Resistant enterococci – mechanisms, laboratory detection and control in hospitals

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Introduction
The incidence of nosocomial colonisation and infections due to Enterococcus spp., with those due to Enterococcus faecium the most prevalent, has risen steadily during the 1980s. At the same time, these bacteria have acquired resistance to aminoglycosides, β-lactams and glycopeptides.\textsuperscript{1-3} Enterococci are opportunists and medical advances ensure an increasing population of patients vulnerable to nosocomial endogenous and exogenous infections with enterococcal bacteraemia, particularly caused by \textit{E faecium}, a marker for serious underlying disease.\textsuperscript{4,5} Clusters of colonisation and infections due to enterococci have occurred among patient subsets susceptible as a result of immunosuppressive disease or treatment and epidemiological studies combined with molecular typing have correlated patient colonisation with dissemination of susceptible and resistant strains.\textsuperscript{6} The success of enterococci as nosocomial pathogens is ensured by their habitat and resistance to desiccation, heat, some disinfectants, and most antimicrobial agents.\textsuperscript{7} Their normal habitat is the large intestine where they may encounter antimicrobial agents excreted by this route, exchange genetic material with other bacterial genera and whence they contaminate the environment.

Intrinsic and acquired antimicrobial resistances
Enterococci possess relative intrinsic resistance to β-lactams and aminoglycosides but are susceptible to glycopeptides although their effect when used alone is usually bacteriostatic. Treatment of serious bacterial infections, such as endocarditis, requires a synergistic bacterial combination of a cell wall active agent, a penicillin or glycopeptide, and an aminoglycoside.\textsuperscript{2,8,9} Acquisition by enterococci of high level gentamicin resistance (minimum inhibitory concentration (MIC) ≥ 500 μg/ml), glycopeptide resistance and β-lactamase activity is ominous, compromising one or both arms of a synergistic regimen and single agent treatment of less serious infection. When such resistance is encountered, unproven agents or regimens may have to be used.\textsuperscript{6} The dissemination of genes encoding glycopeptide resistance may also herald acquisition of vancomycin resistance by other Gram positive bacteria, such as \textit{Staphylococcus epidermidis}, penicillin multiply resistant \textit{Streptococcus pneumoniae} and, as has already been demonstrated in vitro, \textit{Staphylococcus aureus}.\textsuperscript{10}

HIGH LEVEL AMINOGLYCOSIDE RESISTANCE
Aminoglycoside modifying enzymes (AMEs) are the most important mechanisms mediating high level resistance (HLR). Each AME may confer HLR to more than one aminoglycoside and a single enterococcal strain may acquire several AMEs.\textsuperscript{8,9} Although most AMEs are plasmid mediated and transferable, \textit{E faecium} differs from other enterococci in its production of a chromosomally mediated AME. This species specific enzyme, although not conferring HLR, abolishes synergy between cell wall active agents and all aminoglycosides except streptomycin, amikacin and gentamicin.\textsuperscript{10,11} Enterococci may acquire a plasmid or transposon mediated AME exhibiting both 2'-phosphotransferase and 6'-acytyltransferase activities, which confers HLR to gentamicin and all other commercially available aminoglycosides except streptomycin.\textsuperscript{12,13} Against strains of \textit{E faecium} which acquire this enzyme, because of its chromosomal AME (vide supra), only streptomycin achieves synergy. However, both \textit{Enterococcus faecalis} and \textit{E faecium} may acquire another AME, a 6'-adenyltransferase, which confers HLR to streptomycin. This enzyme is usually co- transferred with a 3'-phosphotransferase which confers HLR to kanamycin; this enzyme also abolishes synergy with amikacin without conferring HLR.\textsuperscript{8,11}

β-LACTAMASE PRODUCTION
β-lactamase production in \textit{E faecalis} was first described in 1983. The enzyme is a typical penicillinase—transferable, constitutively produced, completely cell bound, and inhibited by clavulanate.\textsuperscript{14} Nucleotide sequencing of the enterococcal β-lactamase gene, \textit{blaZ}, confirms that the enzyme is indistinguishable from some staphylococcal type A β-lactamases. The gene has been shown to have a chromosomal location in some strains of \textit{E faecalis} and may be incorporated in a transposon-like element.\textsuperscript{15} β-lactamase production has since been demonstrated in \textit{E faecium}.\textsuperscript{16} Although most β-lactamase producing enterococci have exhibited HLR to gentamicin, these traits are not inseparable.\textsuperscript{18}
HIGH LEVEL PENICILLIN RESISTANCE

Strains of enterococci with unusually high penicillin and ampicillin MICs, in the absence of \(\beta\)-lactamase production, are increasingly being reported.\(^{19,20}\) High-level penicillin resistance is thought to result from overproduction of the slow-reacting penicillin binding protein (PBP) 5, a normal PBP of enterococci which can substitute for other PBPs.\(^2\) High-level penicillin resistance may result in loss of synergy with gentamicin, although for some strains this may be overcome with high concentrations of penicillin.\(^21\)

GLYCOPEPTIDE RESISTANCE

Following its introduction in the 1950s, vancomycin was used sparingly due to impurity of early preparations and common side effects. The agent was usually reserved for patients allergic to \(\beta\)-lactams. Since 1970, it has been prescribed extensively for \(S\) aureus infections in patients with end-stage renal failure or haemodialysis. It is not removed during dialysis and can be conveniently administered weekly. Much of its increased consumption in the past decade is accounted for in the treatment of intravenous line infections due to \(S\) epidermidis. Vancomycin resistance to vancomycin remained insignificant clinically for almost 30 years until 1988 when two reports of \(E\) faecalis and \(E\) faecium were published.\(^22,23\) Subsequently, enterococci with inducible low-level vancomycin resistance, but remaining teicoplanin susceptible, and strains with constitutive low-level vancomycin resistance have been encountered. Although referred to as VanA, VanB and VanC phenotypes, respectively, strains with resistance patterns not accommodated by this scheme are encountered increasingly.

The \(\text{van}^+\) genotype confers HLR to vancomycin (MICs of 64 to \(>1000\) \(\mu\)g/ml) and teicoplanin (MICs of 16-512 \(\mu\)g/ml). It is encountered most frequently in \(E\) faecium but also occurs in \(E\) faecalis, \(E\) avium, \(E\) durans, \(E\) mundii, \(E\) gallinarum, and \(E\) casseliflavus. The \(\text{van}^+\) gene has been detected in a strain of \(E\) faecium raffinosus but with vancomycin and teicoplanin MICs of only 16 and 1 \(\mu\)g/ml, respectively. VanA strains usually exhibit inducible, self-transferable glycopeptide resistance, associated with synthesis of a 39 kDa cytoplasmic membrane protein. Constitutive mutants with chromosomal \(\text{van}^+\) genes have also been described.\(^{24}\) In the prototype strain, \(E\) faecium BM4147, resistance is carried on a transposon, designated Tn1546, which encodes seven polypeptides: VanA, VanH, VanX, VanY, VanZ, VanR, and VanS, the first three of which are required for glycopeptide resistance.\(^25\) The VanA and VanH proteins act in concert to replace the D-Ala-D-Ala terminus of the peptidoglycan precursor pentapeptide with the depsipeptide D-Ala-D-Lac to which glycopeptides cannot complex. VanX and VanY act to reduce the production of the peptidoglycan precursor bearing the normal D-Ala-D-Ala terminus. The VanS and VanR proteins comprise a two-component regulatory system for transcription of the genes required for glycopeptide resistance. The function of the \(\text{vanZ}\) gene product has yet to be determined.\(^25\)

The VanB phenotype also confers inducible resistance to vancomycin. The vancomycin MICs are usually lower than those of the VanA phenotype, but may range from 4-1024 \(\mu\)g/ml, and strains usually, though not always, remain susceptible to teicoplanin.

Three species of enterococci possess low-level constitutive vancomycin resistance (VanC phenotype): \(E\) gallinarum, \(E\) casseliflavus, and \(E\) flavescentis. Recently, vancomycin-dependent \(E\) faecalis and \(E\) faecium have been described.\(^{26,27}\) The mechanism has not been elucidated.

**Laboratory aspects**

Important differences exist among the species of enterococci with regard to penicillin, aminoglycoside and glycopeptide resistance patterns (vide supra). For this reason and for epidemiological information enterococci causing serious infections should be fully identified; a minimum requirement is differentiation of the species isolated most frequently from clinical material, \(E\) faecalis and \(E\) faecium. Commercially available kits are useful, supplemented by tests from other schemes,\(^{28}\) or the advice of a reference laboratory may be sought.

HIGH LEVEL AMINOGLYCOSIDE RESISTANCE

Enterococci implicated in serious infections must be screened for high level aminoglycoside resistance which predicts lack of synergy with cell wall active agents.\(^2\)\(^{14}\) Only gentamicin and streptomycin need be tested routinely. Because the 3'-phosphotransferase AME abolishes synergy between amikacin and cell wall active agents without conferring HLR to that aminoglycoside, HLR to kanamycin must be sought if the intention is to use amikacin (vide supra).\(^2\)\(^{14}\) Screening avoids MIC determinations, the inclusion of aminoglycosides in in vitro synergy tests and unnecessary administration of aminoglycosides. Simple and reliable methods are available for screening.\(^29\) Disc diffusion testing with 120 \(\mu\)g gentamicin and kanamycin discs and 300 \(\mu\)g streptomycin discs is cheap and reliable. Provided the standard procedure is followed, a zone diameter of 6 mm indicates HLR and most other strains have zones of \(>10\) mm; isolates giving zones of 7-9 mm should be checked by a different method. Agar dilution using brain heart infusion broth containing 500 \(\mu\)g/ml gentamicin or 2000 \(\mu\)g/ml streptomycin is an alternative. For multipoint testing an inoculum of 10° colony forming units (cfu) per spot is optimal. Broth microdilution using gentamicin at 500 \(\mu\)g/ml or streptomycin at 1000 \(\mu\)g/ml and an inoculum of 10° cfu/ml is sensitive for detecting HLR to these agents. Although the E test (AB Biodisk, Solina, Sweden) detects HLR to gentamicin, detection of HLR to streptomycin is more problematic unless a low range strip (1024 \(\mu\)g) is used or incubation is prolonged to 48 hours.\(^30,31\)
GLYCOPEPTIDE RESISTANCE
High level glycopeptide resistance is readily detected using 30 μg discs. In contrast, low level vancomycin resistance is easily missed by laboratories using disc diffusion susceptibility testing unless the disc contains 5 μg vancomycin, ideally with *E. faecalis* ATCC 51299 (a VanB strain), as control.32 Screening for resistance with brain heart infusion agar containing 6 μg/ml vancomycin is sensitive. Multipoint inoculation with 107 to 109 cfu per spot is recommended.39 Inoculation with a swab, however, has little effect on the results. The E test identifies vancomycin resistance in enterococci.31 For enterococci causing serious infections, the MICs of vancomycin and teicoplanin must be determined.

HIGH LEVEL PENICILLIN RESISTANCE
Disc diffusion using a 10 μg disc identifies ampicillin resistance whether using the Kirby Bauer method or disc diffusion with *E. faecalis* ATCC 29212 as control.15 However, these methods do not differentiate enterococci with high level penicillin resistance (MICs ≥ 128 μg/ml) from those with lower level resistance (MICs 16–32 μg/ml). As the latter may be killed by a combination of penicillin and aminoglycoside, the MICs of penicillin or ampicillin should be determined for enterococci causing endocarditis or other serious infections. The E test is a reliable method for measuring MICs.

DETECTION OF β-LACTAMASE
β-lactamase producing enterococci exhibit a notable inoculum effect: penicillin resistance may not be demonstrated unless a high inoculum—for example, 109 cfu/ml—is used. Commercial tests for β-lactamase detection are recommended; however, a strain of β-lactamase producing *E. faecalis* that does not hydrolyse nitrocefin has been reported.34

Control of resistant enterococci in hospitals
Outbreaks of colonisation/infection by enterococci with up to 53% of infections typically occurring on high dependency units, such as intensive care and oncology wards, although general wards may also be involved. Common source hospital outbreaks are infrequent. Sources implicated have included contaminated porcine xenografts, rectal thermometers and a cardiac bypass pump. Glycopeptide resistance is now widespread and the resistance trait causing most concern. A report addressing the problem has been published recently.39

DETECTION OF PATIENTS COLONISED BY VANCOMYCIN RESISTANT ENTEROCOCCI
Laboratories may elect to survey all or a proportion of clinical isolates of enterococci for vancomycin resistance depending on local experience.35 Patients infected with vancomycin resistant enterococci represent the tip of the iceberg and screening other patients with rectal, perineal and mouth swabs will usually reveal carriers and the possibility of clustering or outbreaks occurring.36

MANAGEMENT OF COLONISED PATIENTS
Communication between laboratory and clinician is essential to ensure the best deployment of available isolation facilities. Realistic policies can only be made locally. Patients who are “high dependency”, those with diarrhoea or with positive urine cultures and a urinary catheter in situ should have priority for isolation. On general wards, ambulant self-caring patients with rectal colonisation probably represent a much lower risk for spread. Patients’ records should be flagged in case of readmission and on transfer, the receiving hospital must be informed. In high dependency units, where vancomycin or multiply resistant enterococci are endemic, it may be prudent to admit patients most at risk of exogenous enterococcal infection directly into protective isolation—transplant and oncology patients, patients with multiple trauma and others with high APACHE II scores.

HEALTH CARE WORKERS
Although isolation of epidemic strains from the hands of staff is insufficient evidence for cross-colonisation by this route, implication of staff members by risk factor analyses and patterns of environmental contamination compatible with hand transmission are persuasive. High level, vancomycin resistant *E. faecium* survives well on fingertips and is not reliably removed by soap, though alcohol containing agents are extremely effective. Hibisol (ICI Pharmaceuticals plc) has the disinfectant properties of alcohol, with residual activity alone sufficient to eliminate large inocula of vancomycin resistant *E. faecium*.37

The employment implications for staff found to have bowel carriage of vancomycin resistant enterococci should be considered before embarking on staff screening programmes. Elimination of bowel carriage cannot be guaranteed. Health care workers with bowel carriage alone are unlikely to transmit enterococci unless personal hygiene is wanting or hands become persistently colonised. One outbreak of vancomycin resistant *E. faecalis* was terminated by the exclusion of a nurse with persistent rectal carriage and a colonised, damaged thumbnail.38

THE ENVIRONMENT
Environmental contamination by epidemic enterococci has been demonstrated in several studies and is particularly heavy in the vicinity of patients with diarrhoea.35 In some studies the sites contaminated are compatible with hand contamination. Contamination of microspheres in fluidised microsphere beds, theoretically at least, may cause both intra- and inter-hospital spread of enterococci.40

Terminal disinfection of the immediate environment of colonised patients is recommended.35 Alcohol is suitable for cleaning many surfaces including equipment control panels, bedrails, drip stands, etc. Contamination of floors is probably less important. Dedicated equipment should be used for colonised
patients and should not be shared between patients without cleaning.\textsuperscript{10}

**ANTIMICROBIAL USAGE**

Several antimicrobials have been implicated in enterococcal superinfections. However, it is difficult to establish whether an agent is a risk factor for selection of resistant enterococci or for enterococci regardless of resistance pattern. Cephaparin, aminoglycoside and antimicrobial usage generally are implicated in enterococcal outbreaks, while vancomycin, aminoglycosides and cephaparin have been linked specifically with acquisition of vancomycin resistant enterococci.

Some studies designed to identify risk factors for acquisition of a resistance trait have selected as controls patients acquiring enterococci lacking that trait. The conclusions are that broad spectrum cephalosporins and aminoglycosides seem to be risk factors for acquisition of gentamicin-resistant strains. However, it is difficult to envisage which antimicrobials will select for enterococci, especially E. faecium. Efforts must focus on curtailing antimicrobial consumption generally as well as policing the classes of antimicrobials used.