from decubitus ulcers of patients in this geriatric institution. Before 1999, no MRSA was detected in residents of this facility, and only a few sporadic cases were found in patients admitted to HUSD. Five different clonal types of MRSA were detected among the 17 patients of the geriatric institution, with the five patients who had been admitted to HUSD since 1999 each being infected by a different clonal type of MRSA. Since all these clones had been documented previously in patients admitted to HUSD, it seems reasonable to conclude that these patients acquired MRSA strains during their hospitalisation period. The fact that the other 12 patients had not been hospitalised previously in HUSD suggests that these MRSA clones may have spread subsequently between different patients within the geriatric institution. The high rate of MRSA found highlights the importance of epidemiological analysis in controlling the dissemination of MRSA in chronic care facilities, as in tertiary hospitals.

REFERENCES

1. Barber M. Methicillin-resistant staphylococci. *J Clin Pathol* 1961; **14**: 385–393.
2. Ayliffe GA. The progressive intercontinental spread of methicillin-resistant *Staphylococcus aureus*. *Clin Infect Dis* 1997; **24**(suppl 1): S74–S79.
3. Voss A, Milatovic D, Wallrauch-Schwarz C, Rosdahl VT, Braveny I. Methicillin-resistant *Staphylococcus aureus* in Europe. *Eur J Clin Microbiol Infect Dis* 1994; **13**: 50–55.
4. Parras F, Rodríguez M, Bouza E *et al*. Epidemic outbreak of methicillin-resistant *Staphylococcus aureus* (MRSA) in a general hospital. Preliminary report. *Enferm Infecc Microbiol Clin* 1991; **9**: 200–207.
5. Trilla A, Marco F, Moreno A, Prat A, Soriano E, Jiménez de Anta MT. Clinical epidemiology of an outbreak of nosocomial infection caused by *Staphylococcus aureus* resistant to methicillin and aminoglycosides: efficacy of control measures. Comité de Control de Infecciones. *Med Clin (Barc)* 1993; **100**: 205–209.
6. Bradley SF. Methicillin-resistant *Staphylococcus aureus* in nursing homes. Epidemiology, prevention and management. *Drugs Aging* 1997; **10**: 185–198.
7. O'Sullivan NP, Keane CT. Risk factors for colonization with methicillin-resistant *Staphylococcus aureus* among nursing home residents. *J Hosp Infect* 2000; **45**: 206–210.
8. Talon DR, Bertrand X. Methicillin-resistant *Staphylococcus aureus* in geriatric patients: usefulness of screening in a chronic-care setting. *Infect Control Hosp Epidemiol* 2001; **22**: 505–509.
9. Capitano B, Leshem OA, Nightingale CH, Nicolau DP. Cost effect of managing methicillin-resistant *Staphylococcus aureus* in a long-term care facility. *J Am Geriatr Soc* 2003; **51**: 10–16.
10. Olona-Cabases M, Tico-Falguera N, Ramírez-Garcerán L, Del Valle-Ortiz O, Castello-Verdu T, García-Fernández L. Methicillin-resistant *Staphylococcus aureus*: a four-year experience in a spinal cord injury unit in Spain. *Spinal Cord* 1996; **34**: 315–319.
11. Kloos WE, Bannerman TL. Staphylococcus and Micrococcus. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, eds. *Manual of clinical microbiology*. Washington, DC: American Society for Microbiology, 1999; 271–276.
12. National Committee for Clinical Laboratory Standards. *Performance standards for antimicrobial disk susceptibility tests*, 7th edn. Approved Standard M2-A7. Wayne, PA: NCCLS, 2002.
13. Tenover F, Arbeit R, Goering R *et al*. Interpreting chromosomal DNA restriction patterns produced by pulsed field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 1995; **33**: 2233–2239.
14. Domínguez MA, de Lencastre H, Liñares J, Tomasz A. Spread and maintenance of a dominant methicillin-resistant *Staphylococcus aureus* (MRSA) clone during an outbreak of MRSA disease in a Spanish hospital. *J Clin Microbiol* 1994; **32**: 2081–2087.
15. Emori TG, Banerjee SN, Culver DH *et al*. Nosocomial infections in elderly patients in the United States, 1986–1990. National Nosocomial Infections Surveillance System. *Am J Med* 1991; **91**(suppl 3B): 289S–293S.
16. Bradley SF. *Staphylococcus aureus* infections and antibiotic resistance in older adults. *Clin Infect Dis* 2002; **34**: 211–216.
17. Nicolle LE, Strausbaugh LJ, Garibaldi RA. Infections and antibiotic resistance in nursing homes. *Clin Microbiol Rev* 1996; **9**: 1–17.

RESEARCH NOTE

False synergy between vancomycin and β-lactams against glycopeptide-intermediate *Staphylococcus aureus* (GISA) caused by inappropriate testing methods

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ABSTRACT

The combination of vancomycin and β-lactams is often considered synergistic and has been recommended for the treatment of glycopeptide-intermediate *Staphylococcus aureus* (GISA) infections. In this study, the combination of vancomycin or

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teicoplanin with different β-lactams was tested. When using NaCl 4% w/v, for better expression of heterogeneous resistance to β-lactams, with a longer (48-h) incubation period and a higher (10^7 CFU/mL) inoculum, the association of vancomycin with β-lactams was antagonistic. However, a synergistic effect was observed for teicoplanin under the same conditions.

Glycopeptide-intermediate *Staphylococcus aureus* (GISA) strains have been isolated in our hospital for more than 10 years [1,2], and similar strains are now reported worldwide [3]. These strains, similar to strain Mu3 [3], show low-level resistance to vancomycin and teicoplanin, with MICs not exceeding 3 and 16 mg/L, respectively, on Mueller-Hinton (MH) agar with a standard inoculum, and MICs of 6–8 or 8–32 mg/L, respectively, when tested on Brain Heart Infusion (BHI) agar with a higher inoculum [3]. Most importantly, all these strains display heterogeneous resistance to vancomycin (and teicoplanin), with colonies growing on 8–16 mg/L of vancomycin, in contrast to 2–4 mg/L for fully susceptible strains [3]. Very few strains worldwide are fully resistant to vancomycin, with an MIC of 8 mg/L for a strain (Mu50) isolated in Japan by K. Hiramatsu, and MICs of >128 mg/L for two recent strains isolated in the USA which carry the *vanA* gene [4]. The clinical impact of GISA has not been established; reported clinical failures can be attributed to low vancomycin levels or inappropriate associations [5]. It has been demonstrated that, with appropriate vancomycin dosage and serum level determination, the failure rate for GISA is similar to the failure rate with fully-susceptible strains [6].

Confusing results concerning the association of vancomycin or teicoplanin with β-lactams have been reported, in that synergy or antagonism has been observed with the same bacteria and the same antibiotic associations, but with different testing procedures [7,8]. In most, if not all of these

studies, conditions were not optimal for the expression of heterogeneous resistance to β-lactams. The influence of salt on the MIC of vancomycin alone or in association with β-lactams has been rarely but clearly reported [9]. In view of these previous results, the aim of this study was to determine the activity of vancomycin or teicoplanin in combination with different β-lactams.

Fourteen GISA strains isolated in France during 1992–2000 were tested in comparison to the prototype Japanese strains Mu50 and Mu3, two methicillin-resistant *S. aureus* (MRSA) strains and one methicillin-sensitive *S. aureus* strain susceptible to glycopeptides, and the susceptible reference strain ATCC 25923. Strains Lim2 and 98141 were obtained from M. C. Ploy and N. El Solh (Institut Pasteur, Paris, France), respectively; the 12 other strains were isolated at the Saint-Joseph Hospital, Paris. MICs of either vancomycin or teicoplanin were determined by the Etest method, with or without NaCl 4% w/v, on MH agar and BHI agar with an inoculum of 0.5 × McFarland standard. Plates were incubated for 48 h at 37 °C in air. MICs were also determined under the same conditions in the presence of cefotaxime 8 mg/L and oxacillin 8 mg/L. Alternatively, MICs of oxacillin, amoxycillin–clavulanate (with a fixed clavulanic acid concentration of 2 mg/L) and cefotaxime were determined under the same conditions on plates containing vancomycin or teicoplanin at 2, 4, 6 or 8 mg/L. All tests were performed at least three times.

The interaction between vancomycin or teicoplanin and β-lactams was also studied on BHI agar, with or without NaCl 4% w/v, with various β-lactam disks on plates containing vancomycin or teicoplanin at 2, 4, 6 or 8 mg/L.

Strains were considered to be GISA if the vancomycin MIC was ≥ 4 mg/L or the teicoplanin MIC was ≥ 8 mg/L and the population analysis profile was similar to that of strain Mu3, with colonies growing at vancomycin ≥ 6 mg/L, in contrast to < 4 mg/L for fully susceptible strains.

The results showed that the MICs of oxacillin, amoxycillin–clavulanate and cefotaxime for all GISA strains were ≥ 32 mg/L when tested on MH agar or BHI agar containing NaCl 4% w/v (data not shown). The comparative MICs of vancomycin or teicoplanin on BHI agar in the presence or absence of NaCl 4% w/v are shown in Table 1. When tested on MH agar, the MICs were 0.5–1.5

Table 1. MICs of vancomycin and teicoplanin obtained by the Etest method on BHI agar

Strain number	Strain designation	MIC by Etest			
		VA BHI	VA BHI +NaCl	TEC BHI	TEC BHI + NaCl
1	ATCC 25923	3	3	3	1
2	MU3	4	3	24	8
3	MU50	12	12	32	16
4	LIM2	6	12	24	12
5	12909	6	8	6	6
6	JL77	4	8	16	6
7	BER	4	12	12	8
8	98141	6	12	16	8
9	GIO	4	8	12	6
10	BAC	6	12	16	8
11	PRE	6	8	16	6
12	CAD	4	8	16	8
13	ROD	6	8	12	6
14	COQ	6	8	12	8
15	BER	4	8	8	6
16	MER	6	8	12	6
17	COQ	6	8	12	6
18	RM 13042	3	3	3	2
19	RM 13062	3	3	3	1.5
20	RM 13089	3	3	3	2

Strains 4–17, 14 GISA isolates from France; strains 18–20, two MRSA and one methicillin-sensitive *S. aureus* susceptible to glycopeptides; VA, vancomycin; TEC, teicoplanin; BHI, brain heart infusion.

dilutions lower (data not shown). In the presence of NaCl, the MICs of vancomycin for the French GISA isolates were 0.5–2 dilutions higher, and the MICs of teicoplanin were 0.5–2 dilutions lower. For strains Mu3 and Mu50, there was no increase and, eventually, a 0.5–1 dilution decrease in the MIC of vancomycin. For the glycopeptide-susceptible strains, the shift in the MIC was minimal.

Fig. 1 shows the results of the interaction of vancomycin with cefotaxime for strain Mu3 in the absence (A) or presence (B) of NaCl 4% w/v. A moderate to strong antagonism was observed

according to the test conditions. Similar results were obtained with all the French GISA isolates.

In the presence of cefotaxime (8 mg/L) in the agar, the MICs of vancomycin were 2–6 mg/L in the absence of NaCl and 3–8 mg/L with NaCl 4% w/v in the agar. For teicoplanin, the MICs were 6–16 mg/L (no NaCl) or 2–4 mg/L (NaCl 4% w/v), respectively.

For strain Mu50, addition of NaCl did not improve expression of the heterogeneous methicillin resistance (Mu50 is a *mecA*-positive strain, but is penicillinase-negative). In contrast to other GISA strains, the association of vancomycin with β-lactams was antagonistic in the absence of NaCl, but not in the presence of NaCl. For teicoplanin, the association with β-lactams was synergistic; this synergy was enhanced in the presence of NaCl.

As indicated by Hiramatsu [3], all GISA strains have undergone multiple small adjustments in their cell-wall metabolism, but they are not identical. GISA strains have a prolonged generation rate, grow as small colonies, need multiple nutrients and are more or less unstable. Incubation for at least 48 h is required before final interpretation of MIC results [3–5,7,10]. Numerous previous studies have demonstrated clearly that the addition of NaCl 2–5% w/v is necessary for the expression of resistance to β-lactams in many heterogeneous MRSA strains. It is unclear why this procedure is used normally only for the detection of resistance and not for MIC determinations. When tested correctly, MRSA strains are
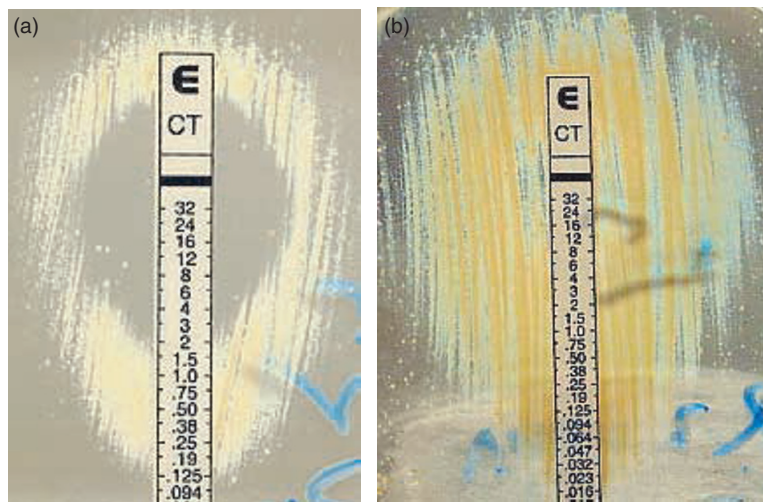
Fig. 1. MIC of cefotaxime for strain Mu3 on BHI agar containing vancomycin 2 mg/L: (a) without NaCl; (b) with NaCl 4% w/v. In the absence of NaCl (a), there is clear antagonism over a limited concentration range 0.12–2 mg/L. However, the presence of a few colonies at concentrations > 2 mg/L corresponds to what is usually observed when testing β-lactams on MRSA strains at sub-optimal conditions. In the presence of NaCl 4% w/v (b) there is clear antagonism for cefotaxime concentrations of ≥ 32 mg/L

always resistant to all β-lactams without any exception.

The reason why NaCl increases resistance to vancomycin, as described previously [9], is not known, nor is the reason for the decrease in the MIC of teicoplanin, which to our knowledge has never been reported previously. Antagonism between β-lactams and vancomycin is very clear in the presence of NaCl 4% w/v (Fig. 1b), and this is particularly true for strain Mu3. For some other strains, the persistance of a few colonies within the β-lactam inhibition zone indicates that at least part of the population is not inhibited by high concentrations of β-lactams in the presence of vancomycin. Moreover, because of the short half-life of most β-lactams, it is anticipated that only low β-lactam levels are present to interact with glycopeptides for quite a long period. However, at high vancomycin concentrations, and for a very limited MIC range (0.5–1 mg/L), the interaction between vancomycin and β-lactams can be considered as synergistic.

In contrast to vancomycin, the interaction between teicoplanin and β-lactams is more often synergistic, as has been demonstrated previously for teicoplanin-resistant isolates of *Staphylococcus epidermidis* [11]; in the presence of NaCl there is enhanced synergy, depending on the precise concentration of the β-lactam (data not shown).

The precise clinical significance and reasons for these results are unknown, but it can be concluded that vancomycin should not be used in association with β-lactams for the treatment of GISA infections.

REFERENCES

1. Mainardi JL, Shlaes DM, Goering RV, Shlaes JH, Acar JF, Goldstein FW. Decreased teicoplanin susceptibility of methicillin-resistant strains of *Staphylococcus aureus*. *J Infect Dis* 1995; **171**: 1646–1650.
2. Quincampoix JC, Carbonne A, Loyer D *et al.* Follow-up of a nosocomial outbreak of methicillin-resistant strains of *Staphylococcus aureus* (MRSA) with decreased susceptibility to teicoplanin (DST) [abstract C167]. In: *Program and abstracts of the 37th Interscience Conference on Antimicrobial agents and Chemotherapy*. Toronto, Canada, Washington DC. American Society for Microbiology 1997.
3. Hiramatsu K. Vancomycin-resistant *Staphylococcus aureus*: a new-model of antibiotic resistance. *Lancet Inf Dis* 2001; **1**: 147–155.
4. Anonymous. Vancomycin-resistant *Staphylococcus aureus*-Pennsylvania 2002. *Morb Mortal Wkly Rep.* 2002; **51**: 902.
5. Goldstein F, Kitzis MD. Vancomycin resistant *S. aureus*: no apocalypse now. *Clin Microbiol Infect* 2003; **9**: 761–765.
6. Trad S, Ben Ali A, Garrouste M *et al.* Pneumopathies à *S. aureus* de sensibilité intermédiaire aux glycopeptides (GISA): la vancomycine (Vanco) reste le traitement de reference [abstract 291/P2]. In: *Program and abstracts of 22eme Réunion Interdisciplinaire de Chiomthérapie Anti-Infectieuse*. Paris, France. Association pour l'Organisation des Réunions Interdisciplinaires de Chimiothérapie Anti-infectieuse 2002.
7. Aritaka N, Hanaki H, Cui L, Hiramatsu K. Combination effect of vancomycin and β-lactams against a *Staphylococcus aureus* strain, Mu3, with heterogeneous resistance to vancomycin. *Antimicrob Agents Chemother* 2001; **45**: 1292–1294.
8. Guerin F, Buu-Hoï A, Mainardi JL *et al.* Outbreak of methicillin-resistant *Staphylococcus aureus* with reduced susceptibility to glycopeptides in a parisian hospital. *J Clin Microbiol* 2000; **38**: 2985–2988.
9. Wong SS, Ho PL, Woo PC, Yuen KY. Bacteremia caused by staphylococci with inducible vancomycin heteroresistance. *Clin Inf Dis* 1999; **29**: 760–767.
10. Cui L, Ma X, Sato K *et al.* Cell wall thickening is a common feature of vancomycin resistance in *Staphylococcus aureus*. *J Clin Microbiol* 2003; **41**: 5–14.
11. Mokhbat J, Acar JF, Goldstein FW. Synergistic effect of imipenem plus teicoplanin against methicillin-resistant, teicoplanin-resistant, coagulase-negative Staphylococci [abstract 125]. In: *Program and abstracts of the 31st Interscience Conference on Antimicrobial Agents and Chemotherapy*. Chicago, IL, Washington DC. American Society for Microbiology 1991.

RESEARCH NOTE

Use of quantitative and objective enzyme immunoassays to investigate the possible association between *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* antibodies and asthma

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