Antibiotic resistance in enterococci

Enterococci—generally identified as bile-tolerant, Lancefield group D streptococci that grow in the presence of 6-5% NaCl—have always been regarded as maverick streptococci. Indeed, their atypical behaviour has lately been recognized by assigning them to a separate genus, Enterococcus spp. (Schleifer & Kilpper-Bälz, 1984). Medically-important representatives include E. faecalis, E. faecium and two relatively rare pathogens, E. durans (often considered a variant of E. faecium) and E. avium.

Traditionally, enterococci have been looked upon as rather feeble pathogens, setting aside their propensity to cause endocarditis and their involvement in urinary tract infection. Elsewhere, they have been considered merely as fellow-travellers in mixed infections rather than as true aetiological agents of disease. This optimistic view of enterocoocal infection is being increasingly challenged, partly on the grounds that modern clinical practice is providing unprecedented freedom for the proliferation of otherwise innocuous organisms, but also because the bacteria themselves may be more virulent than has been hitherto supposed (Hoffmann & Moellering, 1987).

In a recent survey of 814 episodes of bacteremia encountered in Nottingham hospitals over a four-year period, enterococci were identified as the sole causative organism in only 18 cases (2.1%); nearly half the patients with enterococcal bacteremia died, but in only three cases was death directly associated with bacteremia (Ispahani, Pearson & Greenwood, 1987). The susceptibility of E. faecium is generally lower than that of E. faecalis (Moellering et al., 1979) and is less predictable; fortunately, E. faecium is also much less commonly encountered in infection.

Penicillins

Enterococci are usually much less susceptible to penicillins than are other streptococci and they also succumb much more slowly to the bactericidal action of these agents. The organisms would therefore be classed as natively 'tolerant' to penicillins (Tuomanen, Durack & Tomasz, 1986). The susceptibility of E. faecium is generally lower than that of E. faecalis (Moellering et al., 1979) and is less predictable; fortunately, E. faecium is also much less commonly encountered in infection.

Ampicillin is the mainstay of treatment, but benzylpenicillin is also active. The mistaken belief that ampicillin is much more active than benzylpenicillin perhaps arises from the habitual practice of comparing zones observed with a 10-μg ampicillin disc with those seen and 1984 (McGowan, 1989). Any increase in the prevalence of enterococcal infection probably reflects the growth in popularity of cephalosporins at the expense of penicillins in the treatment of seriously ill patients (Morrison & Wenzel, 1986).

In no way is the idiosyncratic behaviour of enterococci better exemplified than in their response to antibiotics. The organisms are ordinarily resistant to aminoglycosides, sulphonamides, cephalosporins (save cefathiamidine; Chen & Williams, 1983), monobactams, many penicillins (e.g. isoxazolylpenicillins, ticarcillin and carbenicillin) and the older quinolones. They are also frequently found to be resistant to chloramphenicol, tetracyclines and macrolides (Kaye, 1982). Thus, among the commonly used antimicrobial agents, the choice lies between those penicillins that retain activity (notably benzylpenicillin and the amino-substituted penicillins, such as ampicillin and the acylureido series), vancomycin (or teicoplanin), trimethoprim and the newer fluoroquinolones. There is also the possibility of exploiting the potentiation of aminoglycoside activity in enterococci by use of cell wall active agents such as penicillins or vancomycin.
with 2 units (1.2 \( \mu \g) \) of penicillin; in minimum inhibitory concentration titrations the difference in activity is seldom greater than twofold. The activity of acylureidopenicillins is similar to that of benzylpenicillin (Rolinson, 1986).

Enterococci do not possess a permeability barrier to penicillins and differences in susceptibility to these drugs among isolates have generally been attributed to alterations in penicillin-binding proteins (Williamson et al., 1983). \( \beta \)-Lactamase production in streptococci remained unknown for 40 years, and it came as a considerable surprise when a strain of \textit{Streptococcus faecalis} was described that was able to inactivate penicillin completely (Murray & Mederski-Samoraj, 1983). The \( \beta \)-lactamase involved was plasmid-associated and was inhibited by clavulanic acid; hybridization studies with cloned penicillinase genes from \textit{Staphylococcus aureus} point to the fact that the genetic information for production of the enzyme had been acquired from staphylococci (Murray et al., 1986). Several similar isolates have been found in different geographical locations in the United States (Moellering, 1988) but \( \beta \)-lactamase production in enterococci appears to be presently rare (Patterson, Masecar & Zervos, 1988a).

\textbf{Aminoglycosides}

High-level resistance to aminoglycosides, which carries the penalty of the abolition of synergy with penicillins, has provided another unpleasant surprise. Although ribosomal resistance to streptomycin, conferring virtually total insusceptibility, was well-known and high-level resistance to kanamycin and amikacin had been documented, high level resistance to gentamicin and tobramycin was high-level resistance to kanamycin and amikacin had been documented, high level resistance to gentamicin and tobramycin was unknown in 1977 (Calderwood et al., 1977). High-level resistance to aminoglycosides is caused by plasmid-encoded aminoglycoside modifying enzymes and is associated with a number of different types of plasmid (Patterson et al., 1988a). Geographical distribution appears to be patchy, although one alarming report from the USA describes 55\% of enterococcal isolates that exhibited the resistance trait (Zervos et al., 1987a). High-level resistance to gentamicin can co-exist with \( \beta \)-lactamase production (Patterson et al., 1988b) but the strains are not necessarily resistant to streptomycin which may thus show synergy with penicillins, providing, of course, that the organisms do not produce \( \beta \)-lactamase (Moellering, 1988). High level resistance to aminoglycosides can be detected by use of a single antibiotic break-point (500 mg/l appears to be suitable for gentamicin; Zervos et al., 1987b) or with a high content (120 \( \mu \g) \) disc (Sahm & Torres, 1988).

\textit{Trimethoprim}

The susceptibility status of enterococci in respect of trimethoprim has been the subject of some dispute, largely because these bacteria can use exogenous supplies of both thymidine and thymine (to which thymidine is converted by thymidine phosphorylase present in lysed horse-blood) and susceptibility tests may be unreliable unless the culture medium is rigorously controlled (Hamilton-Miller & Purves, 1986). Goodhart (1984) has claimed that enterococci reported as susceptible to trimethoprim may not respond \textit{in vivo} because the organisms can also use exogenous folates. However, Hamilton-Miller & Purves (1986) have pointed out that such a general statement betrays a taxonomic confusion, since the more common enterococcal species, \textit{E. faecalis}, unlike \textit{E. faecium}, cannot, in fact, assimilate folic or folinic acids. Moreover, concentrations of folates in urine are unlikely to be sufficient to account for treatment failure (Hamilton-Miller, 1988).

Similar confusion exists over the interaction of trimethoprim with sulphonamides against enterococci. Since these organisms are inherently resistant to sulphonamides one would expect that synergy with trimethoprim would not occur, but several studies, notably that of Crider & Colby (1985) have suggested that ratio-dependent synergy may be demonstrated, at least with \textit{E. faecalis}.

Since many pitfalls exist in the determination of susceptibility of enterococci to trimethoprim as well as possible interactions with sulphonamides, the actual prevalence of resistance and synergy is hard to assess from the literature.

\textbf{Quinolones}

Streptococci, including enterococci, are solidly resistant to nalidixic acid and the older quinolones, but the more recent piperazine-substituted fluoroquinolones exhibit a modest degree of activity. Among presently available derivatives, ciprofloxacin displays the best activity \textit{in vitro} (King & Phillips, 1986) although a
number of more potent compounds, such as CI-934 (King & Phillips, 1986) PD 127,391 (King, Boothman & Phillips, 1988) and T-3262 (Takahata, Otsuki & Nishino, 1988) are under development. In-vitro studies suggest that the degree of susceptibility of enterococci to presently-available fluoroquinolones is likely to be sufficient to allow successful treatment of urinary tract infection (Muranaka & Greenwood, 1988) but not systemic disease. Even in the case of the more active ciprofloxacin MICs are uncomfortably close to concentrations achievable in plasma, and since small increments in resistance readily occur (Muranaka & Greenwood, 1988; Piddock & Wise, 1988) it would be unwise to rely on this drug for the treatment of serious enterococcal infection.

Glycopeptides
I have left consideration of glycopeptides to the last, since the story of resistance to these agents is most puzzling and, in some ways, the most worrying. Vancomycin was first discovered in 1956 and was marketed in 1958 (Griffith, 1984). Despite 30 years of use, resistance to vancomycin among organisms within its Gram-positive spectrum was virtually unknown.

The development of the related glycopeptide, teicoplanin, in the 1980s prompted numerous comparisons with vancomycin in many laboratories, but a review of this literature carried out in 1987 revealed no strain of E. faecalis for which the MIC exceeded 8 mg vancomycin/l or 4 mg teicoplanin/l (Greenwood, 1988). Indeed, it was optimistically predicted that the unprotected superficial target of glycopeptides might be incapable of mutational variation, since the highly-conserved acyl-D-alanyl-D-alanine conformation was thought to be essential for normal wall growth (Reynolds, 1985). Isolated reports of vancomycin-resistant enterococci in the literature (Toala et al., 1969; Harwick, Kalmanson & Guze, 1973) were tacitly thought to arise from probable misidentification of strains of Leuconostoc or Pediococcus, genera known to be natively resistant to vancomycin (Colman & Efstratiou, 1987; Ruoff et al., 1988).

A rash of well-documented reports of vancomycin-resistant isolates of E. faecalis and E. faecium from England (Uttley et al., 1988) and France (Leclercq et al., 1988, 1989; Shlaes et al., 1989a,b) thus came as something of a bombshell. These strains exhibited resistance to high concentrations (> 64 mg/l) of vancomycin and (usually, but not uniformly) teicoplanin. Most of the strains were resistant to glycopeptides at concentrations in excess of 1000 mg/l. The report from Dulwich Public Health Laboratory and the Antibiotics Reference Laboratory at Colindale (Uttley et al., 1988) documented an outbreak in a renal unit in which 55 isolates of vancomycin-resistant enterococci were obtained from 22 patients during a 12-month period. A policy of using vancomycin and cefazidime for the management of acute undiagnosed sepsis had been instituted three months before the start of the outbreak. The French isolates were similarly obtained from patients in a haematology unit in which vancomycin was included in a regimen for the empirical treatment of febrile neutropenic patients (Leclercq et al., 1988).

The patients in whom vancomycin-resistant enterococci were found had not necessarily received vancomycin themselves.

Resistance to glycopeptides in these strains is transferable to other enterococci, but fortunately not to staphylococci (Leclercq et al., 1989). The wisdom of the ingenious genetic manipulations aimed at smuggling the resistance genes into Staphylococcus aureus, as reported at the recent Fourth European Congress of Clinical Microbiology in Nice, must be questioned. The resistant strains do not inactivate vancomycin and no target site modifications have so far been described. The mechanism of resistance thus remains obscure, although Shlaes et al. (1989a) have described an inducible membrane protein which seems to be associated with the resistance phenotype.

Luckily, resistance to ampicillin and vancomycin, as well as high-level resistance to aminoglycosides, appears presently to be uncommon in the UK, although prevalence may be masked to some extent by the assumption of sensitivity. Thus, few laboratories test enterococcal isolates routinely for high-level gentamicin resistance, except, perhaps, indirectly in synergy tests for the small number of patients who present with enterococcal endocarditis.

The occurrence of an outbreak of vancomycin-resistant enterococci is, however, a timely reminder of what microbes are capable of when they are made to fight for survival under intensive antibiotic pressure. During the quarter of a century that vancomycin was used as a second or third line agent, resistance was virtually unknown. The rise to prominence of multiresistant staphylococci has led to the elevation of vancomycin to the status of a front-line antibiotic in some units and this has
undoubtedly contributed to the development of resistance. That it has occurred in enterococci emphasizes the resilience and adaptability of these non-conformist micro-organisms.

The lesson has been preached many times, but must be repeated once again: in the battle for survival, the versatile microbes are fighting a guerrilla war with superior numbers and resistance will eventually prevail unless antimicrobial agents are used with circumspection and common sense.

References


