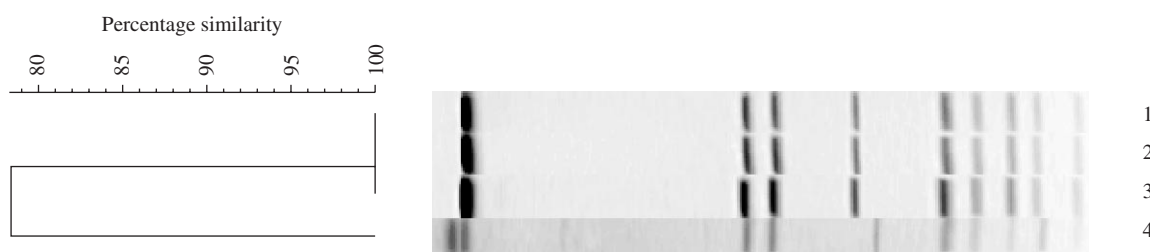


Figure 1. PFGE patterns and dendrogram demonstrating relatedness of Oxford *Staphylococcus* (lane 1) and three clinical isolates of *S. aureus* (lanes 2–4).

the UK with potential to cause community-acquired disease 60 years following the primary isolation of the Oxford *Staphylococcus*. From an evolutionary perspective, it is noteworthy that members of this clonal lineage have subsequently acquired methicillin resistance [specifically following acquisition of the staphylococcal chromosome cassette *mec* (SCC*mec*) type IV element] and emerged as a community-associated MRSA strain (ST30-MRSA-SCC*mec*IV) which has been found in the Southwest Pacific and some other regions, including the UK.^{4,6}

The Oxford *Staphylococcus* has been used as a control organism for some 60 years and we are not aware of any infections resulting from acquisition of this organism by laboratory personnel. Although the provenance of the control strain is unclear, it is tempting to speculate that it was originally isolated from a pyogenic infection associated with the presence of the PVL genes. It may be argued that the organism may have become attenuated following repeated subculture over the years following its initial isolation. Nevertheless, these observations serve as an alert to microbiologists: good laboratory practice should be adhered to where this organism is handled, particularly following the report of infection due to PVL-positive MRSA occurring in a microbiologist working with such organisms.⁷ Attention should be paid to the covering of cuts and wounds and/or wearing of gloves to minimize any risk of infection among laboratory workers. Crucially, healthcare personnel should remain vigilant to the possible occurrence of SSTIs in individuals handling the Oxford *Staphylococcus* and, where *S. aureus* is identified as the aetiological agent, the isolate should be fully characterized to determine its PVL status and relatedness to the control strain.

Acknowledgements

We thank Dr Robert Hill (Antibiotic Resistance Evaluation Unit, Centre for Infections, London) for provision of antimicrobial susceptibility data.

Transparency declarations

None to declare.

References

1. Abraham EP, Chain E, Fletcher CM *et al.* Further observations on penicillin. *Lancet* 1941; ii: 177–89.
2. Heatley NG. A method for the assay of penicillin. *Biochem J* 1944; **38**: 61–5.
3. Dunne WM. Pantone-Valentine leukocidin genes in a laboratory quality control strain of *Staphylococcus aureus*. *J Clin Microbiol* 2006; **44**: 287.
4. Holmes A, Ganner M, McGuane S *et al.* *Staphylococcus aureus* isolates carrying Pantone-Valentine leukocidin genes in England and

Wales: frequency, characterization and association with clinical disease. *J Clin Microbiol* 2005; **43**: 2384–90.

5. Prevost G, Couppie P, Prevost P *et al.* Epidemiological data on *Staphylococcus aureus* strains producing synergohymenotropic toxins. *J Med Microbiol* 1995; **42**: 237–45.

6. Robinson DA, Kearns AM, Holmes A *et al.* Re-emergence of early pandemic *Staphylococcus aureus* as a community-acquired methicillin-resistant clone. *Lancet* 2005; **365**: 1256–8.

7. Nordmann P, Naas T. Transmission of methicillin-resistant *Staphylococcus aureus* to a microbiologist. *New Engl J Med* 2005; **352**: 1489–90.

Journal of Antimicrobial Chemotherapy

doi:10.1093/jac/dkl256

Development of decreased susceptibility to daptomycin and vancomycin in a *Staphylococcus aureus* strain during prolonged therapy

Paul G. Mariani¹†, Helio S. Sader^{2*} and Ronald N. Jones^{2,3}

Meritcare Hospital, Fargo, ND, USA; ²JMI Laboratories, North Liberty, IA 52317, USA; ³Tufts University School of Medicine, Boston, MA, USA

Keywords: *S. aureus*, resistance, glycopeptides, cyclic lipopeptides

*Corresponding author. Tel: +1-319-665-3370; Fax: +1-319-655-3371; E-mail: helio-sader@jmilabs.com

†Present address. Division of Infectious Diseases, University of Miami Miller School of Medicine, Miami, FL, USA

Sir,

We document the development of decreased susceptibility to daptomycin and vancomycin in *Staphylococcus aureus* during a prolonged and complicated therapy with these two antimicrobials. Unlike other reports, the vancomycin MIC value remained at 1 mg/L after several prolonged regimens of vancomycin, but only increased after further prolonged treatment with daptomycin.

A 64-year-old man was admitted to the intensive care unit with methicillin-resistant *S. aureus* (MRSA) bacteraemia and treated with a 17 day course of vancomycin 1 g intravenous (iv) every 12 h. On the 22nd hospital day (HD) the patient became febrile and repeat blood cultures revealed MRSA. A transthoracic-echocardiogram did not reveal evidence of endocarditis. The patient was treated with a 1 month course of

Correspondence

vancomycin 1 g iv every 12 h, and blood cultures became negative. On the 60th HD the patient had a repeat febrile episode with blood cultures demonstrating MRSA and was again treated with vancomycin. On the 99th HD, a white blood cell tagged scan showed infection of the right prosthetic hip with an aspiration culture-positive for MRSA, susceptible to vancomycin (MIC 1 mg/L) and daptomycin (MIC 0.5 mg/L). The patient's course was complicated by myocardial infarction and an episode of acute cholecystitis; therefore, surgery was postponed until the 102nd HD and the patient continued to receive vancomycin as suppressive therapy. Intra-operatively the patient developed a disseminated intravascular coagulation further complicated by non-oliguric acute renal failure, and daptomycin 650 mg daily was initiated. The patient's acute medical problems stabilized over the ensuing 4 weeks. On the 150th HD, the patient suddenly developed a fever and MRI demonstrated a 32 cm thigh abscess. The patient was again taken to surgery and an acetabular biopsy grew a vancomycin-intermediate (MIC 4 mg/L), daptomycin-non-susceptible (MIC 8 mg/L) *S. aureus*, *Citrobacter freundii* and three species of *Candida* (*Candida albicans*, *Candida glabrata* and *Candida parapsilosis*). Antimicrobial therapy was changed to dalfopristin/quinupristin 850 mg/day, gatifloxacin 200 mg/day and fluconazole 400 mg/day. The patient subsequently developed a catheter-associated *Enterococcus faecalis* bacteraemia and expired shortly thereafter.

Three strains of *S. aureus* were isolated from the patient including a blood culture on HD 1, a surgical wound culture from HD 99 and a bone culture from HD 148. The strains were tested by Clinical and Laboratory Standards Institute (CLSI) M7-A7 broth microdilution methods in validated, dry-form panels manufactured by TREK Diagnostics, Inc. (Cleveland, OH, USA).¹ Daptomycin susceptibility was tested as recommended in Mueller-Hinton broth supplemented with 50 mg/L calcium.¹ Disc diffusion methods were performed according to the CLSI M2-A9 method¹ and heteroresistance to vancomycin (hVISA) was evaluated by Etest (AB BIODISK, Solna, Sweden) as previously described by other investigators.²

The three strains tested from this patient exhibited variations in the antimicrobial susceptibility results of daptomycin and vancomycin (Table 1). Strains #1 and #2 showed identical daptomycin (0.5 mg/L) and vancomycin (1 mg/L) MIC results, while strain #3 exhibited a daptomycin MIC of 8 mg/L and a vancomycin MIC of 4 mg/L. Interestingly, dalbavancin MIC values also increased from 0.06 mg/L for strains #1 and #2 to 0.25 mg/L for strain #3 (4-fold increase). Disc diffusion zone diameter results for daptomycin showed a 3–4 mm decrease for strain #3 (15 mm) compared with the first two isolates (18–19 mm), validating observed higher MIC values. The vancomycin disc zone diameter results did not change (16 mm) as the MIC increased from 1 to 4 mg/L. Interestingly, strains #1 and #2 were resistant to penicillin (β -lactamase-positive; MIC at 16 mg/L) and strain #3 was β -lactamase-negative (penicillin MIC at 0.12 mg/L). Conversely, strain #1 was susceptible to rifampicin and strains #2 and #3 were resistant to this antimicrobial.

hVISA was observed in strain #3, which showed an MIC value of 12 mg/L for both vancomycin and teicoplanin when tested by Etest using the high inoculum test variation.² All three *S. aureus* strains demonstrated an identical molecular typing pattern by automated ribotyping (Riboprinter™ Microbial Characterization System, Qualicon, Wilmington, DE, USA) and PFGE.

Table 1. Broth microdilution MIC results (unless noted) for 19 antimicrobial agents tested against three *S. aureus* isolates

Antimicrobial agent	MIC (mg/L) strain # (hospital day/culture type)		
	1 (1/blood)	2 (99/surgical wound)	3 (148/bone)
Daptomycin	0.5	0.5	8
Vancomycin	1	1	4 (12) ^a
Teicoplanin	≤2	≤2	≤2 (12) ^a
Dalbavancin	0.06	0.06	0.25
Oxacillin	>2	>2	>2
Penicillin	16	16	0.12 ^b
Clindamycin	>2	>2	>2
Erythromycin	>8	>8	>8
Telithromycin	>4	>4	>4
Quinupristin/dalfopristin	0.5	0.5	0.5
Chloramphenicol	8	8	4
Ciprofloxacin	>4	>4	>4
Gentamicin	≤2	≤2	≤2
Linezolid	2	2	1
Mupirocin	≤4	≤4	≤4
Rifampicin	≤0.25	>2	>2
Tetracycline	≤2	≤2	≤2
Tigecycline	0.12	0.25	0.06
Trimethoprim/ sulfamethoxazole	≤0.5	≤0.5	≤0.5

^aValue in parentheses represents MIC result when using Etest strips with elevated bacterium inoculum.

^b β -Lactamase test negative.

We describe an *in vivo* increase of daptomycin and vancomycin MIC values for an MRSA strain causing prosthetic joint infection with MRSA bacteraemia following prolonged therapy with these agents. Isolates taken after several prolonged courses of vancomycin and before institution of daptomycin were found to be susceptible to these two antimicrobials by reference broth microdilution, disc diffusion and Etest methods. However, after prolonged daptomycin therapy the strain developed decreased susceptibility to both daptomycin and vancomycin.

Limited information is available on the utility of daptomycin for osteomyelitis. In an experimental rabbit model of MRSA osteomyelitis, treatment success with daptomycin was analogous to that of vancomycin. Eradication was similar for both drugs, and the isolates from the persisting organisms in the model did not demonstrate any antimicrobial resistance.³

Although these antimicrobials have distinctly different mechanisms of action, a recent study from Japan⁴ demonstrated that staphylococcal cell wall thickness caused by prolonged exposure to vancomycin may act as a common obstacle to daptomycin and vancomycin penetration of the cell and increase the MIC of both antimicrobials. In the case report presented here, however, vancomycin and daptomycin MIC values remained low (1 and 0.5 mg/L, respectively) after prolonged use of vancomycin, but increased (4 and 8 mg/L, respectively) after prolonged use of daptomycin. In addition, the dalbavancin

MIC also increased 4-fold (from 0.06 to 0.25 mg/L), since it is a semi-synthetic derivative of the natural glycopeptide produced by a 3,3-dimethylaminopropyl amide substitution on the peptide carboxyl group.⁵ This agent is similar to other glycopeptides in its mechanism of action by binding to the terminal alanyl-alanine of nascent peptidoglycan chains.⁵

Since extended use of daptomycin resulted in elevated MIC values of these glycopeptides (vancomycin and daptomycin), it is possible that exposure to daptomycin, like vancomycin, may cause thickening of the bacterial cell wall, thus decreasing susceptibility to a number of peptides. Another recent study also showed that prolonged exposure to vancomycin prior to daptomycin treatment increases the risk of emergence of a daptomycin non-susceptible subpopulation.⁶ Further studies are necessary to better understand the cell wall alterations initiated by prolonged daptomycin exposure and their effect on the activities of structurally related peptides (glycopeptide and lipoglycopeptide agents).

Finally, daptomycin seems to offer a bactericidal alternative for treatment of MRSA infections that are unresponsive to vancomycin; however, this case illustrates the adaptability of *S. aureus* necessitating clinicians to be aware of the possible daptomycin resistance associated with therapy following vancomycin failure.

Transparency declarations

H. S. S. and R. N. J. have received research/education grants in the last 3 years from AB BIODISK, Abbott, AlamX, Arpida, AstraZeneca, Avexa, Basilea, Bayer, Becton Dickinson, Boehringer-Ingelheim, bioMerieux, Bristol-Myers Squibb, Cadence, Cerexa, Chiron, Cognigen, Cubist, Daiichi, Elan, Elanco, Enanta, GlaxoSmithKline, Intrabiotics, Johnson & Johnson, LG Chemicals, Merck, Micrologix, Novartis, Optimer, Ordway, Oscient, Osmotics, Peninsula, Pfizer, Replidyne, Schering-Plough, Sequoia, Serenex, Shionogi, Theravance, TREK Diagnostics, Vicuron, Wyeth.

References

1. Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: Sixteenth Informational Supplement M100-S16*. CLSI, Wayne, PA, USA, 2006.
2. Wootton M, Howe RA, Hillman R *et al*. A modified population analysis profile (PAP) method to detect hetero-resistance to vancomycin in *Staphylococcus aureus* in a UK hospital. *J Antimicrob Chemother* 2001; **47**: 399–403.
3. Mader JT, Adams K. Comparative evaluation of daptomycin (LY146032) and vancomycin in the treatment of experimental methicillin-resistant *Staphylococcus aureus* osteomyelitis in rabbits. *Antimicrob Agents Chemother* 1989; **33**: 689–92.
4. Cui L, Tominaga E, Neoh HM *et al*. Correlation between reduced daptomycin susceptibility and vancomycin resistance in vancomycin-intermediate *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2006; **50**: 1079–82.
5. Malabarba A, Goldstein BP. Origin, structure, and activity *in vitro* and *in vivo* of dalbavancin. *J Antimicrob Chemother* 2005; **55** Suppl 2: ii15–20.
6. Sakoulas G, Alder J, Thauvin-Eliopoulos C *et al*. Induction of daptomycin heterogeneous susceptibility in *Staphylococcus aureus* by exposure to vancomycin. *Antimicrob Agents Chemother* 2006; **50**: 1581–5.

Journal of Antimicrobial Chemotherapy

doi:10.1093/jac/dkl232

Advance Access publication 3 June 2006

Ritonavir-dependent fluconazole boosting of nelfinavir: a report of three cases

S. Garazzino*, M. Tettoni, A. Calcagno, A. D'Avolio, S. Bonora and G. Di Perri

Department of Infectious Diseases, University of Turin, Turin, Italy

Keywords: pharmacokinetics, drug interactions, therapeutic drug monitoring

*Corresponding author. Tel: +39-333-2466363; Fax: +39-011-4393977; E-mail: silvia.garazzino@inwind.it

Sir,

The use of ritonavir-boosted dual protease inhibitor (PI) combinations is increasingly considered in HIV-infected patients with a history of multiple therapeutic failures.¹ Although pharmacokinetic (PK) studies on several of such combinations provided valuable information on how to cope with drug–drug interactions between concurrently administered PIs, substantial uncertainty persists on the best management of double-boosted PI-based regimens.² In this setting, the introduction of fluconazole as additional boosting agent was found to restore the otherwise reciprocally reduced concentrations of co-administered lopinavir/ritonavir and amprenavir, and higher PI PK exposure was also documented when fluconazole was added to ritonavir/saquinavir and ritonavir/tipranavir.^{3–5} In a pre-registration study it was found that in patients exposed to nelfinavir, but not to ritonavir, the addition of fluconazole resulted in a 27% reduction of nelfinavir oral clearance.⁶

Fluconazole is a weak inhibitor of CYP3A4 and a strong inhibitor of both CYP2C9 and CYP2C19. Fluconazole boosting properties on nelfinavir might be attributable primarily to the inhibitory action on CYP2C19, as the same isoenzyme is responsible for nearly 50% of nelfinavir clearance through the transformation into the nelfinavir metabolite M8.^{7,8} The latter is probably the major metabolic difference between nelfinavir and the other PIs, whose metabolism depends upon CYP3A4 to a greater extent, and it might account for the significant lesser sensitivity of nelfinavir to the boosting effect of ritonavir.

In the therapeutic drug monitoring (TDM) database of the Department of Clinical Infectious Diseases of the University of Torino, Italy, where TDM is carried out on a regular basis, three patients receiving both nelfinavir and fluconazole were found. In one case the antiretroviral regimen also included lopinavir and ritonavir as booster, while the other two patients were given nelfinavir and fluconazole without ritonavir. In all such cases fluconazole was introduced and administered for 4 weeks at a dosage of 100 mg twice daily in order to treat a concomitant oropharyngeal candidiasis. No co-medications were administered in the study period. Plasma samples for PK analysis were