SPECIAL ARTICLE

Susceptibility of enterococci and epidemiology of enterococcal infection in the 1980s

INTRODUCTION

Enterococci *sensu stricto* form part of the normal gut flora (1) and may be found in the mouth, vagina and anterior urethra (2). They are opportunist pathogens which can cause serious infection including endocarditis. Nosocomial enterococcal infection appears to be increasing both in the UK (Public Health Laboratory Service [PHLS] Communicable Disease Surveillance Centre [CDSC], unpublished) and the USA (3) and to correspond to usage of broad spectrum β-lactam antimicrobial agents (4–7) and invasive surgical devices (8, 9). At the same time, the incidence of enterococci resistant or tolerant to previously commonly employed antimicrobial agents or their synergistic combinations is increasing and is compromising therapy of serious enterococcal infection. Strains of enterococci with high-level resistance to streptomycin and kanamycin (minimal inhibitory concentrations [MICs] > 2000 mg/L) were first reported in 1970 (10, 11) and rapidly became widespread (8, 12–14). Transferable, high-level resistance to gentamicin first reported in *Enterococcus faecalis* from France in 1979 (15) is now prevalent in the species (MIC > 1000 mg/L) and has been reported from centres in Europe, including the UK, Japan, the USA, Canada, Thailand, Chile and Australia (8, 14, 16–23). In several centres in the USA β-lactamase production has been detected in strains of *E. faecalis*, all but one of which has been reported to have transferable high-level gentamicin resistance in addition (24, 25). Resistance to β-lactams mediated by variations in the affinities and profiles of penicillin binding proteins (PBPs) among strains of *E. faecium* has been demonstrated (26–28). Most recently high-level, self-transferable resistance to vancomycin and teicoplanin in strains of *E. faecalis* and *E. faecium* in the UK, France, Germany and Spain (22, 29–36) has been documented. Most of the vancomycin-resistant strains of *E. faecalis* also have transferable high-level gentamicin resistance. The significance of *in vitro* tolerance of cell wall active antimicrobial agents by enterococci (37) is a further possible complication to the therapy of enterococcal infections. Studies of animal models of enterococcal endocarditis support the view that it may be clinically important (38). The large number of new β-lactam compounds introduced in the last few years have provided few significant advances in therapy. The newer cephalosporins have little or no activity against enterococci. Indeed these agents, having activity against a wide range of bacteria but not the enterococci, may have encouraged enterococcal colonization and super-infection (39, 40). In the light of these developments it is timely to appraise the current status of antimicrobial susceptibility amongst enterococci, to review possible approaches to the management of enterococcal infection and its changing epidemiology.

Antimicrobial susceptibility and resistance

Compared with streptococci, most strains of enterococci are relatively resistant to penicillin and ampicillin (41), *E. faecium* more so than *E. faecalis* (42, 43). Minimal inhibitory concentrations required to inhibit 90 % of strains (MIC 90s) of *E. faecalis* are typically 2–4 mg/L of penicillin and 0·5–2 mg/L ampicillin (44–46). β-Lactamase producing-strains of *E. faecalis* have MICs of penicillin and ampicillin of 6·25 and 3·1 mg/L at a standard inoculum of 10^5 colony forming units. The MICs are inoculum dependent and resistance may not be detected unless screening for β-lactamase production is performed. The MICs of penicillin and ampicillin for these strains are reduced to 1·6 mg/L by addition of the β-lactamase inhibitors clavulanic acid or sulbactam. The genes coding for β-lactamase production (and high-level gentamicin resistance) are located on at least two distinct self-transferable plasmids (25). The possible clinical significance of these strains is supported in an animal model of endocarditis but the problem of appropriate therapy for such infections has not been resolved (47, 48) though ampicillin-sulbactam or daptomycin seem worth further evaluation (49). The broad-spectrum penicillins azlocillin, mezlocillin and piperacillin (44, 50) show good activity against enterococci with MIC 90s of $\leqslant 0\cdot8$–16 mg/L but their activity against β-lactamase positive strains is reduced (51). Carbenicillin and ticarcillin are poorly active against enterococci (46).

The relative resistance of *E. faecalis* to penicillins compared with the streptococci is intrinsic and due to cell wall structure. *Enterococcus faecium* is commonly more resistant to penicillins than *E. faecalis*, typical MIC 90s ranging from 4–8 mg/L and in some series up to 38 mg/L (41). The decreased susceptibility of strains of *E. faecium* is associated with differing profiles and reduced affinities of their PBPs (26–28). Resistance of enterococci to virtually all cephalosporins is also associated with an inherent low affinity of their PBPs for these compounds (28). Minimal inhibitory concentrations usually exceed 32 mg/L. There are some *in vitro* exceptions, e.g. cefpirome (52). However, MICs are close to breakpoints and there is evidence that the composition of the test media can affect results and yield falsely low MIC results for cephalosporins (53). A few cephalosporin/amino-glycoside combinations may be synergistic when tested against enterococci *in vitro* but are ineffective *in vivo* (54).

Most strains of *E. faecalis*, including β-lactamase producers (51) and those with high-level vancomycin resistance (personal observation) are susceptible to the carbapenem imipenem with MICs of 1–4 mg/L. In strains lacking high-level resistance to aminoglycosides, synergy can be demonstrated with imipenem/gentamicin combinations (55). Strains of *E. faecium* are resistant to imipenem having MICs of 16–> 32 mg/L. (personal observation).

Most enterococci are susceptible to the glycopeptide antimicrobial agents vancomycin and teicoplanin if susceptibility is defined as an MIC $\leqslant 4$ mg/L. However, the usefulness of these drugs is compromised by the fact that their effect is bacteriostatic in most circumstances (56) and resistance to vancomycin at high-level is beginning to emerge in both *E. faecalis* and *E. faecium* (22, 29–36). Reports include one of a large cluster of severe infections due to vancomycin-resistant *E. faecalis* and *E. faecium* having vancomycin MICs in the range 64–> 2000 mg/L

(22, 36). High-level vancomycin resistances are transferable by conjugation to susceptible enterococci, and a number of other Gram-positive species (57), some vancomycin resistance genes are plasmid-borne and the involvement of a conjugative transposon has been postulated (36). Among these strains the expression of vancomycin resistance is inducible by growth in low concentrations of vancomycin or teicoplanin and coincides with the appearance of a new membrane protein (34, 35, 58, 59). Many of the *E. faecalis* strains also have transferable high-level gentamicin resistance and resistance to several other antimicrobial agents including erythromycin and chloramphenicol.

Enterococci are relatively resistant to aminoglycosides at clinically achievable serum concentrations (MICs 4–64 mg/L). In some centres in the USA more than 50% of strains of *E. faecalis* have high-level gentamicin resistance (7). In one recent survey in the UK, 7–10% of all strains and 30% of those isolated from blood cultures were found to be highly resistant (14). In a survey in a South-East London hospital, 10 of 13 (77%) high-level, vancomycin-resistant *E. faecalis* strains had high-level gentamicin resistance compared with 32% of 76 vancomycin-susceptible strains (unpublished observations). Two of 25 strains of Smyth and co-workers (14) and four of 76 vancomycin-susceptible strains referred to above were normally susceptible to streptomycin, suggesting that in some instances penicillins might be combined with streptomycin for *E. faecalis* infections requiring synergistic therapy. However, strains with high-level resistance to gentamicin are usually also resistant at high level to other aminoglycosides. High-level aminoglycoside resistance is most commonly mediated by one or more amino-glycoside-modifying enzymes, the genes coding for which are usually carried on self-transferable plasmids (17, 19, 60). High-level resistance to streptomycin may also be due to ribosomal alterations (61) but this latter resistance mechanism is probably uncommon in clinical practice. Strains with high-level aminoglycoside resistance are clinically important since they are no longer susceptible to the synergistic bactericidal effect of combinations of aminoglycoside and β-lactam antimicrobial agents. *Enterococcus faecium* is resistant to the synergistic effects of penicillin or ampicillin with kanamycin, netilmicin, sisomicin or tobramycin. This is attributable to possession of a non-transferable, low-level acetyl-transferase which confers moderate resistance to these aminoglycosides but not to gentamicin (43). Strains of *E. faecium* having high-level resistance to gentamicin have not been reported in clinical practice though Chen and Williams (19) have produced resistant *E. faecium* transconjugants in mating experiments with *E. faecalis*. Penicillin/ampicillin with gentamicin is therefore the synergistic combination of choice for infection due to *E. faecium*.

Quinolone antimicrobial agents are not usually regarded as agents of choice for the therapy of enterococcal infections. Ciprofloxacin among the licensed quinolones is the most active against enterococci. However, MICs are close to the upper breakpoint for susceptibility of 4 mg/L and thus a twofold dilutional error would make the organism appear resistant *in vitro*. Despite this, the use of ciprofloxacin has contributed to bacteriological cure in some circumstances (14, 36). Enterococci are not universally susceptible and the emergence of resistance during therapy has been reported on at least one occasion (36).

Individual strains of both *E. faecalis* and *E. faecium* may be susceptible to a

14-2

number of antimicrobial agents active against Gram-positive species including chloramphenicol, erythromycin, fusidic acid, rifampicin, tetracycline and tri-methoprim. Several of these compounds exert bacteriostatic rather than bactericidal effects and relatively high concentrations, and therefore dosages, are required to inhibit growth. Transferable plasmid and/or transposon mediated resistance has also been reported amongst enterococci for chloramphenicol, erythromycin and tetracycline (62–65). Thus these agents are poor therapeutic alternatives for difficult enterococcal infections.

Among new antimicrobial agents, the lipopeptide daptomycin may have a role in future in the therapy of infection due to enterococci, including the various emerging resistant strains. Daptomycin has excellent *in vitro* activity against enterococci including β-lactamase-producing, high-level gentamicin-resistant and vancomycin-resistant strains. Minimal inhibitory concentrations are less than 4 mg/L (22, 29, 36, 66). Both vancomycin-sensitive and vancomycin-resistant enterococci have been shown to be capable of one-step mutation to MIC's of 4–16 mg/L (29, 36, 67). Bactericidal activity has been demonstrated in time-kill curves at four times the MIC. Regrowth occurred at lower concentrations (66). In animal models of endocarditis and pyelonephritis, use of daptomycin has contributed to cure (68–70). However, clinical studies are required to validate these preliminary observations. A lipopeptolide, MDL 62198 (ramoplanin), has MICs for enterococci similar to daptomycin (36). Tosufloxacin, a new quinolone, is particularly potent against Gram-positive species and MIC 90s for *Enterococcus* spp. are half (1 mg/L) those of ciprofloxacin (71). However, human pharma-cokinetic properties of tosufloxacin have not been published so its role is speculative.

Antimicrobial therapy of enterococcal infections

The isolation of enterococci does not always require antimicrobial chemo-therapy. Simple drainage or removal of invasive devices may be sufficient for cure. In some clinical contexts, e.g. polymicrobial abdominal and pelvic infections, the pathogenic role of enterococci is obscure. In certain well defined clinical situations, such as urinary tract infection, cholecystitis, endocarditis and a variety of infections in the immunocompromised patient, these organisms undoubtedly behave as pathogens and require treatment. In uncomplicated urinary tract infection (UTI) in younger women and more complicated UTI in older persons, e.g. following urinary catheterization but excluding those with renal parenchymal involvement, ampicillin or amoxycillin alone may suffice. In the patient allergic to penicillins, ciprofloxacin, nitrofurantoin or trimethoprim may be substituted, as indicated by *in vitro* susceptibility testing. Serious infection including endocarditis, pyelonephritis or sepsis in an immunocompromised patient requires therapy with penicillin/ampicillin plus an aminoglycoside or vancomycin/teicoplanin plus an aminoglycoside. The aminoglycoside usually recommended is gentamicin which is mandatory if the organism is *E. faecium*. The recommendation to use potentially nephrotoxic/ototoxic drugs must be tempered with regular monitoring of serum levels. Synergy *in vitro* is consistently achieved with penicillin, ampicillin or vancomycin plus gentamicin for normally susceptible enterococci (54). Synergy is due to increased uptake of gentamicin following inhibition of cell wall synthesis by

the penicillin (72). The synergy between vancomycin and gentamicin is due to a similar effect (54). Where there is penicillin allergy, the synergistic combination of vancomycin and gentamicin may be substituted. High-level resistance to gentamicin and/or vancomycin presents major therapeutic problems. A combination of ampicillin and ciprofloxacin has been reported to be synergistic (73) or indifferent *in vitro* but to have contributed to successful therapy for two patients with endocarditis caused by *E. faecalis* strains having high-level gentamicin resistance (14). It is worth remembering that a few strains of *E. faecalis* with high-level gentamicin resistance remain susceptible to streptomycin which may be substituted for gentamicin. Other combinations are possibly inappropriate in *in vitro* tests: ampicillin and rifampicin are reported to be antagonistic (74), rifampicin and vancomycin are indifferent (75) and combining rifampicin with fusidic acid resulted in the emergence of resistance to both agents (76). Rifampicin and trimethoprim in combination may be worth further investigation (77), as may rifampicin and ciprofloxacin. The latter two antimicrobial agents have been reported to be effective combination therapy for the treatment of methicillin-resistant *Staphylococcus aureus* infections and colonizations (78). If serious infections due to β-lactamase producing *E faecalis* occur, therapy might include combinations of β-lactams with β-lactamase inhibitors. In infections with strains of *E. faecium* resistant to vancomycin, penicillins and gentamicin achieve synergy even in the presence of relatively high MICs for penicillins (79). Other aminoglycoside/penicillin combinations are ineffective.

Where indicated on clinical grounds, endocarditis prophylaxis for instrumentation of the gastro-intestinal or genito-urinary tract should include antimicrobial agents reliably active against the enterococci. Amoxycillin plus a single dose of gentamicin have been recommended (80). This recommendation may require modification if individual patients are known to be infected/colonized with resistant strains or if such strains are prevalent in the hospital population.

Epidemiology

The acquisition of enterococcal infection has been assumed to be almost exclusively endogenous (2). However, the apparent increase in the incidence of nosocomial enterococcal infection and the emergence of novel antimicrobial resistances among the enterococci have prompted a reappraisal of the epidemiology and significance of these organisms in nosocomial infections. Evidence for a true increase of enterococcal infection as opposed to an increased awareness comes from a number of sources (3, 4, 6, 9), including bacteraemia and meningitis reports to the PHLS Communicable Disease Surveillance Centre (CDSC unpublished). This increase may be due to the relative resistance of enterococci to a wide range of antimicrobial agents including extended-spectrum cephalosporins and aminoglycosides. These impart a selective advantage to the organisms, particularly in high-dependency units where such agents are used widely. The increasing number of in-patients compromised either by immune-suppression or invasive devices and who may be exposed to multiple antimicrobial therapy, form a discrete population at risk of colonization and superinfection with enterococci. Clustering of such patients may increase the possibilities for nosocomial enterococcal transmission. The faecal reservoir probably plays an important role.

Bowel and perineal colonization may lead to infection of catheters or other compromised sites with subsequent opportunities for wider dissemination both in the individual and between patients. Apparent outbreaks have been reported (7, 22, 36, 81–83). Sampling of the environment and nurse's hands during one cluster of vancomycin-resistant enterococcal infections failed to reveal any obvious routes of transmission, though duration of hospitalization did seem to be associated with faecal carriage (36). In contrast, in an epidemiological study of nosocomial infection by gentamicin-resistant *E. faecalis*, these enterococci were isolated from both staff hands and the environment (37). The lack of suitable typing schemes for enterococci is at present limiting the study of their transmission and renders epidemiological analysis of outbreaks and clusters extremely difficult. Little is known of the hospital epidemiology of infections due to enterococci of standard susceptibility. Most interest has focused on strains showing one or more relatively unusual antimicrobial resistances because they provide markers for the identification of possible clusters. The plasmid content of strains of high-level gentamicin-resistant *E. faecalis* has been used to draw conclusions as to their relatedness. Zervos and co-workers (8) reported strains of *E. faecalis* recovered from ten patients during a 2-month period in one ward area which were identical with respect to plasmid profile. The plasmids had identical transfer properties, donor and transconjugant resistances and *Eco* R1 and *Hin*d III digest patterns. Similarly, variations in the same properties in a larger series of high-level gentamicin-resistant *E. faecalis* from ten different hospitals in nine diverse geographical areas of the USA were used to argue against clonal dissemination as the cause of the increased frequency of isolation of such strains (21). The genetic determinants for gentamicin resistance appeared on a variety of different conjugative and non-conjugative plasmids.

Though detailed studies of plasmid properties may be useful markers for distinguishing strains, differences in plasmid content *per se* do not necessarily provide information on the relatedness of the genetic determinants of anti-microbial resistance in enterococci. Transposons carrying genes conferring resistance to one or more antimicrobial agents such as Tn917 (erythromycin resistance) (84) and Tn916 (tetracycline and minocycline resistance) (85) are widely distributed amongst enterococci on different plasmids and also in the bacterial chromosome (86). LeBlanc and colleagues (87) used DNA from plasmid pJH1 (63) which confers resistance to erythromycin, kanamycin, streptomycin and tetracycline to prepare probes for the examination of 91 Group D streptococcal strains which had the erythromycin, kanamycin, streptomycin resistance phenotype. These 91 strains came from both human and animal sources over a wide geographical area and had a variety of plasmid profiles. Despite the apparent heterogeneity of the strains, nearly 70 % contained DNA that hybridized to each of the three resistance determinants from pJH1. Thus, although the plasmid content of enterococci may be heterogeneous the genetic determinants of many of the antimicrobial resistances observed are remarkably similar if not identical. To draw epidemiological conclusions on the relatedness of nosocomial enterococcal strains based primarily on the demonstration of particular resistance genes (83) without considering the genetic location of such genes may be misleading.

CONCLUSIONS

Enterococci appear to have become significant nosocomial pathogens. The microbiologist should be alert to their possible role in serious infection in immunocompromised patients especially those who have received organ grafts, those nursed in high-dependency units including neonatal intensive care units, and patients prescribed long courses of broad-spectrum β-lactams. The use of gut sterilization and decontamination regimens in leukaemic patients and those nursed on intensive care units respectively may create further sub-sets of patients vulnerable to superinfection with enterococci. Enterococci recovered from clinical material and deemed to be "significant" should be speciated both for epidemiological purposes and because species is relevant to antimicrobial therapy, particularly combined therapy. Strains of *E. faecalis* should be tested for susceptibility to a 200 μg gentamicin disk and have an MIC determination if resistance is detected. Where high-level gentamicin resistance is present, streptomycin susceptibility should be sought in case this aminoglycoside can be substituted for gentamicin to achieve a synergistic combination with a penicillin. β-Lactamase screening of *E. faecalis* should also now be considered as a routine, otherwise possible resistance to penicillins may emerge undetected. Enterococci susceptible to ampicillin should no longer be assumed to be susceptible to vancomycin. There is evidence that cross-infection may occur with high-level vancomycin and/or gentamicin resistant enterococci. Colonized/infected patients should be nursed in isolation. Once resistant enterococci colonize or infect, long-term faecal carriage may be a consequence. Affected patients notes should be flagged to alert staff on subsequent admissions. There is now a need to evaluate new antimicrobial agents, e.g. daptomycin, tosufloxacin and alternative combinations for the management of multiply resistant enterococci especially *E. faecalis* and to develop typing schemes to improve epidemiological analysis.

R. C. GEORGE
Antibiotic Reference Laboratory,
Division of Hospital Infection,
Central Public Health Laboratory,
61 Colindale Avenue,
London NW9 5HT

A. H. C. UTTLEY
Public Health Laboratory,
Dulwich Hospital,
East Dulwich Grove,
London SE22 8QS

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