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ARTICLE

Detection of new mutations conferring resistance to linezolid in glycopeptide-intermediate susceptibility *Staphylococcus hominis* subspecies *hominis* circulating in an intensive care unit

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Abstract Glycopeptides and linezolid are the most widely used antibiotics to treat infections by methicillin-resistant *Staphylococcus* spp. We report the presence of various isolates of methicillin-resistant *S. hominis* subsp. *hominis* with resistance to linezolid and reduced susceptibility to glycopeptides. We studied ten blood culture isolates of *S. hominis* subsp. *hominis* from nine patients admitted to our hospital. Etest was used to study susceptibility to antibiotics commonly prescribed against staphylococci. Domain V region of the 23S rRNA gene was amplified and sequenced to detect possible mutations that confer resistance to linezolid. Pulsed-field gel electrophoresis (PFGE) was used for the clonality study of isolates. All isolates were resistant to oxacillin, gentamicin, levofloxacin, cotrimoxazole, and linezolid, and susceptible to tigecycline and daptomycin. Nine of the isolates were resistant to erythromycin and clindamycin, and showed heterogeneous resistance to glycopeptides. C2190T, G2603T, and G2474T mutations were detected in domain V of the 23S rRNA gene. PFGE showed the presence of two different clones. This report alerts to the possible appearance of clinical strains of methicillin-resistant staphylococci with intermediate resistance to glycopeptides, resistance to linezolid, and multiple resistance to other second-line antibiotics.

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Introduction

The antibiotic pressure in some hospital departments, especially Intensive Care Units (ICUs), the inappropriate use of antibiotics, and the difficulty in detecting certain resistance phenotypes have led to a major worldwide increase in bacterial resistance rates [1] and favored the development of multiple resistances in microorganisms such as *Staphylococcus* spp. [2]. In this genus, the association of methicillin-resistance with resistance to aminoglycosides, fluoroquinolones, tetracyclines, and macrolides, among other antibiotics, is well documented [3].

There have been increasing reports of methicillin-resistant staphylococci, mainly *S. aureus* (MRSA), with reduced susceptibility to glycopeptides (glycopeptide-intermediate-resistant *S. aureus*, GISA) [4] and even of isolates resistant to vancomycin and teicoplanin (glycopeptide-resistant *S. aureus*, GRSA) [5]. This emerging phenomenon has also been described in other species of coagulase-negative staphylococci [6]. Hence, it is especially important to correctly detect isolates with heterogeneous resistance to glycopeptides (heteroresistant glycopeptide intermediate *S. aureus*, hGISA) [7]. Critically, hGISA isolates might be

precursors of GISA strains [8], associated with therapeutic failure [9]. All of these developments have limited the success of hospital therapies, increasing the morbidity and mortality of infected patients [10].

This situation has prompted the introduction of novel antibiotics against multiresistant Gram-positive microorganisms, e.g., quinupristin-dalfopristin, linezolid, tigecycline, and daptomycin, which have proven effective against GISA, hGISA, and GRSA isolates [11, 12].

Ever since linezolid was approved for the treatment of Gram-positive bacteria in our country in 2001, its pharmacokinetic and pharmacodynamic properties have led to its increasing use for the main indications, i.e., nosocomial pneumonia, community-acquired pneumonia, and complicated skin and soft tissue infections [13]. It inhibits the synthesis of proteins by binding to the domain V region of the 23S rRNA gene [14]. Mutations in this domain have been associated with resistance to linezolid in such species as *S. aureus*, coagulase-negative staphylococci, *Enterococcus faecalis*, and *E. faecium* [15–17]. G2576T mutation is the most frequent cause of resistance to linezolid found in clinical isolates of *Enterococcus* spp. and *Staphylococcus* spp. [18]. Other reported mutations include G2447T and T2500A in *S. aureus*, C2534T in *S. epidermidis*, G2505A in *E. faecium*, and C2512T, G2513T, and C2610G in *E. faecalis* [19, 20]. Resistance to linezolid is also produced by the action of Cfr-rRNA methyltransferase, which is known to confer resistance to phenicols, lincosamides, pleuromutilins, and streptogramin A [21].

We report the presence of various methicillin-resistant isolates of *S. hominis* subsp. *hominis* with resistance to linezolid and a reduced susceptibility to glycopeptides in blood cultures from ICU or Emergency Area patients at our third-level university hospital in Southern Spain.

Materials and methods

Bacterial isolates and patients

We studied ten clinical isolates of *S. hominis* subsp. *hominis* from nine patients (eight in the ICU and one in the Emergency Area) admitted to our hospital between November 2007 and March 2008. In all cases, *S. hominis* subsp. *hominis* was isolated in blood cultures (BacT/ALERT 3D; bioMérieux, Marcy-l'Etoile, France). Isolates were identified by using the WIDER system (Francisco Soria Melguizo, Madrid, Spain), confirming the result by 16S rRNA gene sequencing.

Data were gathered (see Table 1) on the date of ICU (or Emergency Area) admission, date of positive blood cultures, number of blood cultures in which *S. hominis* was identified with respect to the total number of blood cultures, diagnosis causing ICU/Emergency Area admission, antibiotic treatments before and since admission, and the presence of other microorganisms obtained from different samples, when applicable.

In six patients (In Table 1, P4 to P9), *S. hominis* was isolated in only one blood culture out of multiple serial extractions during their hospital stay. It was isolated from a blood culture taken from one of these patients (patient P9) in the Emergency Area but not in subsequent blood cultures taken in the ICU. *S. hominis* was isolated in two blood cultures taken from patient P3 on different days (isolates S-6911 and S-7063). *S. hominis* was isolated in three blood cultures taken on the same day from patient P1 and in four blood cultures taken on the same day from patient P2. However, only one isolate from each of these two patients could be phenotypically and genotypically analyzed. The others were not preserved because they were the first two patients and the clinical significance of *S. hominis* had not yet been recognized.

Antimicrobial susceptibility testing

Susceptibility of the isolates to antibiotics commonly used against staphylococci (Table 2) was measured by Etest (AB Biodisk, Solna, Sweden) with 0.5 McFarland inoculum in Mueller-Hinton agar (bioMérieux), following the manufacturer's recommendations. Clinical and Laboratory Standards Institute (CLSI) [22] criteria were applied to define the cutoff points for all antibiotics except for tigecycline. This was assigned a minimum inhibitory concentration (MIC) cutoff value of ≤0.5 μg/ml, in accordance with recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [23].

In isolates with MIC values ≥4 μg/ml for vancomycin or ≥8 μg/ml for teicoplanin, the Etest macromethod was used [24] to detect any heterogeneous resistance to glycopeptides [7]. Briefly, 100 μl of an inoculum suspension to 2.0 McFarland was pipetted onto a standard BHI agar plate (bioMérieux). Etest vancomycin and teicoplanin MIC gradient strips (AB Biodisk) were then applied, incubating the plates at 35°C, and reading them at 24 and 48 h, taking the result at 48 h as the endpoint. An isolate was considered to show heterogeneous resistance to glycopeptides when its MIC value was ≥8 μg/ml for vancomycin and ≥8 μg/ml for teicoplanin or ≥12 μg/ml for teicoplanin [7].

Following CLSI recommendations [22], *S. aureus* ATCC 29213 was used as the control in all susceptibility studies.

PCR amplification of the domain V region and DNA sequencing

Domain V region of the 23S rRNA gene spanning 2011 to 2699 bp (*E. coli* numbering) was amplified. Oligonucleotide

Table 1 Profile of the nine patients with the presence of *Staphylococcus hominis* in the blood culture

Patient	Date of ICU admission (dd/mm/yy)	Date of sampling (dd/mm/yy)	Isolate code	No. of positive samples/total blood cultures	Hospital department	Diagnosis	Linezolid treatment before ICU admission	Linezolid treatment during ICU admission	Glycopeptide treatment before ICU admission	Glycopeptide treatment during ICU admission	Other antibiotic treatments during ICU admission	Isolates of other microorganisms in other locations
P1	13/11/07	16/11/07	S-6567	3/20	ICU	Hemorrhagic shock[a]	No	No	No	Yes, vancomycin 19 days	Meropenem	MRSA *E. faecalis*
P2	18/11/07	22/11/07	S-6639	4/4	ICU	Medicament overdose/ nosocomial pneumonia	No	Yes, 2 days	No	Yes, vancomycin to replace linezolid	Piperacillin-tazobactam	No
P3	05/12/07	13/12/07 26/12/07	S-6911 S-7063	2/32	ICU	Septic shock[a]	No	Yes, 7 days	No	Yes, teicoplanin, 6 days	Meropenem Levofloxacin Piperacillin-tazobactam Clindamycin Tigecycline	*E. coli*
P4	01/01/08	09/01/08	S-117	1/16	ICU	Pneumonia	No	No	No	No	Ceftriaxone Levofloxacin Azithromycin	No
P5	08/01/08	09/01/08	S-127	1/4	ICU	Fever of unknown origin	No	No	No	No	Levofloxacin	No
P6	10/02/08	12/02/08	S-532	1/2	ICU	Atypical pneumonia	No	No	No	Yes, 8 days	Levofloxacin Ceftriaxone	No
P7	19/02/08	21/02/08	S-650	1/36	ICU	Diverticulitis/ enterovesical fistula	No	Yes, 2 days	No	No	Meropenem Fluconazole Caspofungin Ciprofloxacin	*P. aeruginosa* *C. albicans*
P8	08/03/08	10/03/08	S-855	1/10	ICU	Unstable angina	No	No	No	No	No	No
P9	24/03/08 25/03/08	24/03/08	S-1018	1/4 0/4	Emergency area ICU	Acute peritonitis (intestinal ischemia)	No	No	No	No	Imipenem	No

[a] Non-survivor

Table 2 Minimum inhibitory concentration (MIC) values (in μg/ml) obtained by Etest in Mueller-Hinton agar at 24 h of incubation at 37°C for the antibiotics assayed in the ten clinical isolates of *Staphylococcus hominis*

Isolate	Oxacillin	Vancomycin	Teicoplanin	Linezolid	Daptomycin	Tigecycline	Gentamicin	Levofloxacin	Erythromycin	Clindamycin	Cotrimoxazole
S-6567	>256	2	12	24	0.25	0.19	16	4	>256	2	>32/608
S-6639	>256	2	12	24	0.25	0.25	16	4	>256	2	>32/608
S-6911	>256	2	12	32	0.19	0.25	16	4	>256	2	>32/608
S-7063	>256	1.5	0.5	32	0.25	0.38	>1,024	>32	0.25	0.5	>32/608
S-117	>256	2	12	32	0.19	0.25	16	4	>256	2	>32/608
S-127	>256	2	12	32	0.19	0.25	16	4	>256	2	>32/608
S-532	>256	2	12	24	0.19	0.25	16	4	>256	2	>32/608
S-650	>256	2	12	24	0.25	0.25	16	4	>256	2	>32/608
S-855	>256	2	12	24	0.19	0.25	16	4	>256	2	>32/608
S-1018	>256	2	12	24	0.19	0.19	16	4	>256	2	>32/608

primers 5′-TGGGCACTGTCTCAACGA and 5′-ATCCCGGTCCTCTCGTACTA were used to amplify a 690-bp fragment. Polymerase chain reaction (PCR) conditions were 5 min at 95°C; 35 cycles at 95°C (1 min), 55°C (1 min), and 72°C (2 min); and a final 5 min at 72°C. Amplified DNA products were resolved by electrophoresis in agarose (1% wt/vol) gels containing ethidium bromide. Finally, the PCR product was purified (Wizard PCR Preps DNA Purification System; Promega, Madison, WI, USA), processed with a DNA sequencing kit, and analyzed in an automatic DNA sequencer (Applied Biosystems, Foster City, CA, USA). DNA sequences obtained were aligned with the corresponding nucleotide sequences from a linezolid-susceptible *S. aureus* reference strain (GenBank accession number X68425).

PFGE

We used a modification of the protocol of Murchan et al. [25], carrying out two procedures, digesting agarose blocks with SmaI and then with ApaI, and loading them into two agarose gels at 1% wt/vol. Electrophoresis was done in 0.5X Tris-Borate-EDTA buffer in a CHEF-DRII apparatus (Bio-Rad Laboratories, Hemel Hempstead, U.K.) under the following conditions: pulses of 0.1 to 30 s, 6 V/cm for 24 h at a temperature of 12 to 14°C. The gels were stained with ethidium bromide, visualized with ultraviolet light, and photographed. Fragment patterns were interpreted as described by Tenover et al. [26].

Results

Table 2 lists the MIC values obtained for the ten isolates in the antibiotic susceptibility study using Etest in Mueller-Hinton agar. All were resistant to oxacillin (MIC>256 μg/ml), gentamicin (MIC≥16 μg/ml), levofloxacin (MIC≥4 μg/ml), cotrimoxazole (MIC>32/608 μg/ml), and linezolid (MIC≥24 μg/ml) (Fig. 1a), and susceptible to daptomycin (MIC≤0.25 μg/ml) and tigecycline (MIC≤0.38 μg/ml). Nine of the isolates were resistant to erythromycin (MIC>256 μg/ml) and with intermediate resistance to clindamycin (MIC=2 μg/ml), whereas the remaining isolate (S-7063) was susceptible to both antibiotics.

All isolates except for S-7063 showed an MIC value of 2 μg/ml for vancomycin and of 12 μg/ml for teicoplanin in the Etest in agar Mueller-Hinton (Fig. 1b, c). Subsequent application of the Etest macromethod in BHI yielded MIC values of 8 μg/ml for vancomycin and 12 μg/ml for teicoplanin (Fig. 1d, e), confirming the presence of strains with heterogeneous resistance to vancomycin in these nine isolates.

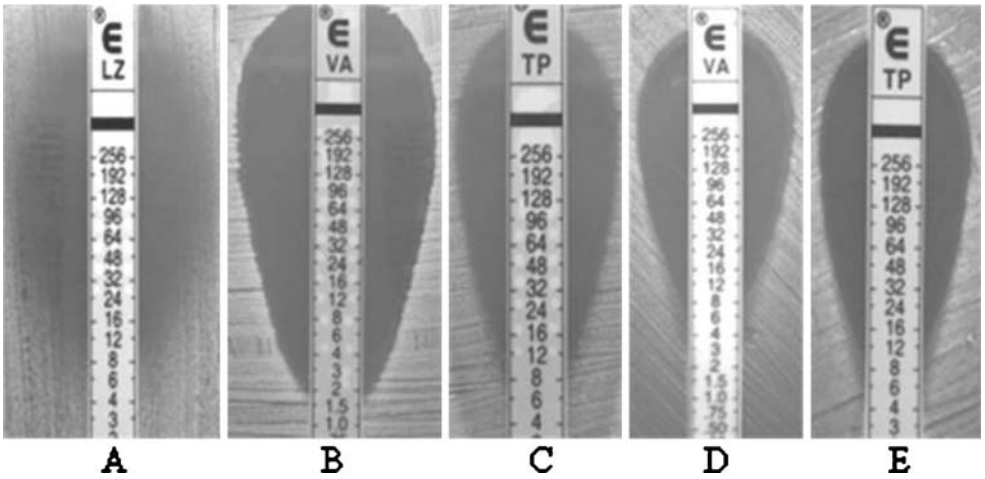


Fig. 1 Etest results for linezolid (**a**: suspension to 0.5 McFarland in Mueller-Hinton agar at 24 h of incubation) and for vancomycin and teicoplanin (**b** and **c**: suspension to 0.5 McFarland in Mueller-Hinton agar at 24 h of incubation; **d** and **e**: suspension to 2.0 McFarland in BHI agar after 48 h of incubation) in one of the studied isolates of *Staphylococcus hominis* (S-855)

Figure 2 depicts amplification of the 690-bp fragment of the domain V region of the 23S rRNA gene, performed in all isolates. Subsequent sequencing of this fragment revealed the presence of C2190T and G2603T mutations in nine of the isolates (S-6567, S-6639, S-6911, S-117, S-127, S-532, S-650, S-855, and S-1018) and of C2190T and G2474T mutations in isolate S-7063 (Fig. 3).

PFGE with SmaI digestion (Fig. 4) showed that all isolates except for S-7063 had the same electrophoretic profile, showing only three bands. A subsequent PFGE with ApaI digestion (Fig. 5) showed a pattern for these nine isolates that confirmed their epidemiologic relationship. Isolates in lines 1 to 6 and line 8 showed the same profile (profile A) and isolates in lines 7 and 9 were considered as two subtypes: profile A_1 and A_2, respectively. Isolate S-7063 in line 10 showed a completely different profile (profile B).

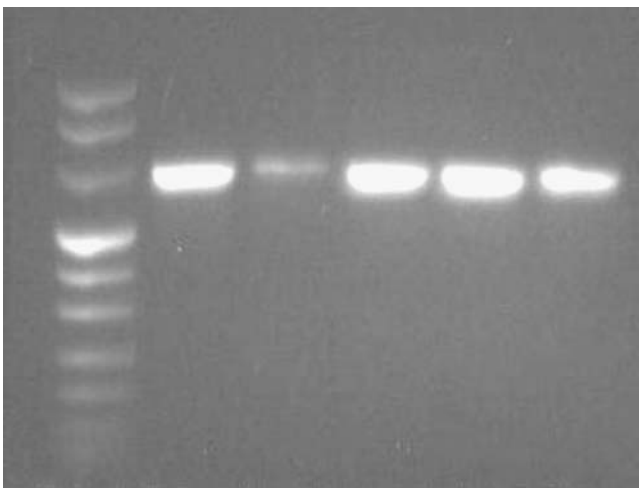


Fig. 2 Detection of the 690-bp fragment of domain V of the 23S rRNA gene by agarose gel electrophoresis (lane 1, molecular weight marker VIII, Roche Diagnostics, Spain)

Discussion

Coagulase-negative staphylococci, which can form part of normal skin flora, are also a frequent cause of infections in hospitalized patients. They are commonly implicated in catheter infections, infectious endocarditis, prosthesis infections, and osteoarticular infections, among others. Their treatment with glycopeptides or linezolid, especially in ICUs, has considerably increased the consumption of these antibiotics in recent years. As with other families of antibiotics, it has been reported that the increase in their use is related to an increase in the detection of resistant isolates [19, 27].

This is the first case since the introduction of glycopeptides and linezolid in our hospital of a circulating strain of methicillin-resistant *Staphylococcus* spp. with resistance to linezolid and a reduced susceptibility to glycopeptides.

The previous administration of linezolid [28] or glycopeptides [24] is known to be associated with the development of resistance in coagulase-negative staphylococci. There have also been reports of infection or colonization by linezolid-resistant coagulase-negative staphylococci in patients with no previous exposure to the antibiotic [28] and of environmental contamination by methicillin-resistant staphylococci with reduced susceptibility to glycopeptides [29].

In the majority of our patients, the bacterium was identified in only one of several serial blood cultures; therefore, this microorganism can be considered as a contaminant of these blood cultures rather than being responsible for a true bacteremia. Only one patient (P2) showed the microorganism in four blood cultures after presenting with nosocomial pneumonia at several days after ICU admission for medication overdose, but it was not detected in the sputum cultures from this patient.

One possible explanation is that these bacteria were transmitted via the hands of the healthcare staff or were

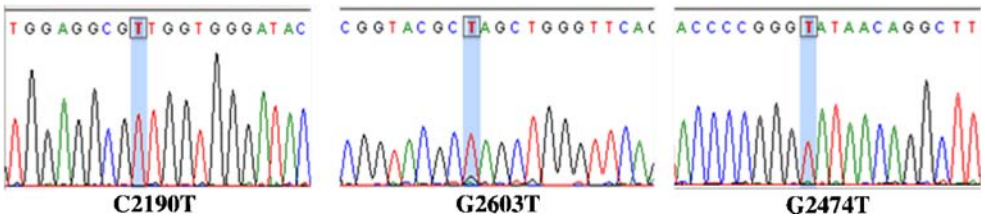
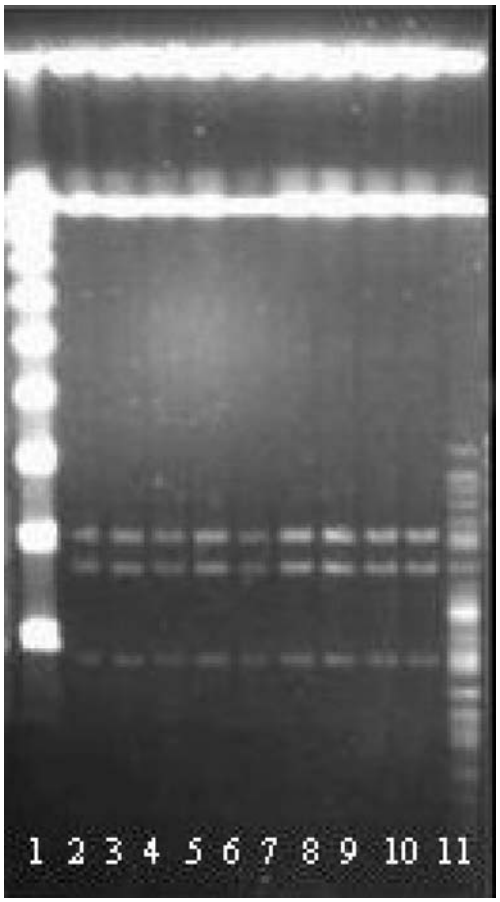


Fig. 3 Mutations detected in the studied isolates of *Staphylococcus hominis*

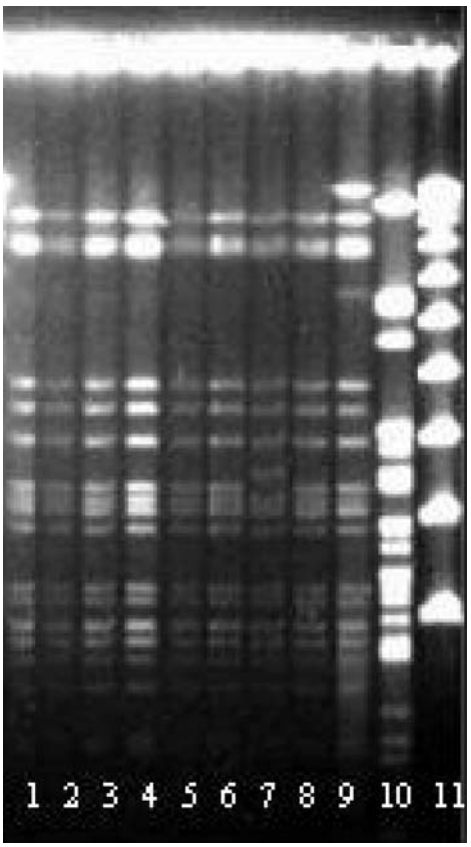
present in the ICU environment [30]. However, prevention measures recommended for the management of MRSA-colonized patients were taken [31], and none of the ICU staff or patients screened for nasal carriage were colonized with the resistant strain and no environmental contamination was detected. The origin of the outbreak remains unknown.

Various mutations in the domain V region of the 23S rRNA gene have been associated with resistance to linezolid in staphylococci, but only G2576T and G2603T mutations has been described in clinical isolates [18, 32]. G2576T mutation was not detected in our isolates. However, we report, for the first time, the presence of two



Lane 1: Ladder marker
Lane 2: isolate S-117
Lane 3: isolate S-127
Lane 4: isolate S-532
Lane 5: isolate S-650
Lane 6: isolate S-855
Lane 7: isolate S-1018
Lane 8: isolate S-6567
Lane 9: isolate S-6639
Lane 10: isolate S-6911
Lane 11: isolate S-7063

Fig. 4 Pulsed-field gel electrophoresis (PFGE) of ten *Staphylococcus hominis* digesting with SmaI



Lane 1: isolate S-117
Lane 2: isolate S-127
Lane 3: isolate S-532 } Profile A
Lane 4: isolate S-650
Lane 5: isolate S-855
Lane 6: isolate S-1018
Lane 7: isolate S-6567 Profile A_1
Lane 8: isolate S-6639 Profile A
Lane 9: isolate S-6911 Profile A_2
Lane 10: isolate S-7063 Profile B
Lane 11: Ladder marker

Fig. 5 PFGE of ten *Staphylococcus hominis* digesting with ApaI

mutations simultaneously in linezolid-resistant *Staphylococcus* spp.

The clinical significance of this circulating strain was not initially understood, since *S. hominis* was isolated with other microorganisms frequently implicated in nosocomial infections and, therefore, is considered a contaminant, and because *S. hominis* was isolated in only one culture from some of these patients.

Hospitalized patients, especially ICU patients, can be infected by colonized patients or healthcare personnel. The possible selection over time of isolates of the more virulent *S. aureus* that are MRSA, GISA, and/or resistant to linezolid poses a potentially severe challenge to current anti-Gram-positive therapies. Although the prevention of dissemination is important, we believe that the rational use of antibiotics in preventing the selection of drug-resistant pathogens is as important.

References

1. Shorr AF, Lipman J (2007) Resistance in the intensive care unit: whose problem is it and how can intensivists help? Crit Care Med 35:299–301
2. MacKenzie FM, Bruce J, Struelens MJ et al (2007) Antimicrobial drug use and infection control practices associated with the prevalence of methicillin-resistant *Staphylococcus aureus* in European hospitals. Clin Microbiol Infect 13:269–276
3. Ruiz de Gopegui E, Oliver A, Galmés MI et al (2005) Consolidation of a *Staphylococcus aureus* multiresistant clone not related to the Iberian in a hospital from Mallorca. Enferm Infecc Microbiol Clin 23:140–144
4. Hiramatsu K, Hanaki H, Ino T et al (1997) Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. J Antimicrob Chemother 40:135–136
5. Chang S, Sievert DM, Hageman JC et al (2003) Infection with vancomycin-resistant *Staphylococcus aureus* containing the *vanA* resistance gene. N Engl J Med 348:1342–1347
6. Chiew YF, Charles M, Johnstone MC et al (2007) Detection of vancomycin heteroresistant *Staphylococcus haemolyticus* and vancomycin intermediate resistant *Staphylococcus epidermidis* by means of vancomycin screening agar. Pathology 39:375–377
7. Walsh TR, Bolmström A, Qwärnström A et al (2001) Evaluation of current methods for detection of staphylococci with reduced susceptibility to glycopeptides. J Clin Microbiol 39:2439–2444
8. Walsh TR, Howe RA (2002) The prevalence and mechanisms of vancomycin resistance in *Staphylococcus aureus*. Annu Rev Microbiol 56:657–675
9. Ariza J, Pujol M, Cabo J et al (1999) Vancomycin in surgical infections due to methicillin-resistant *Staphylococcus aureus* with heterogeneous resistance to vancomycin. Lancet 353:1587–1588
10. Charles PGB, Ward PB, Johnson PDR et al (2004) Clinical features associated with bacteremia due to heterogeneous vancomycin-intermediate *Staphylococcus aureus*. Clin Infect Dis 38:448–451
11. Cha R, Brown WJ, Rybak MJ (2003) Bactericidal activities of daptomycin, quinupristin-dalfopristin, and linezolid against vancomycin-resistant *Staphylococcus aureus* in an in vitro pharmacodynamic model with simulated endocardial vegetations. Antimicrob Agents Chemother 47:3960–3963
12. Huang YT, Liao CH, Teng LJ et al (2008) Comparative bactericidal activities of daptomycin, glycopeptides, linezolid and tigecycline against blood isolates of gram-positive bacteria in Taiwan. Clin Microbiol Infect 14:124–129
13. Grau S, Aguado JM, Mateu-de Antonio J et al (2007) Economic evaluation of linezolid versus teicoplanin for the treatment of infections caused by gram-positive microorganisms in Spain. J Chemother 19:398–409
14. Swaney SM, Aoki H, Ganoza MC et al (1998) The oxazolidinone linezolid inhibits initiation of protein synthesis in bacteria. Antimicrob Agents Chemother 42:3251–3255
15. Tsiodras S, Gold HS, Sakoulas G et al (2001) Linezolid resistance in a clinical isolate of *Staphylococcus aureus*. Lancet 358:207–208
16. Tarazona RE, Padilla TP, Gómez JC et al (2007) First report in Spain of linezolid non-susceptibility in a clinical isolate of *Staphylococcus haemolyticus*. Int J Antimicrob Agents 30:277–278
17. Werner G, Strommenger B, Klare I et al (2004) Molecular detection of linezolid resistance in *Enterococcus faecium* and *Enterococcus faecalis* by use of 5′ nuclease real-time PCR compared to a modified classical approach. J Clin Microbiol 42:5327–5331
18. Hong T, Li X, Wang J et al (2007) Sequential linezolid-resistant *Staphylococcus epidermidis* isolates with G2576T mutation. J Clin Microbiol 45:3277–3280
19. Kelly S, Collins J, Maguire M et al (2008) An outbreak of colonization with linezolid-resistant *Staphylococcus epidermidis* in an intensive therapy unit. J Antimicrob Chemother 61:901–907
20. Meka VG, Pillai SK, Sakoulas G et al (2004) Linezolid resistance in sequential *Staphylococcus aureus* isolates associated with a T2500A mutation in the 23S rRNA gene and loss of a single copy of rRNA. J Infect Dis 190:311–317
21. Mendes RE, Deshpande LM, Castanheira M et al (2008) First report of cfr-mediated resistance to linezolid in human staphylococcal clinical isolates recovered in the United States. Antimicrob Agents Chemother 52:2244–2246
22. Clinical and Laboratory Standards Institute (CLSI) (2007) Performance standards for antimicrobial susceptibility testing; seventeenth informational supplement. CLSI publication M100-S17. CLSI, Wayne, PA, USA
23. European Committee on Antimicrobial Susceptibility Testing (EUCAST) Steering Committee (2006) EUCAST technical note on tigecycline. Clin Microbiol Infect 12:1147–1149
24. Voss A, Mouton JW, van Elzakker EP et al (2007) A multi-center blinded study on the efficiency of phenotypic screening methods to detect glycopeptide intermediately susceptible *Staphylococcus aureus* (GISA) and heterogeneous GISA (h-GISA). Ann Clin Microbiol Antimicrob 6:9
25. Murchan S, Kaufmann ME, Deplano A et al (2003) Harmonization of pulsed-field gel electrophoresis protocols for epidemiological typing of strains of methicillin-resistant *Staphylococcus aureus*: a single approach developed by consensus in 10 European laboratories and its application for tracing the spread of related strains. J Clin Microbiol 41:1574–1585
26. Tenover FC, Arbeit RD, Goering RV et al (1995) Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 33:2233–2239
27. Kolar M, Urbanek K, Vagnerova I et al (2006) The influence of antibiotic use on the occurrence of vancomycin-resistant enterococci. J Clin Pharm Ther 31:67–72
28. Potoski BA, Adams J, Clarke L et al (2006) Epidemiological profile of linezolid-resistant coagulase-negative staphylococci. Clin Infect Dis 43:165–171
29. Perdelli F, Dallera M, Cristina ML et al (2008) A new microbiological problem in intensive care units: environmental

contamination by MRSA with reduced susceptibility to glycopeptides. Int J Hyg Environ Health 211:213–218
30. Simpson RA, Spencer AF, Speller DC et al (1986) Colonization by gentamicin-resistant *Staphylococcus epidermidis* in a special care baby unit. J Hosp Infect 7:108–120
31. Centers for Disease Control and Prevention (CDC) (2007) Information about MRSA for Healthcare Personnel [electronic guide]. Available online at: http://www.cdc.gov/ncidod/dhqp/ar_mrsa_healthcareFS.html [consulted 29 March 2008]
32. Lincopan N, de Almeida LM, Elmor de Araújo MR et al (2009) Linezolid resistance in Staphylococcus epidermidis associated with a G2603T mutation in the 23S rRNA gene. Int J Antimicrob Agents 34:281–282. doi:10.1016/j.ijantimicag.2009.02.023