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Resistant Nosocomial Enterococcal Infections

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Enterococcus faecalis, formerly classified as *Streptococcus faecalis*, belongs to Lancefield group D and is frequently isolated from nosocomial infections. Of the nosocomial infections reported to the Centers for Disease Control (CDC) in its National Nosocomial Infection Survey (NNIS), enterococci were isolated from 11% of cases. Frequently, *E. faecalis* is recovered as one of several kinds of bacteria causing infections complicating abdominal or pelvic surgery; in such situations, its role is difficult to assess. On the other hand, it is clearly a pathogen when it is isolated from a normally sterile site, such as the urinary tract, serous cavities, the subarachnoid space, or the blood. *Enteroccus* spp are regarded as a separate genus based on DNA homology; they are identified in the clinical microbiology laboratory by their ability to grow in media containing 40% bile, cleave esculin, and grow in broth containing 6.5% NaCl. Most laboratories do not attempt to speciate enterococci. Enterococci currently account for 9% of all nosocomial bacteremias that occur at the University of Michigan Hospital and 14% of all bacteremias that occur at the Ann Arbor Veterans Administration Hospital.

Changes in the Susceptibility of *Enterococcus faecalis*

Enterococcus faecalis exemplifies tolerance to antimicrobics. For serious, life-threatening infections, e.g., infective endocarditis, combination therapy using an aminocyclitol plus a cell-wall active agent is necesary to achieve bactericidal therapy. The bactericidal action of the combination may result primarily from the activity of the aminocyclitol; the cell-wall active agent may in some way act mainly to facilitate penetration into the bacterial cell. Formerly, low-level intrinsic resistance to aminocyclitols was the rule in *E. faecalis*, with high-level resistance a rare occurrence. Initially, streptomycin was

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the aminocyclitol used to treat serious infections, usually, in combination with penicillin G. Isolates resistant to streptomycin were recognized soon after it became the treatment of choice; fortunately, the newly developed aminocyclitols, such as gentamicin, were active against all of the resistant strains.

In 1979, the first high-level gentamicin resistant *E. faecalis* were reported from the Institute Pasteur, and in early 1980, the first of such strains were reported in the United States. Following these reports, screening was initiated at several institutions leading to the recognition of an increasing prevalence of these strains as causes of nosocomial infections. Currently, 13% of *E. faecalis* isolated from normally sterile body sites at the University of Michigan Hospital have high-level resistance to gentamicin, and 55% of all clinical isolates at the Ann Arbor Veterans Administration Hospital display this phenotype. Similarly resistant *E. faecalis* have also been reported from California, Illinois, Pennsylvania, Connecticut, Washington, and Texas. Serious infections, including endocarditis, have been reported to be caused by isolates with high-level resistance. The nature of the resistance has varied from institution to institution with some isolates having patterns similar to the isolates originally reported from France. These isolates are resistant to gentamicin and amikacin but remain susceptible to streptomycin, preserving its utility as a therapeutic option. Unfortunately, the majority of resistant clinical isolates are not inhibited by any of the aminocyclitols.

In addition to resistance to aminocyclitols, rare isolates also produce beta-lactamase. The clinical consequences are not devastating because the beta-lactamase is susceptible to inhibition by clavulanic acid. All of the beta-lactamase-producing strains reported to date have also possessed high-level resistance to gentamicin.

Epidemiology

Infection with *E. faecalis* traditionally has been considered to be acquired from endogenous flora. The sudden increase of high-level gentamicin resistant isolates of *E. faecalis* in a number of institutions prompted reexamination of the epidemiology of infection caused by enterococci, especially as clusters of infection in both geography and time were recognized. Detailed study of the epidemiology of enterococcal infections is limited by the lack of a typing system. However, by using total plasmid content and restriction endonuclease digests of plasmid DNA as indicators of strain identity, identical organisms were recovered from several groups of patients housed on the same nursing unit. It seemed unlikely that all of the patients carried the same organism endogenously and then became infected from their own flora; exogenous acquisition seemed more likely. Exogenous acquisition of *E. faecalis* was documented subsequently in a prospective study in an intensive care unit where high-level gentamicin resistance was endemic. Within the limits of detection by culture, patients newly admitted to the unit were negative; subsequently, while in the unit, they acquired resistant enterococci. Cross-infection was suggested by the ease of recovery of high-level resistant *E. faecalis* from the hands of personnel working in the unit. The reservoir and mode of transmission are remarkably similar to the multidrug resistant Enterobacteriaceae that frequently cause similar problems in the hospital environment.

Mechanisms of Resistance

The high-level resistance to gentamicin in *E. faecalis* in the original isolates described at the Institute Pasteur had resistance genes on a plasmid. This has been true in the isolates from the United States as well. The plasmids have been heterogeneous, varying in molecular mass, restriction patterns, and genes encoding additional resistances. This argues against selection of a resistant clone with subsequent dissemination but rather suggests involvement of transposition in the evolution of these plasmids. The resistance of several isolates from the United States and Europe is the result of inactivating enzymes and is thus similar to the plasmid-mediated resistance found in Gram-negative bacilli. Several different enzymes are produced in the most commonly isolated strains.

The DNA sequence responsible for resistance to gentamicin that resides on pIP800, the plasmid carried by the original French isolates, has been cloned and sequenced. This sequence encodes for a protein that is bifunctional, having 6′-acetyltransferase [AAC (6′)] and 2″-phosphotransferase [APH (2″)] activities. Additional adenylating activity found in the strains predominant in the United States results in inactivation of streptomycin, rendering all commercially available aminocyclitols ineffective.

Detection

The prevalence of high-level resistance to gentamicin in *Enterococcus* spp appears to have increased since it was first reported in 1979. Standard susceptibility testing, including disk diffusion and microtiter MICs, does not discriminate between the usual moderate level of intrinsic resistance and high-level resistance. Although the zone size from Kirby-Bauer disk

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diffusion tests might provide a clue, a poor correlation between MIC and zone size appears to be the rule with resistant *Enterococcus* spp. A single-well microdilution test for screening high-level resistance appears to provide the most useful information, yielding a positive predictive value of over 99% and a specificity of 100%. This test is similar to the single tube screening test devised for detecting resistance to streptomycin; instead of 2000 ug/ml of streptomycin, 500 ug/ml of gentamicin is used. The test provides a yes or no screen; growth correlates with lack of aminocyclitol-penicillin synergy. Screening with the single tube test appears to be useful for blood and other normally sterile body-fluid isolates; testing gentamicin alone is sufficient for initial screening. If the isolates display high-level resistance to gentamicin, additional testing using the single tube test for streptomycin at 2000 ug/ml is useful because occasional isolates are susceptible to streptomycin but resistant to gentamicin.

Table 1. Susceptibility of *Enterococcus faecalie* UM 2906

Antimicrobic	MIC, μg/ml
Gentamicin	2000
Tobramycin	2000
Amikacin	2000
Streptomycin	2000
Neomycin	2000
Netilmicin	500
Spectinomycin	62.5
Penicillin	3.2
Vancomycin	3.2
Erythromycin	100
Tetracycline	100

Therapy

The clinical infections caused by resistant *Enterococcus* spp mirror the spectrum of infections caused by ordinary *E. faecalis*. The approach to therapy for urinary tract infections, soft-tissue infection, and mixed infections that can be drained surgically is no different than for routine isolates. Inhibitory therapy with cell-wall active agents seems to be adequate. Endocarditis and bacteremia complicating nondebrideable foci, such as extensive burns or mediastinitis, are major therapeutic problems. With isolates apparently susceptible to streptomycin, using the 2000 ug/ml screen, streptomycin may be combined with penicillin or vancomycin. Formal synergy studies should be carried out with isolates causing serious infections in order to document the activity of streptomycin. If no aminocyclitol is active, bactericidal therapy cannot be delivered. Search for a replacement for the aminocyclitol component of the traditional combination regimens has not been fruitful so far. High-dose ampicillin has been suggested as one approach; usually, there is clearance of bacteremia with improvement in clinical parameters of infection, followed by relapse when therapy is stopped. A few patients do respond and are apparently cured; success has been reported with vancomycin as well. These successes may represent infections caused by strains of *E. faecalis* that are killed rapidly by cell-wall active agents alone. However, a regimen that is consistently bactericidal against aminocyclitol-resistant *E. faecalis* is needed to control serious infections caused by these emerging pathogens.

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