Journal of Antimicrobial Chemotherapy (2003) **52**, 864–868
DOI: 10.1093/jac/dkg457
Advance Access publication 16 October 2003

JAC

Antibacterial susceptibility of a vancomycin-resistant *Staphylococcus aureus* strain isolated at the Hershey Medical Center

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Received 14 August 2003; returned 22 August 2003; revised 26 August 2003; accepted 28 August 2003

Staphylococcus aureus **strain HMC3 isolated at the Hershey Medical Center, was resistant to vancomycin (VRSA) through the presence of the *vanA* resistance gene; it also contained *mecA*, *erm*(A), *erm*(B), *tet*(K) and *aac*(6′)-*aph*(2″), conferring resistance to licensed β-lactams, macrolides, tetracycline and aminoglycosides. HMC3 also had alterations in GyrA and GrlB and was resistant to available quinolones. Experimental drugs with low MICs (<2 mg/L) for VRSA HMC3 included cephalosporins BAL9141 and RWJ-54428; glycopeptides oritavancin and dalbavancin; the lipopeptide daptomycin; the glycolipodepsipeptide ramoplanin; new fluoroquinolones WCK 771 A, WCK 1153, DK-507k and sitafloxacin; and the DNA nanobinder GS02-02. These agents were all bactericidal as were trimethoprim/sulfamethoxazole and teicoplanin (MIC 4 mg/L). Oxazolidinones linezolid and ranbezolid; the injectable streptogramin quinupristin/dalfopristin; DNA nanobinders GS2-10547 and GS02-104; peptide deformylase inhibitors NVP-PDF713 and GS02-12; tetracycline derivative tigecycline; the antifolate iclaprim; mupirocin and fusidic acid were all active *in vitro* but bacteriostatic.**

Keywords: *S. aureus*, VRSA, antimicrobial susceptibilities, mechanisms of resistance

Introduction

Fifteen years after the initial report of *Enterococcus faecium* with a transferable *vanA* gene and 6 years after the first description of vancomycin-intermediate *Staphylococcus aureus*, two vancomycin-resistant *S. aureus* (VRSA) strains containing the *vanA* gene cluster were isolated in the US between June and September 2002.[1,2]

We tested antimicrobial susceptibilities and mechanisms of resistance of the VRSA strain isolated at the Hershey Medical Center in September 2002, as well as of a cured vancomycin-susceptible derivative of this VRSA, using a variety of clinically used and experimental antibacterials.

Materials and methods

Bacterial strains

Vancomycin-resistant *S. aureus* strain HMC3 was isolated at the Hershey Medical Center from a heel wound in a 70-year-old male patient.[2] A spontaneously cured vancomycin-susceptible derivative of this VRSA, *S. aureus* strain HMC3-1, was obtained by serial passage of the VRSA in drug-free medium.

Antimicrobials

Oritavancin (LY 333328) was from Lilly Research Laboratories (Indianapolis, IN, USA); dalbavancin from Vicuron Pharmaceuticals, Inc. (King of Prussia, PA, USA); ramoplanin from the Genome Therapeutics Corp. (Waltam, MA, USA); BAL9141 from Basilea Pharmaceutica AG (Basle, Switzerland); RWJ-54428 from Johnson & Johnson (Raritan, NJ, USA); daptomycin from Cubist Pharmaceuticals, Inc. (Lexington, MA, USA); tigecycline from Wyeth-Ayerst Laboratories (Pearl River, NY, USA); ranbezolid from Ranbaxy Laboratories (New Delhi, India); WCK 771 A, WCK 919, and the latter's constituent enantiomers (WCK 1152 and WCK 1153) from Wockhardt Research Center (Aurangabad, India); DK-507k and sitafloxacin from Daiichi Pharmaceutical Co., Ltd. (Tokyo, Japan); iclaprim from Arpida AG (Münchenstein, Switzerland); NVP-PDF713 from the Novartis Pharmaceutical Corp. (Summit, NJ, USA); and GS02-02, GS02-12, GS2-10547 and GS02-104 from GeneSoft Pharmaceuticals Inc. (South San Francisco, CA, USA). Other drugs were obtained from their manufacturers.

MIC determinations and time–kill studies

MICs were determined using the macrobroth dilution method.[3] Time–kill studies were carried out as described previously.[4]

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Table 1. Bacteriostatic/bactericidal effects of selected antibacterials towards VRSA strain HMC3

Class	Drug and concentration[a]	Viability counts[b]			
		3 h	6 h	12 h	24 h
Glycopeptides	teicoplanin				
	4×MIC	–1	–2	>–3	>–3
	2×MIC	–1	–2	>–3	>–3
	MIC (4 mg/L)	–1	–1	–1	<–1
	dalbavancin				
	4×MIC	–2	>–3	>–3	>–3
	2×MIC	–2	>–3	>–3	>–3
	MIC (0.5 mg/L)	–2	>–3	>–3	<–1
	oritavancin				
	4×MIC	>–3	>–3	>–3	>–3
	2×MIC	–2	>–3	>–3	>–3
	MIC (0.25 mg/L)	–1	>–3	>–3	>–3
Glycolipodepsipeptide	ramoplanin				
	4×MIC	>–3	>–3	>–3	>–3
	2×MIC	>–3	>–3	>–3	>–3
	MIC (0.125 mg/L)	–2	>–3	–1	–2
Lipopeptide	daptomycin				
	4×MIC	>–3	>–3	>–3	>–3
	2×MIC	>–3	>–3	>–3	>–3
	MIC (0.5 mg/L)	>–3	>–3	>–3	>–3
β-Lactams	BAL9141				
	4×MIC	–2	>–3	>–3	>–3
	2×MIC	–2	>–3	>–3	>–3
	MIC (1 mg/L)	–1	–2	–1	–1
	RWJ-54428				
	4×MIC	–2	–2	>–3	>–3
	2×MIC	–2	–2	>–3	>–3
	MIC (0.5 mg/L)	–2	–2	>–3	<–1
Quinolones	sitafloxacin				
	4×MIC	>–3	>–3	>–3	>–3
	2×MIC	>–3	>–3	>–3	>–3
	MIC (1 mg/L)	<–1	<–1	<–1	<–1
	DK-507k				
	4×MIC	>–3	>–3	>–3	>–3
	2×MIC	>–3	>–3	>–3	>–3
	MIC (1 mg/L)	–2	>–3	>–3	>–3
	WCK 1153				
	4×MIC	–2	>–3	>–3	>–3
	2×MIC	–1	>–3	>–3	>–3
	MIC (1 mg/L)	<–1	–2	–2	<–1
	WCK 771 A				
	4×MIC	–2	>–3	>–3	>–3
	2×MIC	–2	>–3	>–3	>–3
	MIC (0.5 mg/L)	<–1	–2	–2	<–1
Streptogramin	quinupristin/dalfopristin				
	4×MIC	<–1	<–1	–1	–2
	2×MIC	<–1	<–1	–1	–2
	MIC (1 mg/L)	<–1	<–1	–1	–2
Oxazolidinones	linezolid				
	4×MIC	<–1	–1	–2	–2
	2×MIC	<–1	<–1	<–1	–1
	MIC (1 mg/L)	<–1	<–1	<–1	<–1
	ranbezolid				
	4×MIC	–1	–1	–2	–2
	2×MIC	<–1	–1	–2	–2
	MIC (1 mg/L)	<–1	<–1	<–1	<–1

Table 1. (*Continued*)

Class	Drug and concentration[a]	Viability counts[b] 3 h	6 h	12 h	24 h
DNA nanobinders	GS02-02				
	4 × MIC	–1	–1	–2	>–3
	2 × MIC	–1	–1	–2	>–3
	MIC (0.25 mg/L)	–1	–1	–1	<–1
	GS2-10547				
	4 × MIC	–1	–1	–2	–2
	2 × MIC	<–1	–1	–1	–2
	MIC (0.25 mg/L)	<–1	–1	<–1	<–1
	GS02-104				
	4 × MIC	–1	–1	–2	–2
	2 × MIC	<–1	–1	–2	–2
	MIC (0.06 mg/L)	<–1	–1	–1	<–1
PDF inhibitors	NVP-PDF713				
	4 × MIC	<–1	<–1	–1	<–1
	2 × MIC	<–1	<–1	–1	<–1
	MIC (0.5 mg/L)	<–1	<–1	–1	<–1
	GS02-12				
	4 × MIC	<–1	<–1	<–1	<–1
	2 × MIC	<–1	<–1	–1	<–1
	MIC (0.25 mg/L)	<–1	<–1	<–1	<–1
Tetracycline	tigecycline				
	4 × MIC	<–1	–1	<–1	<–1
	2 × MIC	<–1	–1	<–1	<–1
	MIC (0.12 mg/L)	<–1	<–1	<–1	<–1
Miscellaneous	trimethoprim/sulfamethoxazole				
	4 × MIC	<–1	–2	>–3	>–3
	2 × MIC	<–1	–2	>–3	–2
	MIC (0.25 mg/L)	<–1	–1	>–3	<–1
	mupirocin				
	4 × MIC	<–1	–1	<–1	<–1
	2 × MIC	<–1	–1	<–1	<–1
	MIC (0.125 mg/L)	<–1	<–1	<–1	<–1
	fusidic acid				
	4 × MIC	–1	–1	–1	–1
	2 × MIC	<–1	<–1	<–1	<–1
	MIC (4 mg/L)	<–1	<–1	<–1	<–1

[a]4 × MIC, four times the MIC; 2 × MIC, two times the MIC.
[b]–1, 90% killing; –2, 99% killing; –3, 99.9% killing.

Determination of resistance mechanisms and DNA manipulation

The presence of antibiotic resistance genes in strains HMC3 and HMC3-1 was examined by PCR.[5–7] Alterations in quinolone resistance determining regions (QRDRs) in *gyrA*, *gyrB*, *grlA* and *grlB* genes were studied as described previously.[8] After amplification, PCR products were purified from excess primers and nucleotides using a QIAquick PCR Purification Kit (Qiagen, Inc., Valencia, CA, USA) and sequenced directly using a CEQ 8000 Genetic Analysis System (Beckman Coulter, Inc., Fullerton, CA, USA). Pulsed-field gel electrophoresis of total DNA was carried out as previously described.[9]

To locate the *vanA* gene, an internal PCR fragment of *vanA*, labelled using the ECL nucleic acid labelling and detection system, was used as a probe for hybridization to digested and separated genomic DNA, which had been transferred to nylon membranes under a vacuum.

Results

Mechanism of resistance

VRSA strain HMC3 contained the *vanA* gene on a 135 kb *Sma*I genomic DNA fragment (data not shown); it also contained *mecA*, *erm*(A), *erm*(B), *tet*(K) and *aac*(6′)-*aph*(2″). A spontaneous vancomycin-susceptible derivative HMC3-1 lacked *vanA*, *erm*(B) and *aac*(6′)-*aph*(2″). Variations in the size of the *Sma*I fragment carrying the *vanA* gene were observed among seven separate VRSA isolates from the same patient, suggesting instability of the resistance element (data not shown).

For both HMC3 and HMC3-1, analysis of QRDRs of *gyrA*, *gyrB*, *grlA* and *grlB* showed substitution of serine by leucine at position 84 in GyrA and substitution of glutamic acid by lysine at position 471 in GrlB, but no alterations in either GyrB or GrlA.

Antimicrobial susceptibility

The vancomycin MIC for strain HMC3 was 32 mg/L, though it remained susceptible to teicoplanin (4 mg/L). MICs of the experimental glycopeptides oritavancin and dalbavancin and the glycolipodepsipeptide ramoplanin were low (0.125–0.5 mg/L). Teicoplanin, dalbavancin, oritavancin and ramoplanin were bactericidal against HMC3 after 6–12 h in the 1–2 × MIC range. Regrowth was observed at the MIC after 24 h with dalbavancin (Table 1).

Two experimental anti-MRSA cephalosporins, BAL9141[10] and RWJ-54428,[11] each had low MICs for HMC3 and were bactericidal at 2 × MIC after 6–12 h (Table 1). The strain was highly resistant to macrolides, telithromycin and clindamycin (>64 mg/L), but susceptible to quinupristin/dalfopristin, although this was only bacteriostatic. The MICs of oxazolidinones linezolid and ranbezolid were 1 mg/L and they were bacteriostatic. Though marketed fluoroquinolones were not active, experimental quinolones sitafloxacin, WCK 771 A,[12] WCK 1153[12] and DK-507k,[13] each had MICs in the 0.5–1 mg/L range and were bactericidal in the 1–2 × MIC range after 3–6 h (Table 1). DNA nanobinders are a new class of antibiotics that bind to the minor groove of DNA and inhibit DNA function and transcription;[14] DNA nanobinders GS02-02, GS2-10547 and GS02-104 all had low MICs for VRSA strain HMC3, and GS02-02 was bactericidal (Table 1). Peptide deformylase (PDF) inhibitors interfere with removal of the formyl moiety of nascent polypeptides by PDF.[15] PDF inhibitors NVP-PDF713 and GS02-12 had low MICs for VRSA strain HMC3, and were bacteriostatic. The VRSA was resistant to aminoglycosides and tetracycline, intermediate to chloramphenicol, and susceptible to tigecycline, iclaprim, trimethoprim/sulfamethoxazole, mupirocin and rifampicin (<0.06 mg/L), with a fusidic acid MIC of 4 mg/L. Tigecycline, mupirocin, fusidic acid and iclaprim were all bacteriostatic. Trimethoprim/sulfamethoxazole had a low MIC (0.25 mg/L) for the VRSA and was bactericidal at a concentration of 4 × MIC after 12 h (Table 1).

In comparison with HMC3, derivative HMC3-1 was susceptible to vancomycin (4 mg/L) and showed a lower dalbavancin MIC (0.06 mg/L); however, activities of teicoplanin, oritavancin and ramoplanin were unchanged. HMC3-1 also became susceptible to gentamicin (0.5 mg/L). Activities of other drugs tested against HMC3 and HMC3-1 were identical.

Discussion

Within a short period of time, two distinct, non-clonal strains of *S. aureus* resistant to vancomycin were isolated in the USA, first in Detroit, MI and shortly afterwards in Hershey, PA.[1,2] Although both isolates possessed the *vanA* gene cluster, they have different glycopeptide resistance phenotypes; the Detroit strain of VRSA being highly resistant to both vancomycin and teicoplanin, and with an oritavancin MIC of 4 mg/L, whereas the Hershey VRSA strain HMC3 is susceptible to teicoplanin, and has low oritavancin and dalbavancin MICs.

The level of expression of *vanA* in VRSA strain HMC3 is currently under investigation in our laboratory. However, this study indicates that the *vanA* gene cluster is not a stable genetic element in strain HMC3; serial passage of the strain in the absence of vancomycin led to loss of the *vanA* cluster and, consequently to restoration of vancomycin susceptibility. A decrease in dalbavancin MIC was also noted in strain HMC3-1, though MICs of teicoplanin, oritavancin and ramoplanin were unaffected. Vancomycin susceptibility was accompanied by emergence of gentamicin susceptibility, attributable to loss of the *aac*(6′)-*aph*(2″) gene; *erm*(B) was also lost. Loss of these resistance genes may result from the instability of transposon Tn*1546*, which is often carried by conjugative plasmids with a broad host range in Gram-positive bacteria.

Emergence of a truly vancomycin-resistant *S. aureus* seriously threatens the most important treatment option available to clinicians for infections resulting from methicillin-resistant *S. aureus*. VRSA strain HMC3 is resistant to most available bactericidal drugs; it was bacteriostatically inhibited by linezolid, tigecycline, iclaprim and the PDF inhibitors. Though quinupristin/dalfopristin is typically rapidly bactericidal against Gram-positive cocci, it was bacteriostatic against strain HMC3, perhaps because of the presence of constitutively expressed *erm* genes. Antibiotics bactericidal against strain HMC3 included cephalosporins BAL9141 and RWJ-54428; quinolones WCK 771 A, WCK 1153, sitafloxacin and DK-507k; the DNA nanobinder GS02-02; glycopeptides oritavancin and dalbavancin; glycolipodepsipeptide ramoplanin; and lipopeptide daptomycin.

Acknowledgements

This work was supported in part by grants from Cubist Laboratories (Lexington, MA, USA) and Johnson & Johnson Research Laboratories (Raritan, NJ, USA).

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