Indian Journal of Medical Microbiology, (2005) 23 (4):214-9

Review Article

ENTEROCOCCAL RESISTANCE – AN OVERVIEW

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Abstract

Nosocomial acquisition of microorganisms resistant to multiple antibiotics represents a threat to patient safety. Here, we review the antimicrobial resistance in *Enterococcus*, which makes it important nosocomial pathogen. The emergence of enterococci with acquired resistance to vancomycin has been particularly problematic as it often occurs in enterococci that are also highly resistant to ampicillin and aminoglycoside thereby associated with devastating therapeutic consequences. Multiple factors contribute to colonization and infection with vancomycin resistant enterococci ultimately leading to environmental contamination and cross infection. Decreasing the prevalence of these resistant strains by multiple control efforts therefore, is of paramount importance.

Key words*: Enterococci, antimicrobial resistance, therapeutic options, control efforts*

Enterococci, though commensals in adult faeces are important nosocomial pathogens.[1-3] Their emergence in past two decades is in many respects attributable to their resistance to many commonly used antimicrobial agents (aminoglycosides, cephalosporins, aztreonam, semisynthetic penicillin, trimethoprim-sulphamethoxazole)[4,5] and ease with which they appear to attain and transfer resistant genes,[6] thus giving rise to enterococci with high level aminoglycoside resistance (HLAR), β-lactamase production and glycopeptide resistance.

The most common nosocomial infections produced by these organisms are urinary tract infections (associated with instrumentation and antimicrobial administration), followed by intra-abdominal and pelvic infections. They also cause surgical wound infections, bacteraemia, endocarditis, neonatal sepsis and rarely meningitis.[2,6,7] *E.faecalis* is the most common cause (80-90%)of infection followed by *E.faecium* (10-15%).[7] However, emergence of enterococci with multi drug resistance particularly to vancomycin is predominantly seen in *E.faecium* [8] followed by increase in frequency of its recovery from infection. As vancomycin resistant enterococci (VRE) also have ampicillin resistance and HLAR, they are the most difficult to treat. Thus, this entity merits a complete description of antimicrobial resistance, current possibilities for treatment and variety of measures that may limit the proliferation of resistance within a health care environment.

Antimicrobial Resistance

Enterococci have a remarkable ability to survive in an environment of heavy antibiotics. Indeed, it is the resistance of these organisms to multiple antimicrobial agents that makes them such feared opponents. Antimicrobial resistance in enterococci is of two types: inherent/ intrinsic resistance and acquired resistance. Intrinsic resistance is species characteristics and thus present in all members of species and is chromosomally mediated. Enterococci exhibits intrinsic resistance to penicillinase susceptible penicillin (low level), penicillinase resistant penicillins, cephalosporins, lincosamides, nalidixic acid, low level of aminoglycoside and low level of clindamycin.[1] Although most enterococci are susceptible to co-trimoxazole *in vitro*, this combination does not work *in vivo*, because enterococci are able to incorporate preformed folic acid which enables them to bypass the inhibition of folate synthesis produced by co-trimoxazole.[2] On the other hand, acquired resistance results from either mutation in DNA or acquisition of new DNA. Examples of acquired resistance include resistance to penicillin by β-lactamases, HLAR, vancomycin, chloramphenicol, erythromycin, high level of clindamycin, tetracycline and fluroquinolone resistance.[1]

Resistance to β-Lactams

Intrinsic resistance

Enterococci begin with intrinsic resistance to most β-lactam antibiotics because of low affinity penicillin binding proteins (PBPs), which enable them to synthesize cell wall components even in the presence of modest concentration of most β-lactam antibiotics.[1,2] While most isolates of *E.faecalis* can be inhibited by concentration of penicillin achievable in the plasma (MIC of 1 to 8 μgm/mL) this is usually not the case with *E.faecium* (MIC of 16 to 64 μgm/mL). Higher level of resistance in *E.faecium* has been attributed to over production of low affinity PBP-5, a protein that can take over the function of all PBPs.[9] Moreover, concentration of

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Received : 30-05-05
Accepted : 14-06-05

ampicillin that are needed to inhibit enterococci are about half that of penicillin.[3] Thus, in general, ampicillin is more effective than penicillin *in vitro.*[10]

Tolerance

In addition, enterococci are "tolerant" to the activity of β-lactams, that is, enterococci are inhibited but not killed by these agents. This property is an acquired characteristic. Enterococci quickly develop tolerance after exposure to as few as five doses of penicillin. As most enterococci are tolerant to cell wall active agents, penicillin or glycopeptide, alone often fail to cure serious infections like endocarditis and meningitis which require bactericidal therapy and this is achieved by synergistic effect of penicillin/ampicillin plus aminoglycoside: standard treatment for serious infection.[2,3]

β-lactamase enzyme

Enterococci, exclusively strains of *E. faecalis*, expressing β-lactamase enzyme and having high level resistance to penicillin (HLPR) and ampicillin (MIC ≥ 256 μgm/mL) have been reported from various locations.[11-16] Its production is plasmid mediated and enzyme is constitutively produced. Because amount of β-lactamase production by enterococci may be insufficient for detection by routine antibiotic susceptibility testing, isolates from serious infection such as bacteraemia should be screened specifically for β-lactamase production.[17] Recommended and reliable method for β-lactamase production is chromogenic cephalosporin, nitrocefin.[18] *E. faecalis* strains producing β-lactamase are not susceptible to anti-staphylococcal penicillins but are susceptible to ampicillin, amoxicillin and piperacillin combined with drugs that inhibit penicillinase such as clavulanic acid, sulbactam and tazobactam.[3,14]

With a single known exception, isolates of *E.faecium* do not produce penicillinase yet confer high level resistance (HLR).[8,19] This, HLPR of *E.faecium* may be extreme example of intrinsic resistance associated with low affinity PBPs or may represent acquired resistance.[1]

Aminoglycoside Resistance

Intrinsic resistance

Enterococci exhibit low level resistance to all aminoglycosides (MIC 8 to 256 μgm/mL) which appears to be due to low uptake of these agents. However, aminoglycoside uptake is enhanced when enterococci are exposed to β-lactams.[1] This synergy underlies the long standing practise of combining both classes of antibiotics to treat serious enterococcal infections as combination overcomes the intrinsic resistance exhibited by enterococci and a synergistic effect is usually achieved since the intracellular penetration of aminoglycoside is facilitated by cell wall active agent.

Acquired resistance

Combination of penicillin plus streptomycin produced bactericidal killing of enterococci, until unfortunately, enterococci developed HLR to streptomycin.[20-24] But these isolates were not highly resistant to gentamicin thereby leading to widespread use of gentamicin plus penicillin for serious infections. Subsequently, however enterococci developed HLR to gentamicin (HLGR) that caused resistance to synergism between gentamicin and penicillin.[8,11,13,16,19,25-27] This acquired resistance is highly specific and renders bacteria resistant to high levels of aminoglycosides and as a result resistance to synergism. HLAR is defined as occurring when drug concentration of ≥ 2000 mgm/mL are required for inhibition of organism. HLAR is being conducted by series of aminoglycoside modifying enzymes (AME) coded by plasmid and are transferable. The most frequently encountered enzyme include a) dual function 2'phosphotransferase and 6'acetyl transferase conferring HLR to all available aminoglycoside (kanamycin, gentamicin, amikacin, netilmicin, tobramycin) except streptomycin; b) 3'phosphotransferase coding for HLR to kanamycin and penicillin-amikacin synergy without HLR to gentamicin; c) 6'adenyl transferase which produces HLR to streptomycin but does not inactivate other useful aminoglycosides.[1] Although no single enzyme can inactivate all available aminoglycosides, 30% of VRE strains can produce multiple enzyme types and are thus highly resistant to all known aminoglycosides.[9]

Screening for HLAR

As routine disc diffusion does not detect HLAR,[28] a formal MIC determination which shows that the MIC is ≥ 2000 μgm/mL is definitive for HLR and resistance to synergism. However, performing full MICs routinely is quite cumbersome and time consuming. Thus, several alternative methods have been proposed for detection of HLAR. These methods are: agar screening, high content disc and broth dilution.

Agar screening

Concentration of ≥ 2000 μgm/mL for streptomycin and other aminoglycosides and 500 μgm/mL for gentamicin are recommended as break points.[6,10] The 500 μgm/mL dilution (rather than ≥ 2000 μgm/mL) is used for screening gentamicin since some strains exhibiting MICs of 500-1000 μgm/ml also resist synergy of killing. Inocula of 10^4 and 10^6 cfu per spot gives best result regardless of medium used.[29] Growth of a single colony on agar dilution plates indicates resistance.

High content disc

Discs of 120 μgm of gentamicin, kanamycin and 300 μgm of streptomycin are recommended for disc diffusion test. Resistance is indicated by no zone and susceptibility by a zone of ≥ 10mm, strains with zone of 7mm-9mm should be tested

by dilution methods. For amikacin disc test results, considerable overlap occurs between zone size ranges of susceptible and resistant isolates. Thus, amikacin cannot be used to determine *E. faecalis* susceptibility to amikacin-penicillin synergy. In contrast, kanamycin disc more accurately predicts amikacin-penicillin synergy than does amikacin. Thereby, kanamycin proves to be an accurate and reliable substitute.[30]

Broth dilution test

Single concentration of 1000 μgm/mL of streptomycin[10] and 500 μgm/ml gentamicin by microdilution are recommended as break points.[31] An inoculum of 10^5 cfu/mL is recommended for testing since inoculum size has been found to be important factor in reliable detection of HLR.[29]

Glycopeptide Resistance

Considerable consternation greeted the first report of appearance of VRE in 1980s[32] followed by its rapid spread[8,25,26,33-35] thereby becoming a significant clinical problem. Three phenotypes of glycopeptide resistance have been reported in enterococci: Van A phenotype, with inducible high level resistance to both vancomycin (MIC ≥ 64 μgm/mL) and teicoplanin (MIC ≥ 16 μgm/mL), Van B with variable levels of inducible resistance to vancomycin (MIC 8 to 64 μgm/mL) and sensitive to teicoplanin (MIC ≤ 1 μgm/mL), Van C phenotype with intrinsic, constitutive low level resistance to vancomycin (MIC ≥ 8 and ≤ 32 μgm/mL) and susceptibility to teicoplanin (MIC ≤ 1 μgm/mL).[7] Van A and Van B are usually associated with *E.faecalis* and *E.faecium* whereas Van C are seen in *E.gallinarum* and *E.casseliflavus* strains. Van A is more widely distributed and thus the predominant type of resistance reported.[3] Moreover, vancomycin resistance has appeared preferentially in *E.faecium*, which is inherently more resistant to multiple drugs making therapy extremely problematic.[4] Clinically, vancomycin resistance has been associated with more frequent episodes of recurrent bacteraemia, persistent isolation of enterococci from primary sites of infection, increased frequency of endovascular infection and increased mortality.[10]

Colonization and infection

Faecal carriage of VRE is recognized to be frequently associated with serious clinical infection and it is likely that colonization of gastrointestinal tract occurs as a prelude to clinical infection. Risk factors for colonization and invasive disease include both heavy use of antimicrobial agents (especially vancomycin, third generation cephalosporins and antimicrobial agents with activity against anaerobes etc.) and variety of non antimicrobial factors including prior GI colonization with VRE, increased length of hospital stay, older age, proximity to case, care for a case by health care worker with GI colonization with VRE and immunosuppressive opponents[2,5,8,36-38] (Table1). Furthermore, these colonized patients contaminate themselves as well as environment, thereby having potential for transfer of VRE from environment to patients.[8,9] As VRE survives for long periods of time on dry surfaces[38] it is a successful environment contaminant causing some outbreaks.[25]

Table 1: Risk factors for VRE colonization or infection

Antimicrobial agents	Vancomycin
	Cephalosporins
	Aminoglycosides
	Aztreonam
	Antianaerobic drugs (metronidazole, clindamycin)
	Multiple antibiotics: risk increases as number of prior antibiotics increases
Nonantimicrobial factors	GI colonization
	Length of stay
	Un-isolated ICU days
	Older age
	Proximity to case
	Care for case
	Neutropenia
	Transplantation
	Severity of illness
	Haematological malignancies

Screening methods for detection of VRE

In the face of increasing rate of colonization with VRE and in the light of increasing concerns about the possible effect of this organism on patients with high risk of infections screening methods have been introduced for detection of VRE. The reliable and recommended agar screen method includes using brain heart infusion (BHI) agar with 6 μgm of vancomycin per mL.[17] Inoculum of 10^5 - 10^6 cfu is spotted and plate incubated at 35^0 C for 24 hours. Growth indicates resistance and no growth indicates susceptibility.[6] Two *Enterococcus* selective broths for isolation of VRE from colonized patients are also available. These are enterococcal broth with bile esculin azide and sodium azide with 6 μgm vancomycin (EBVA) and M- *Enterococcus* broth with sodium azide and triphenyl tetrazolium with 6 μgm vancomycin (M-EVA).[39] Similarly, antibiotic gradient method (E test) is also able to detect VRE.[9,10] Once suspected, based on a screening method, vancomycin resistance should be confirmed by using a different method.

Antibiotic synergism

Enhanced killing, called synergism is defined for enterococci as ≥ 100 fold increase in killing by the drug combination over the killing accomplished by most active of the two drugs when tested separately and resistance to synergy is ≤ 100 fold increase in killing.[30] The use of an aminoglycoside and penicillin in combination for severe

enterococcal infections is standard for management. By mechanism of synergy, penicillin facilitates the entry of aminoglycoside into the bacterial cell but does not cause an irreversible defect by itself. Synergistic effect depends on subsequent susceptibility of bacteria to aminoglycoside. Therefore, the enzyme that inactivates aminoglycoside also makes the organism resistant to synergism.[10]

Two most common methods used for determining synergy are the checkerboard technique and time kill test.[10] These are, however, too cumbersome, time consuming and labor intensive for routine use in many laboratories. As in enterococci synergy resistance is most frequently mediated by HLAR,[40] alternative method used to accurately differentiate between isolates that are resistant or susceptible to synergy is using high content aminoglycoside disc (120 μgm of gentamicin disc and 300 μgm of streptomycin disc). It is a convenient technique for laboratories to screen clinically *E. faecalis* strains for synergy resistance. Because of greater resistance of *E.faecium* to both b-lactam and aminoglycoside antibiotics, the disc agar diffusion results can be applied only to *E. faecalis* isolates and not to *E. faecium* strains.[30]

Therapeutic options for multiply resistant enterococci

Enterococci have a vast potential for acquiring and disseminating resistant genes.[6] As a result of this, they are currently causing significant therapeutic difficulties. Strains resistant to penicillin by β-lactamase production respond to gentamicin plus ampicillin-sulbactam or ampicillin – clavulanate or vancomycin. β-lactam resistance without β-lactamase production responds to vancomycin plus gentamicin.[41] Management of clinical HLAR enterococcal infection is quite limited. The common regimen include monotherapy with vancomycin, ampicillin, penicillin, mezlocillin or piperacillin. However, relapse or primary failure occurs as penicillin or ampicillin or vancomycin alone produces a bacteriostatic rather than bactericidal effect. Currently, there is no ideal therapy which yields bactericidal activity for serious infections caused by VRE. Above all, assessing the efficacy of therapy remains difficult because VRE is often associated with severe underlying illnesses and can be a part of polymicrobial infection. Fortunately, most VRE, particularly *E.faecalis* are moderately susceptible to ampicillin. If the MIC for ampicillin is ≤ 64 μgm /mL recommended therapy is high dose ampicillin or ampicillin-sulbactam combined with gentamicin or streptomycin (unless there is HLR). If VRE is highly resistant to ampicillin (MIC ≥ 64 μgm / mL) or both to gentamicin and streptomycin, then no drug regimen will be uniformly useful.[3] Some of these strains remain susceptible to tetracycline, erythromycin, chloramphenicol, fluoroquinolones, novobiocin or rifampicin and used as monotherapy or usually combining 2 or 3 antibiotics.[2,4] Even when a single agent or a combination of agents show *in vitro* activity against a particular VRE strain, overall therapeutic efficacy may be < 70 %.[9] Teicoplanin can be used in patients exhibiting the Van B phenotype preferably in combination with streptomycin or gentamicin (if not resistant).[2] Two newer agents with activity against VRE are quinopristin-dalfopristin and linezolid which are approved. Quinopristin-dalfopristin is streptogramin combination with *in vitro* bacteriostatic activity against *E.faecium* but not against *E.faecalis* or other enterococcal species.[3,9,41] Favourable clinical responses have been obtained in approximately three quarters of patients treated with these agents.[2,41] Limited clinical experiments suggest that linezolid (member of oxazolidinone class) is atleast as efficacious as quinopristin-dalfopristin.[41] Experimental agents with *in vitro* activity against VRE include glycopeptide (LY 333328), clinafloxacin, minocycline, ketolides, glycylcyclines, evernimomycin and daptomycin (Table2).[2,3,9]

The choice of antibiotics should not only depend on antimicrobial quantitative microbiological susceptibility data, but also on the type of infection being treated (endocarditis versus urinary tract infection), the severity of this infection and clinical response to the regimen chosen.

Table 2: Resistant pattern and treatment options

β-lactamase production	Gentamicin + ampicillin-sulbactam / amoxicillin-clavulanate /imipenem / vancomycin
β-lactam resistance without β-lactamase production	Gentamicin + vancomycin
HLGR	Streptomycin sensitive – streptomycin + ampicillin / vancomycin
	Streptomycin resistant – No proven therapy (continous infusion ampicillin, prolonged treatment)
Vancomycin resistance	ampicillin (MIC ≤ 64) - high dose ampicillin / ampicillin sulbactam + gentamicin or streptomycin (if no HLAR)
	ampicillin (MIC ≥ 64) or HLAR + β- lactam resistance - no uniformly bactericidal drugs available
	Newer drugs:
	Quinopristin-dalfopristin (only *E.faecium*)
	Linezolid (all enterococci)
	Investigational: Evernimomycin, daptomycin, LY333328
	For Van B - Teicoplanin

In UTI – consider nitrofurantoin, fosmomycin, ampicillin, amoxicillin or a quinolone [3,6]

Control efforts

Due to lack of uniformly effective antimicrobial therapy for patients infected with multiply resistant enterococci, limiting the dissemination of these organisms is of paramount significance. Primarily, the use of those antimicrobial agents that select for their isolations (cephalosporins,[42] antianaerobic drugs[37]) must be limited. Recommendation to reduce cross contamination by multiply resistant enterococci include surveillance for colonization, identification of colonized and infected patients, isolation of colonized patients, the use of gowns and gloves by health care worker (barrier method), hand washing with an antiseptic after gloves removal and avoid contact with environmental surfaces after gloves removal. Medical equipments (stethoscopes,blood pressure cuffs etc.) must be dedicated to HLR patients. Environmental decontamination is also required with effective disinfectants (isopropyl alcohol, sodium hypochlorite, phenolic and quarternary ammonium compounds).[3-5,9]

Thus, multifactorial control efforts can effect a decrease or atleast prevent an increase in the number of patients colonized or infected by these organism.

Conclusions

During past two decades, enterococci resistant to multiple antimicrobial agents have been recognized, including strains resistant to vancomycin, β-lactams and aminoglycosides, making it a formidable nosocomial pathogen. Such strains pose therapeutic dilemmas for clinicians. Thus, it is crucial for laboratories to provide accurate antimicrobial resistance patterns for enterococci so that effective therapy and infection control measures can be initiated.

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