REFERENCES


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 β-lactams was tested for synergistic activity against 10 isolates of GISA. False synergy was observed in 8/10 isolates.

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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 (10⁷ CFU/mL) inoculum, the association of vancomycin with β-lactams was antagonistic. However, a synergistic effect was observed for teicoplanin under the same conditions.

**Keywords** β-Lactams, GISA, glycopeptides, susceptibility testing, *Staphylococcus aureus*

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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
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

<table>
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<th>Strain number</th>
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<th>MIC by Etest</th>
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<td>ATCC 25923</td>
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<td>20</td>
<td>RM 13089</td>
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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.

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


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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