Detection of glycopeptide resistance in clinical isolates of Gram-positive bacteria

The glycopeptide antibiotics, vancomycin and, to a lesser extent, teicoplanin, are used both for the treatment and prophylaxis of serious infections caused by multi-resistant, Gram-positive bacteria. In particular, vancomycin is used extensively to treat severe, systemic infections, such as endocarditis or continuous ambulatory peritoneal dialysis (CAPD) peritonitis, caused by methicillin-resistant strains of Staphylococcus aureus or coagulase-negative Staphylococcus spp. (Kucers & Bennett, 1987; Eykyn, 1988).

Despite occasional reports of resistant strains, reviewed by Johnson et al., 1990, vancomycin resistance did not present a significant problem in the thirty years following the introduction of this antibiotic into clinical use (Cooper & Given, 1986). However, during the 1980s, resistance to vancomycin and/or teicoplanin was found in several bacterial genera which can cause, and indeed have caused, serious infections in humans.

High-level resistance to glycopeptide antibiotics is now recognized as an intrinsic characteristic of some species of Lactobacillus, Leuconostoc, Pediococcus and Erysipelothrix (Colman & Efstratiou, 1987; Johnson et al., 1990). Although these genera may cause serious infections, the numbers of such infections are relatively few and the organisms are often susceptible to a range of antibiotics. For these reasons, vancomycin resistance in these organisms does not usually cause therapeutic problems. In contrast, the emergence of glycopeptide resistance in both Enterococcus spp. (Courvalin, 1990; Johnson et al., 1990; Guiot, Peertmans & Sebens, 1991) and Staphylococcus spp. (Schwalbe, Stapleton & Giligan, 1987; Aubert et al., 1990; Johnson et al., 1990; Kaatz et al., 1990; Veach et al., 1990; Sanyal et al., 1991) has caused wider clinical concern. These organisms are often multi-resistant and vancomycin is more frequently regarded as the treatment of choice for infections caused by them.

In enterococci, both high-level (minimum inhibitory concentration, MIC, > 64 mg/L) and low-level (MIC 16–64 mg/L) vancomycin resistance have been reported (Courvalin, 1990; Johnson et al., 1990; Guiot et al., 1991). High-level vancomycin resistance is usually associated with cross-resistance to teicoplanin (MIC 8- > 128 mg/L), but isolates exhibiting low-level resistance to vancomycin remain sensitive to teicoplanin in vitro (MIC < 2 mg/L). The therapeutic significance of this in-vitro susceptibility to teicoplanin is unclear at present.

Coagulase-negative staphylococci which have low-level resistance to teicoplanin, but which are sensitive to vancomycin have been described (Johnson et al., 1990). In addition, we are aware of a single report of the emergence of teicoplanin resistance (MIC rising from 1 to 8 mg/L) in S. aureus during teicoplanin therapy (Kaatz et al., 1990). This strain remained sensitive to vancomycin. Although rarer, low-level vancomycin resistance (MIC 8–16 mg/L) has also been reported in single isolates of coagulase-negative staphylococci (Schwalbe et al., 1987; Aubert et al., 1990; Johnson et al., 1990; Veach et al., 1990; Sanyal et al., 1991). In contrast to low-level vancomycin resistant enterococci, such staphylococci may display cross-resistance to teicoplanin (Schwalbe et al., 1987; Aubert et al., 1990; Veach et al., 1990; Sanyal et al., 1991). High-level glycopeptide resistance has not been reported in staphylococci to date.

In the clinical laboratory, an agar incorporation breakpoint method is likely to be the most reliable means of detecting vancomycin resistant organisms. A breakpoint concentration of 4 mg/L vancomycin is recommended by both the British Society for Antimicrobial Chemotherapy (BSAC) Working Party (1988) and the National Committee for Clinical Laboratory Standards (NCCLS; 1990a) for distinguishing 'susceptible' from 'resistant' strains. The MICs of vancomycin and teico-
plasmid for isolates which grow at the breakpoint concentration may be determined subsequently to distinguish between high- and low-level glycopeptide resistance.

The composition of the medium used for MIC determination does not affect significantly the results obtained for vancomycin (Fehningham et al., 1987; Utley et al., 1989; Chomarat, Espinouse & Flandrois, 1991; Sanyal et al., 1991). In contrast, the basal medium, the presence of nutritional supplements, the size of the inoculum and the incubation period each have a more pronounced effect on results obtained for teicoplanin (Fehningham et al., 1987; Chomarat et al., 1991). Coagulase-negative staphylococci appeared two to three-fold more susceptible to teicoplanin when MICs were determined on Diagnostic Sensitivity Test agar than on Iso-SensiS test agar (Fehningham et al., 1987). Similarly, the addition of blood to either medium caused a slight increase in MICs obtained (Fehningham et al., 1987).

Disc susceptibility testing may provide a suitable alternative for the detection of high-level glycopeptide resistance in enterococci and genera exhibiting intrinsic resistance. In our experience, isolates with high-level resistance show no zone of inhibition around either a 30 μg vancomycin or teicoplanin disc (Johnson et al., 1991). However, Sahm & Olsen (1990) found that disc tests performed according to NCCLS criteria (NCCLS, 1990b), categorized as sensitive all enterococci with vancomycin MICs of < 2048 mg/L unless an increased inoculum or prolonged incubation period was employed.

Low-level glycopeptide resistance may not be detected by disc testing. The current NCCLS interpretative criteria recommend the use of 30 μg vancomycin discs and zone sizes of ≥ 12 mm (equivalent to MIC ≤ 4 mg/L) for susceptibility (or moderate susceptibility for enterococci) and ≤ 9 mm (equivalent to MIC ≥ 32 mg/L) for resistance (NCCLS, 1990b). Swenson and colleagues (Swenson, Hill & Thornsberry, 1989) found that these criteria failed to classify correctly some enterococci with vancomycin MICs of 8–32 mg/L. This group recommended the use of modified criteria with zone sizes of ≤ 14 mm indicating resistance and ≥ 15 mm indicating susceptibility. However, isolates with MICs of 8 mg/L may still be missed using these modified criteria (Swenson et al., 1989). It is unclear at the present time how the NCCLS criteria (NCCLS, 1990b) will affect detection of low level resistance in staphylococci. For testing teicoplanin, 30 μg discs are also recommended with interpretative zone sizes of ≤ 10 mm (equivalent to MIC > 8 mg/L) for resistance and ≥ 14 mm (equivalent to MIC ≤ 4 mg/L) for susceptibility (Barry, Thornsberry & Jones, 1986; Barry et al., 1987). In contrast the French Society for Microbiology recommend a zone size of ≥ 17 mm for susceptibility to teicoplanin (MIC ≤ 4 mg/L) (Courvalin et al., 1990).

As low-level vancomycin resistant isolates show only slightly reduced zones around a 30 μg vancomycin disc and may be classified as sensitive by NCCLS criteria (Swenson et al., 1989; Sahm & Olsen, 1990), critical comparison of zone sizes with those of control strains is necessary. We have found, using the Stokes' method (Stokes & Ridgway, 1987) with NCTC 6571 as a control, that the use of a 5 μg vancomycin disc greatly facilitates detection of low-level resistance in both coagulase-negative staphylococci (Sanyal et al., 1991) and enterococci (authors' unpublished observations). Such isolates appear sensitive to vancomycin by the Stokes' method when a 30 μg vancomycin disc is used.

There were no significant differences between results obtained by Stokes' tests performed on Iso-SensiS or Diagnostic Sensitivity Test agars (using 30 or 5 μg vancomycin discs and 30 μg teicoplanin discs). The addition of 5% lysed horse blood to either medium caused a reduction in zone sizes. However, zone sizes for the control strain were affected equally and there was no alteration in the category of susceptibility assigned (Sanyal et al., 1991; authors' unpublished observations). Similarly, the addition of blood to the test medium had no significant effects on the results of vancomycin disc tests performed according to NCCLS criteria (Sahm & Olsen, 1990).

Variations in the classification of susceptibility to teicoplanin and vancomycin of coagulase-negative staphylococci by disc diffusion methods have been studied. The results obtained for teicoplanin were affected by inoculum density, the basal medium and the length of incubation whilst those for vancomycin were not (Chomarat et al., 1991).

An automated method of susceptibility testing (AMS-Vitek) has been evaluated for the detection of vancomycin resistant strains of enterococci (Sahm & Olsen, 1990). However, this method failed to classify as resistant enterococci for which vancomycin MICs were 128–256 mg/L when determined by an agar incorporation method.
Resistance to glycopeptide antibiotics in intrinsically resistant genera and S. aureus is expressed constitutively (Johnson et al., 1990; Kaatz et al., 1990). In contrast both high- and low-level glycopeptide resistance in enterococci are inducible phenotypes, requiring the production of novel cytoplasmic membrane proteins (Courvalin, 1990; Johnson et al., 1990). Sahm & Olsen (1990) investigated the effect of this inducibility on detection of enterococcal vancomycin resistance in vitro. Pre-incubation of strains in the presence of vancomycin affected the results of disc susceptibility tests and tests performed by automated methods, but this effect was not seen for all strains tested. Agar incorporation and broth micro-dilution techniques were unaffected by pre-incubation of strains in the presence of vancomycin (Sahm & Olsen, 1990). These workers concluded that while all methods detected strains with the highest level of vancomycin resistance tested (MIC 2048 mg/L), agar incorporation or broth micro-dilution methods were more effective than disc tests or automated methods for detecting strains with lower levels of vancomycin resistance (Sahm & Olsen, 1990).

Resistance to glycopeptide antibiotics in Gram-positive bacteria appears to remain relatively uncommon. However, the extent to which under-reporting and failure to detect resistance are factors in this is not known. The recognition of glycopeptide resistance in clinical isolates is essential for effective patient management. The rapid detection of such isolates enables alternative therapies to be instituted. It should be remembered that the correct identification of high-level resistant organisms may affect significantly the therapy chosen. In particular some organisms of intrinsically resistant genera such as Pediococcus spp., many of which remain susceptible to commonly used antibiotics, are often misidentified as enterococci which are more commonly multi-resistant (Facklam, Hollis & Collins, 1989). It may also be prudent to nurse in isolation patients from whom glycopeptide-resistant organisms are isolated in order to decrease the risks of cross-infection.

It is of interest that in recent reports of vancomycin resistant coagulase-negative staphylococci (Schwalbe et al., 1987; Aubert et al., 1990; Veach et al., 1990; Sanyal et al., 1991) and teicoplanin-resistant S. aureus (Kaatz et al., 1990), the resistant strains were isolated from patients who had received multiple or prolonged courses of glycopepti

des. This suggests that cultures from such patients and those from units where glycopeptide usage may be high (e.g. renal units with large numbers of CAPD patients) should be screened carefully for resistance.

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References


Management of biliary tract infections: potential role of the quinolones

During the past decade, the search for new antimicrobials with greater potency and expanded spectrum of activity has produced the fluorinated quinolones, which have undergone a remarkable expansion and currently stand as a major tool in antimicrobial chemotherapy. Consistently, fluoroquinolones exhibit both antibacterial and pharmacokinetic properties that should enable them to have a significant role in the management of biliary tract infections (BTI).

The factors governing the efficacy of antibiotics in BTI are primarily the spectrum of activity against the common pathogens encountered in this type of infection, the bactericidal activity in bile, and their pharmacokinetic properties, particularly the distribution in tissues and extent of biliary excretion.

Escherichia coli, Klebsiella spp., Proteus spp., and Enterococcus faecalis comprise more than 70% of the aerobic biliary pathogens. E. coli alone or as part of mixed bacterial infection is the primary pathogen involved, being isolated in about 50% of infections (Maddocks, Hilsen & Taylor, 1973; Pitt, Postier & Cameron, 1982). Pseudomonas aeruginosa is often encountered during endoscopic procedures on the biliary tract, usually as a result of exogenous contamination. In 17-50% of cases there is polymicrobial aerobic infection. In addition, it must be stressed that anaerobes, especially Bacteroides spp. and Clostridia spp., may be isolated in up to 40% of samples of infected bile (Nielson & Justesen, 1976; England & Rosenblatt, 1977), usually as part of mixed aerobic-anaerobic infections. The wide variety of organisms encountered in BTI makes the choice of an appropriate antibiotic difficult. Even the newer drugs do not display satisfactory activity against all the potential pathogens. Against the Enterobacteriaceae, quinolones exhibit high and similar potencies for most species, including Proteus and Enterobacter spp., as well as Bacteroides spp. and Clostridia spp., and are generally resistant to quinolones. When one compares the in-vitro antimicrobial activity of the most active quinolone, ciprofloxacin, with that of some β-lactams advocated in the treatment of BTI on account of their high biliary excretion, it is considerably more potent than the ureidopeni-