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Relationships between Enterococcal Virulence and Antimicrobial Resistance

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INTRODUCTION

Enterococci, along with approximately 450 other taxa of anaerobic and aerobic bacteria, are part of the normal intestinal flora (80). Prior to identification of multiple-antibiotic-resistant strains in the late 1970s, enterococci were considered relatively innocuous organisms. Over the past two decades, enterococci have been identified as the agents of nosocomial infection with increasing frequency, paralleling the accretion of antimicrobial resistance to most currently approved agents. As a result, enterococci have emerged as one of the leading clinical challenges for physicians when identified as the cause of serious or life-threatening infections.

The emergence of vancomycin-resistant enterococci (VRE) has alarmed the global infectious diseases community for several reasons. First, enterococcal acquisition of vancomycin resistance leaves few options for disease management. Second, conjugation experiments have confirmed vancomycin resistance gene transfer from enterococci to Staphylococcus aureus (85). Third, epidemiological studies in the United States and Europe have identified different selection pressures for VRE proliferation, yet similar and rapid expansion of resistant populations. Finally, the limited successes over the past decade of prevention and control strategies for containing vancomycin resistance (as well as methicillin resistance in staphylococci) highlight the difficulty of limiting the problem once it is established (47).

This article focuses on the relationships between enterococcal virulence and antibiotic resistance, the latter being reviewed in an accompanying manuscript in this issue (21). Enterococcal infections may be due to at least 12 species, including Enterococcus avium, Enterococcus casseliflavus, Enterococcus durans, Enterococcus faecalis, Enterococcus faecium, Enterococcus gallinarum, Enterococcus hirae, Enterococcus malodoratus, Enterococcus munditiis, Enterococcus pseudaoiuom, Enterococcus raffinosus, and Enterococcus solitarius. Additional species such as Enterococcus ecerorum, Enterococcus columbiae, Enterococcus saccharolyticus, Enterococcus dispers, Enterococcus raffinosus, Enterococcus seriolicida, and Enterococcus flavescens have been proposed as additions to this list. Most clinical infections are due to either E. faecalis or E. faecium. Occasionally infections are due to E. gallinarum, E. raffinosus, E. casseliflavus, E. avium, E. pseudaoiuom, E. malodoratus, E. munditiis, E. durans, or E. hirae. Therefore, our focus will be predominantly on E. faecalis and E. faecium. Our goal is to highlight current concepts and controversies as well as gaps that exist in the literature on enterococcal pathogenesis, disease management, and public health.

SHIFTING SPECTRUM OF ENTEROCOCCAL INFECTION

Epidemiologic studies appear to conflict with respect to the association between enterococcal species and disease. Historically, the ratio of infections due to E. faecalis to those due to all other Enterococcus species was approximately 10:1. In re-
cient years, there has been a progressive decline in this ratio of enterococcal bacteremia (P. Traynor, D. F. Sahm, and L. M. Mundy, Seventh Annu. Meet. Soc. Healthcare Epidemiol. Am., abstr. 52, 1997). This microbiologic shift is likely to be explained in part by the emergence of VRE, in particular, the predominance of the species E. faecium among this subset of enterococcal isolates. Data from the National Nosocomial Infection Surveillance (NNIS) survey reveal a rising percentage of VRE since 1989, with rates now approaching 20% of all enterococcal isolates (including all species); an equal proportion of VRE isolates occurring in and out of intensive care units; and E. faecium as the dominant species identified among VRE (although many enterococci are not identified) (19, 48). In a comparison of NNIS pathogens from 1994 through 1998 and January through May 1999, there was a 47% increase in VRE (48).

VRE were first detected in Europe in 1986 and soon after a VanB E. faecalis clinical isolate was reported from St. Louis in the United States (95, 112). The epidemiological parameters that contributed to VRE dissemination now seem distinct for the United States and Europe. In Europe, suspected reservoirs related to animal husbandry and now community ecologies appear to be primary sources of VRE (9, 10, 77, 110). In the United States, VRE reservoirs include hospital staff and patients, including those who have survived hospital stays and reside in skilled nursing facilities; organisms are transmitted by vectors such as stethoscopes, electronic thermometers, sphygmomanometers and health care workers’ hands (11, 14, 74).

In the United States, injudicious use of antimicrobial agents and rising colonization pressure (proportion of patients colonized with VRE in a defined geographic area) are the largest contributors to selection of vancomycin resistance (15). In Europe, the use of avoparcin as a growth promoter in animal feeds seems to be the major contributor to vancomycin resistance (77). Epidemiologic studies examining glycopeptide use in animal husbandry have provided evidence that vanA-mediated VRE is now ubiquitous in European communities, the organisms readily colonizing intestinal tracts of animals for which avoparcin was used as a feed supplement (1, 10, 64, 65). Subsequent enteric colonization of humans has been documented (9). In contrast, the United States has not permitted the use of avoparcin in animal feed. In this country, VRE emergence and spread appears to result from selection in clinical settings.

From the earliest reports of VRE clinical isolates, most were VanA phenotype strains of E. faecium and were associated with outbreaks in special units with immunocompromised patients on prolonged antimicrobial regimens, with extended lengths of stay and higher severity-of-illness scores (15, 29, 34, 40, 62, 70, 73, 75, 76, 94, 105). In multivariate analyses, length of stay, severity of illness, and colonization pressure were independent predictors of VRE colonization and infection (15). Over time, the enterococcal problem has become endemic, with community-based issues of animal food supplies and nosocomial issues of colonization pressure and severity of illness (15, 34, 70).

**EMERGENCE OF ANTIMICROBIAL RESISTANCE**

The intrinsic resistance of enterococci to many commonly used antimicrobial agents may have allowed them a cumulative advantage for further acquisition of genes encoding high-level resistance to aminoglycosides, penicillins, tetracycline, chloramphenicol, and now vancomycin. There are at least three major reasons for the emergence of multidrug-resistant enterococci: (i) baseline intrinsic resistance to several antimicrobial agents, (ii) acquired resistance via mobility of the resistance genes on plasmids and transposons, and chromosomal exchange, and (iii) the transferability of resistance. These mechanisms are reviewed in detail by Cetinkaya et al. in the accompanying article in this issue (21). In addition, it is important to note that these genetic transfers often occur in the gastrointestinal tracts of humans and animals, many of which have other bacteria under potential selective pressure from therapeutic or subtherapeutic levels of on-going antimicrobial exposure. Finally, the environmental burden of antimicrobial utilization, colonization pressure, and nosocomial transmission of VRE is high in many hospitals and may also be high in the animal health industry, where the organisms appear to be hearty survivors.

From the perspective of E. faecium antimicrobial resistance, there is an association between ampicillin and vancomycin resistance. Ampicillin-resistant E. faecium isolates are most often detected before vancomycin resistance is detected. Together, the genetic linkage in E. faecium between ampicillin, penicillin-binding protein 5, and vancomycin (106) and clinical studies that have shown prior β-lactam use as a leading predisposing factor suggest that antimicrobial agents such as cephalosporins contribute to the emergence of vancomycin-resistant E. faecium (75). The linkage between a β-lactam-resistant penicillin-binding protein and vancomycin resistance does not appear to have occurred yet in E. faecalis, which may account for the sporadic detection of vancomycin-resistant E. faecalis.

The literature to date suggests that clonal dissemination of certain enterococcal strains, increased environmental enterococcal burden secondary to antimicrobial regimens, and limitations of effective infection control measures have together contributed to the rising endemicity and nosocomial outbreaks from VRE. It appears that many clones of E. faecium and E. faecalis never proliferate and that the majority of E. gallinarum and E. raffinosus isolates do not transmit vancomycin resistance genes or proliferate.

Notably, enteric VRE colonization usually precedes infection (115). The lower intestinal tract is the most frequently colonized site, and persistent enteric colonization, especially among those with frequent hospital readmissions, may be a prominent VRE reservoir. Skin contamination in these patients occurs readily (11,14,120). Numerous studies have demonstrated contamination of health care workers’ gloved and ungloved hands. In one recent report, 29% of health care workers still had VRE on their hands after glove removal (S. Badri, N. Sahgal, A. Tenorio, K. Law, B. Hota, M. Matushek, M. Hayden, G. Trenholme, and R. Weinstein, Abstr. 36th Annu. Meet. Infect. Dis. Soc. Am., Abstr. 599, 1998). Complicating the issue of skin colonization are the potential sequelae of enterococcal pseudomembranous colitis with resultant nonjudicious use of antibiotic therapy (7). Therefore, the horizontal transmission of VRE in nosocomial environments can occur readily if Centers for Disease Control and Prevention guidelines to prevent the spread of vancomycin resistance genes are not strictly followed.

**ASSOCIATIONS BETWEEN INFECTIONS AND MORTALITY**

The reported estimates of mortality risk associated with VRE bacteremia vary according to the study design and analysis, patient population, case definition, control selection, and enterococcal species studied (Table 1) (28, 34, 70, 76, 86, 89, 98, 102, 105). The majority of these studies are small and report only crude mortality rates (118). Control populations have included patients with no bacteremia (28, 29) and pa-
Patients with varied enterococcal species (76, 86, 98, 105). In addition, interspecies differences may confound estimates of mortality risk attributable to vancomycin resistance (76). In two recent studies, vancomycin-resistant *E. faecium* infection did not independently increase mortality risk compared with patients who had vancomycin-susceptible *E. faecium* bactemia when adjustments were made for severity of illness (34, 70). Although virulence traits are less well characterized in *E. faecium* compared to *E. faecalis*, neither group of investigators were able to assess the blood isolates for potential virulence traits that may have contributed to mortality (50).

In clinical infectious disease management, two assumptions are frequently made with respect to multidrug-resistant pathogens. The first assumption is that more antimicrobial drug resistances equate with greater virulence. The second assumption is that attributable mortality is linked to the pathogens’ antimicrobial susceptibility profile rather than the availability and prompt initiation of suitable antimicrobial agents. To date, data in support of either position are lacking. As noted above, it was recently observed that crude mortality among bacteremic patients with *E. faecium* does not differ between those with vancomycin-susceptible and vancomycin-resistant isolates when adjustments are made for severity of illness (34, 70). This finding is similar to a comparison of crude mortality among bacteremic patients with methicillin-susceptible *Staphylococcus aureus* versus methicillin-resistant *S. aureus* (MRSA) (42). Speculatively, a two-tailed test for mortality (higher and lower mortality) associated with death from multidrug-resistant organisms may reveal that increased resistance is associated with a reduction in other aspects of fitness, including virulence. However, evidence to the contrary exists, including a study that found a strong association between high-level gentamicin resistance and expression of a known enterococcal cytolytic toxin among a large collection of *E. faecalis* bacteraemia isolates (50).

In this study, infection with cytolytic, high-level gentamicin-resistant strains was associated with a fivefold-increased risk of death despite the uniform vancomycin susceptibility of these isolates (50). Unfortunately, patient severity of illness was not assessed in this cohort to determine if there was an interaction between cytolsin production and severity of illness.

Clearly, further clinical and translational research is needed to dissect such questions for enterococci, including species other than *E. faecalis*. In addition, very little is known about host immune responses to enterococci. Collaborative work that allows the clinical assessment of patients (34, 70) and the molecular characterization of recovered isolates (50) would allow our understanding of enterococcal virulence to advance.

**PATHOGENESIS AND VIRULENCE**

Bacteria colonize the intestine and interact in complex and largely unstudied associations with other bacteria, the intestinal epithelium, and other components of the mucosal interface. Comparatively little is known about the nature of enterococcal colonization of the human gastrointestinal tract except that enterococci occur in relative abundance in human feces (80, 84). Factors that influence enterococcal species-specific selection in colonization of the intestinal tract (i.e., factors favoring colonization by *E. faecalis* over *E. faecium*, etc., or vice versa) are also not well understood.

Enterococcal virulence has been reviewed recently elsewhere (53, 57), so this article focuses on emerging concepts for defining and studying enterococcal virulence. Examples of well-documented virulence traits are discussed to build an understanding of how auxiliary enterococcal traits contribute to the pathogenesis of infection.

Virulence among enterococci appears to have evolved in a mode and tempo that are no different from the emergence of

**TABLE 1.** Recent epidemiologic studies of crude mortality reported for enterococcal bacteremia

<table>
<thead>
<tr>
<th>Species</th>
<th>Reaction to vancomycin</th>
<th>Crude mortality data, no. dead/no. tested (% dead) No. (%)</th>
<th>Study design</th>
<th>Study dates</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. faecium</em></td>
<td>R</td>
<td>16/32 (50)</td>
<td>Case-control; liver transplant patients</td>
<td>1988–1994</td>
<td>87</td>
</tr>
<tr>
<td><em>E. faecium</em></td>
<td>S</td>
<td>? (14)</td>
<td>NNIS data</td>
<td>1989–1993</td>
<td>19</td>
</tr>
<tr>
<td><em>E. faecium</em></td>
<td>S</td>
<td>17/48 (35)</td>
<td>Cohort; liver transplant patients</td>
<td>1991–1994</td>
<td>71</td>
</tr>
<tr>
<td><em>E. faecalis and E. faecium</em></td>
<td>S</td>
<td>1/56 (2)</td>
<td>Retrospective cohort</td>
<td>1991–1996</td>
<td>74</td>
</tr>
<tr>
<td><em>E. faecium</em></td>
<td>S</td>
<td>13/32 (41)</td>
<td>Retrospective cohort</td>
<td>1992–1995</td>
<td>100</td>
</tr>
<tr>
<td>Enterococcus spp.</td>
<td>R</td>
<td>16/21 (76)</td>
<td>Case-control; oncology outbreak</td>
<td>1993</td>
<td>28</td>
</tr>
<tr>
<td>Enterococci</td>
<td>R</td>
<td>116/260 (45)</td>
<td>Historical cohort</td>
<td>1993–1995</td>
<td>68b</td>
</tr>
<tr>
<td>Enterococci</td>
<td>R</td>
<td>1/6 (17)</td>
<td>Prospective cohort</td>
<td>1994</td>
<td>112</td>
</tr>
<tr>
<td><em>E. faecium</em></td>
<td>S</td>
<td>7/23 (30)</td>
<td>Retrospective cohort</td>
<td>1999</td>
<td>33c</td>
</tr>
</tbody>
</table>

* R, resistant; S, susceptible.

<table>
<thead>
<tr>
<th>Study dates</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1991–1994</td>
<td>84</td>
</tr>
<tr>
<td>1991–1993</td>
<td>96</td>
</tr>
<tr>
<td>1991–1994</td>
<td>71</td>
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<td>1991–1996</td>
<td>74</td>
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<tr>
<td>1992–1994</td>
<td>79</td>
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<tr>
<td>1992–1995</td>
<td>100</td>
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<tr>
<td>1993</td>
<td>28</td>
</tr>
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<td>1993–1995</td>
<td>29</td>
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<td>1993–1995</td>
<td>68b</td>
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<tr>
<td>1994</td>
<td>112</td>
</tr>
<tr>
<td>1999</td>
<td>33c</td>
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After controlling for severity of illness, vancomycin resistance was not associated with mortality (odds ratio 1.74, 95% confidence interval 0.5 to 6.12, *P* = 0.39).
pathogenic lineages of other species (32, 100). Wherever it has been studied, genetic lineages within every bacterial species capable of causing human infection arise within the population, with either enhanced or attenuated virulence traits (32). Examples of genetic lineages with enhanced virulence traits of current interest include *Escherichia coli* O157 (4), MRSA (67), and pandemic strains of *Vibrio cholerae* (68, 114). The emergence of genetic lineages with enhanced virulence results from the acquisition of new traits by genetic exchange. This genetic exchange involves a diversity of genetic elements, such as SCCmec, a large, 52-kb novel genetic element encoding methicillin resistance in *S. aureus* (63); pathogenicity islands in a number of species encoding toxins (39), adhesions, and other virulence-associated factors; plasmids associated with virulence factors (79) and transposons (100). These elements enter into the species once or very rarely, resulting in the emergence of unusually pathogenic lineages within the species (32). In comparison to the emergence of pathogens with enhanced virulence, vertical inheritance slowly penetrates into other lineages of the species by further DNA transfer (17). The prediction, then, is that there are traits in enterococci that may confer enhanced abilities to cause disease and that these traits may be associated with a single genetic lineage of the species if recently acquired. Alternatively, such traits may have permeated more deeply into the species by horizontal transfer if acquired comparatively earlier. If confirmed, genetic lineages will be manifested by the observation of multiple clinical isolates of discrete chromosomal lineages bearing this new trait (as determined by molecular genetics). If the trait has permeated the species more deeply, the trait may be found in a diversity of genetic backgrounds.

Among enterococci, traits that have been acquired by some lineages, have permeated the species to various degrees, and are suspected to relate to an enhanced ability to cause disease include antibiotic resistance determinants, a cytolytic toxin, gelatinase, aggregation substance, extracellular superoxide production, and enterococcal surface protein (Table 2) (55, 57). All of these appear to fulfill the formal definition of virulence factor (although some await direct examination), in that they enhance the ability of the organism to cause disease beyond that intrinsic to the species background. Autolysins, best characterized in *E. hirae*, are not known to be virulent in *E. faecalis* and will not be discussed further (30, 92, 101). The cell wall carbohydrate of enterococci has been the subject of study, but there is currently little agreement as to the identity and role of these carbohydrates (3, 49, 119). Other phenotypic and potential virulence factors that require further investigation include hyaluronidase, lipoteichoic acids, fibronectin, and surface carbohydrates.

### Antibiotic Resistance Determinants

When viewing human-associated enterococci in their entirety, it is obvious that the great majority of enterococci exist as commensals in the gastrointestinal tract (80, 84) or in ecologies contaminated by human wastes, with a comparatively minuscule fraction that experience natural selection in the process of causing human infection. Therefore, it seems safe to assume that traits that are widely distributed within human-associated enterococcal species have been selected because of their role in conferring fitness for existence and perpetuation in the overwhelmingly primary ecological system, the gastrointestinal tract. The concept that emerges, then, is that acquired virulence traits operate in a genetic background that has evolved to survive in the highly competitive gastrointestinal tract. The intrinsic ruggedness of the enterococcus undoubtedly contributes to its persistence at sites of infection as well as to the organism’s resistance to antibiotics. However, as factors that confer intrinsic ruggedness appear to have been selected and function primarily to enhance competitiveness as a commensal organism, compromising these traits may compromise the enterococcus’s ability to cause disease as well as its ability to function as a commensal organism. Ubiquitous traits of enterococcal species probably do not represent virulence traits sensu stricto and will not be discussed further in this review. A second important concept that has emerged, and is key to understanding enterococcal infection at the molecular and cellular level, is that nosocomial enterococcal disease is predominantly a two-stage process. There is an initial, usually asymptomatic colonization of the gastrointestinal tract by enterococcal strains possessing various traits, such as antibiotic resistances, cytolytic toxin genes, or possibly aggregation substance or the protease gelatinase upon hospital admission (81, 109). Subsequently this population is expanded, often facilitated by antibiotic elimination of competitors. For a select number of patients, there is subsequent tissue invasion, directly or indirectly, from the expanded gastrointestinal tract reservoir. The prediction from this model is that infection-derived enterococcal isolates will mirror those of the gastrointestinal tract of hospitalized patients but be of unknown relationship to commensal populations within the community. Given this two-stage model of asymptomatic colonization with nosocomial strains followed by tissue invasion, exogenously acquired factors can enhance the virulence of enterococci by functioning at either or both levels. That is, a factor may enhances the ability of a nosocomial strain to outcompete indigenous commensal enterococci, increase the presence of nosocomial strains in the gastrointestinal tract, and as a result increase the statistical likelihood of causing disease as breaches in containment occur. Antibiotic resistances clearly fall into this category, as would new surface proteins that enable the nosocomial organism to colonize a different niche from that occupied by indigenous strains. Theoretically, new fermentation pathways that would enable the organism to localize in new areas of the gastrointestinal tract could perform a similar role, although this prospect has yet to be closely examined.

The second level at which an exogenously acquired factor could enhance enterococcal virulence is at the level of tissue destruction or toxicity, enhancing its ability to breach containment in the gastrointestinal tract or other commensal site and cause symptomatic disease. A factor that enhances disease severity, as opposed to disease probability, would not necessarily appear in increased numbers among clinical isolates, but would be associated with more severe clinical presentation or sequelae.

Several factors confound the relationship between entero-
coccii, virulence, and disease. Enterococci are a leading cause of subacute endocarditis, which typically occurs in older male patients with genitourinary tract infection (78). As these are often acquired in the community, strains causing these infections are of unknown relationship to those that have become endemic within hospitals. Aside from subacute endocarditis, most other enterococcal disease occurs in patients with underlying conditions representing a wide spectrum of severity of illness and immune modulation (70). As host immunosuppression increases, the requirements for particular virulence traits to enhance the likelihood or severity of disease decrease. Because of the sophistication of support currently available to prolong the life of patients with severely debilitating underlying conditions, some fraction of enterococcal disease is undoubtedly attributable to ordinary commensal strains without any special features. Therefore, collections of infection-derived isolates should contain a spectrum of types of strains, from pure commensals to those harboring the most synergistic combinations of various virulence traits.

Several studies have attempted to examine clinical isolates with the goal of identifying traits that enhance the organism’s ability to cause disease beyond those associated with simply being an enterococcus. Interpreting these studies, however, requires careful examination of study design to determine what question the data answer. In one study, serial nonduplicative E. faecalis blood isolates were obtained from patients at a large clinic over a 4-year period in the mid-1980s (50). These isolates were examined for production of a cytolytic toxin, which confers a hemolytic phenotype, as well as for antibiotic resistance. Of 190 isolates, 36% were resistant to high levels of aminoglycosides and 45% were hemolytic. Notably, these traits were not randomly and independently distributed among the 190 strains; 91% of the gentamicin-resistant strains were hemolytic, whereas 19% of the gentamicin-susceptible strains were hemolytic. Furthermore, genetic identity confirmed that 12 of 12 hemolytic, gentamicin-resistant strains were identical (from 10 different patients) versus only two of nine nonhemolytic, gentamicin-susceptible strains (from 8 different patients, with the identical isolates from the same patient taken 7 weeks apart). These data show that neither gentamicin resistance nor the cytolytic toxin conferring the hemolytic phenotype had deeply penetrated the species and imply that the two factors may work synergistically to result in disease. In addition, this outbreak study of virulent enterococcal clones identified virulence traits in a significant proportion of bacteremic patients. Obviously, counting all infections caused by this entire genetic lineage as a single event would underrepresent the problem. A study that focuses on strains of unique chromosomal lineage, however, is well designed to answer the question to what extent a particular trait has permeated the species.

In a large study that examined the relationships between enterococcal disease and enterococcal traits, isolates related by DNA fingerprint were excluded (25). Between 25 and 33% of the presumably unique genetic lineages derived from infection sites and a slightly lower proportion from the gastrointestinal tract of hospitalized patients possessed the trait of cytolytic toxin production (25). This observation is consistent with a limited penetration of the species by the cytolytic toxin determinant. These data are also consistent with enterococcal infection conforming to the two-stage model described above, whereby nosocomial strains first colonize the gastrointestinal tract after hospital admission, then appear at disseminated sites (molecular genetic testing that confirms unrelated isolates in the gastrointestinal tract and sterile body sites may imply that the primary infection occurred from an external source). As data were derived preferentially for isolates that were non-clonal, raw incidence numbers were not provided, and the actual percentage of infections that were caused by cytolytic toxin-expressing strains cannot be determined.

The study by Coque et al. did examine the penetration of other potentially virulence-associated traits into the species E. faecalis (25). It was observed that approximately one-half of the genetic lineages of the species from either infections or feces of hospitalized patients possessed genes for aggregation substance and the protease gelatinase. This suggests either that these traits entered the species earlier than did the cytolytic toxin or that they are associated with elements that are more mobile than that encoding the cytolysin. Aggregation substance is an integral component of the pheromone-responsive plasmid exchange system (23). Therefore, nosocomial strains of E. faecalis may be those best equipped to participate in genetic exchange and may be selected by the presence of antibiotic resistance determinants on such plasmids. The basis for the penetration of the gelatinase determinant into E. faecalis lineages associated with the nosocomial environment is more obscure. Gelatinase, aggregation substance, and cytolysin were all observed among fecal strains from the community in approximately 25% of the genetic lineages examined (25).

Huycke et al. found that bacteremic patients harboring strains expressing the hemolytic cytolysin in a background of gentamicin resistance were at a fivefold-increased risk of death within 3 weeks of a culture-positive blood specimen (50). Although aminoglycoside resistant, all isolates were negative for β-lactamase production and none were vancomycin resistant. Moreover, this risk of mortality was found to be independent of therapy. Collectively, these data imply that the cytolysin contributed to the mortality associated with human enterococcal bacteremia. It is the only study, to our knowledge, to implicate an enterococcal virulence factor in mortality.

Nevertheless, demonstration of a role in virulence for any particular enterococcal trait requires direct testing in well-controlled and well-designed disease models. The majority of investigative research in enterococcal pathogenesis and virulence has focused on E. faecalis adherence and lytic activities because they fit paradigms established for highly virulent pathogens. Nearly nothing is known of the specific interactions between E. faecium and human host tissues. All nonhuman models of disease represent an approximation, with various inherent levels of compromise. Although well established for the study of hypervirulent pathogens, murine LD50 determinations involving bolus injections of 107 to 109 organisms intraperitoneally and measuring demise in hours (40, 99) lack sensitivity for examining the pathogenesis of disease in humans. A major limitation of this approach is that an animal without underlying pathology is burdened with a sudden lethal bolus of large numbers of bacteria (in contrast to the usually indolent course of human infection). Moreover, these large numbers of bacteria are usually prepared in vitro in enriched laboratory medium and as a result are ill adapted to respond to restrictive host environmental cues. Finally, there are relatively few parameters for measurement of virulence factors or their contribution to the pathogenesis of infection in acutely lethal models.

In an attempt to more closely approximate the course and conditions of human disease, models varying in immune competence have been explored. Because of background variability and susceptibility to infection by commensal organisms that colonize the respiratory pathways, these models have not been extensively pursued for studying enterococcal virulence. In addition, polymicrobial infections and adjuvant therapeutics have been confounding variables in many animal models. More recently, the cellular and molecular basis for immune limitation
in the eye began to emerge (104). As this organ provided a readily observable organ system with limited immune responsiveness in an otherwise healthy background, the rabbit endophthalmitis model was developed and used to study the pathogenesis of enterococcal infection (18, 57). Major advantages of this system include the exquisitely small infectious dose, with 10 organisms sufficient to establish infection. After inoculation, infection develops over the course of 3 to 7 days, enabling the enterococci to adapt, expand in numbers, and respond to in vivo environmental cues. Moreover, the course of infection can be studied quantitatively using electrophysiology (electroretinography) as well as by direct observation using the standard tools of ophthalmology; all measures can be made repeatedly over the course of infection, allowing the host-bacterium interaction to be studied by multiple parameters in real time. Other advantages of the endophthalmitis model include the ability to administer intravitreal antibiotic and anti-inflammatory agents as is done for clinical endophthalmitis, providing rapid therapeutic intervention and reducing potential adverse events (60). Offsetting some of these strengths is that, although providing in vivo environmental cues, the eye is not a common site for enterococcal infection and has built-in limitations in host response that may vary from the response encountered by offending organisms at other anatomical sites. However, many enterococcal infections occur in patients with a spectrum of underlying pathologies that similarly limit host response.

As enterococci are also a leading cause of subacute endocarditis, the rabbit endocarditis model using abraded heart valves, via catheter-induced trauma, has also been used to assess virulence. Infused organisms are permitted to colonize the damaged valve, and comparison of various treatment regimens and pathologies can be made.

These models have enabled key findings to be made on the contribution of various virulence factors to the course and severity of enterococcal disease.

Cytolysin

The cytolsin is a novel hemolytic, posttranslationally modified protein toxin that occurs in up to 60% of E. faecalis isolates retrieved from outbreak investigations (36, 55–59, 72). The encoding operon is carried on a plasmid or integrated into the bacterial chromosome (56). In addition to toxin activities, the cytolsin of E. faecalis possesses bacteriocin activity against a broad range of gram-positive bacteria (58). The self-protective mechanism of immunity for the cytolsin-producing strain has recently been described (24). Diagnostically, this toxin causes a beta-hemolytic reaction on human and horse blood agar (but does not hemolysise sheep blood agar, which is frequently used in clinical microbiology laboratories).

A number of independent studies using different model systems have consistently found a role for the E. faecalis cytolsin in the toxicity of enterococcal infection (18). The rabbit endophthalmitis model was used to examine in detail the contribution to virulence of cytolsin, using isogenic strains of E. faecalis differing only in this trait (59, 60). In these studies, E. faecalis caused an infection accompanied by significant inflammatory sequelae even when the organisms were effectively killed by antibiotics (59, 60). These sequelae could be successfully managed by corticosteroid adjutant therapy in combination with antibiotics. However, if the E. faecalis strain produced the cytolytic toxin, the combined beneficial effect of antimicrobial and anti-inflammatory therapy was completely offset by the organotoxic activity of the cytolsin, which completely destroyed the organ even though all other aspects of the infection were successfully managed (59). These studies show that even in an organ with limited immune response, enterococcal disease has an important inflammatory component as well as an organotoxic component if the offending organism is a cytolsin producer.

Similar observations were made in a rabbit endocarditis model (22). These studies found that vegetations due to cytolytic enterococci exhibited a significant increase in lethality compared to those caused by isogenic strains specifically defective in cytolsin production. Interestingly, vegetations induced by the cytolytic strains, although more lethal, were actually smaller. Together, these studies represent important refinements on the original acute lethality observations made in the murine intraperitoneal challenge model (54). Even though the number of organisms used in this challenge was very large (10^8 to >10^10 CFU), the cytolsin was found to significantly enhance both the lethality of bolus injection, as shown by a >100-fold reduction in the LD_{50} and the rate at which deaths occurred.

Aggregation Substance

Aggregation substance is a pheromone-inducible surface protein of E. faecalis which promotes mating aggregate formation during bacterial conjugation (23). As an important component of the bacterial pheromone-responsive genetic exchange system, aggregation substance mediates efficient enterococcal donor-recipient contact to facilitate plasmid transfer (23). In vitro, aggregation substance mediates adhesion to a variety of eukaryotic cell surfaces, such as cultured pig renal tubular cells, and promotes internalization by cultured human intestinal cells (66, 87, 97). Aggregation substance was also studied in the rabbit model of E. faecalis endocarditis and found to be associated with greater vegetation size compared to vegetations caused by isogenic aggregation substance-defective strains, although these infections were not observed to be lethal (22). More recent studies in a similar model suggest that aggregation substance and its cognate ligand, binding substance, may lead to destruction of myocardial and pulmonary tissues (96). In vitro, aggregation substance also promotes direct opsonin-independent binding of E. faecalis to human neutrophils via complement receptor type 3 and other receptors on the neutrophil surface (113) and appears to promote intracellular survival of E. faecalis inside neutrophils (93). In a comparison of ingested, nonopsonized aggregation substance-bearing E. faecalis to ingested, opsonized E. faecalis, there were higher levels of phagosomal pH, extracellular superoxide, phagosomal oxidant production, surface Mac-1 expression, and shedding of L-selectin (93).

In vivo, aggregation substance may contribute to the pathogenesis of enterococcal infection through a number of mechanisms. Aggregation substance is known to be induced by pheromone signals (23) and by serum (66, 71), suggesting that aggregation substance-expressing cells likely form larger aggregates in vivo than cells not expressing this trait. Aggregation contributes to bacterial virulence in other systems (114), and it is presently unknown how the simple act of aggregation may influence phagocytosis and the subsequent fate of the organism. There are also indications that aggregation substance may bind and present its cognate ligand, which is believed in part to relate to teichoic acids, on the surface of the organism, possibly resulting in superantigen activity (96). Finally, aggregation substance increases the hydrophobicity of the enterococcal surface (46), which may induce localization of cholesterol to phagosomes and prevent or delay fusion with lysosomal vesicles (31, 35). It may be this aggregation that also enhances the organ-
ism’s ability to associate with intestinal epithelial cells (87). As most cytolytic strains of \textit{E. faecalis} also express aggregation substance, these factors may well work synergistically, as shown by the results of studies in endocarditis (22). Evidence that these two traits are highly coevolved also comes from the observation that the cytolsin is bactericidal for noncytolytic enterococci, yet cytolsin-coding aggregation substance-bearing plasmids are transferred to these otherwise sensitive recipients efficiently (27). In retrospect, it is not surprising that an aminoglycoside-resistant lineage of \textit{E. faecalis} expressing cytolsin and aggregation substance proved particularly virulent (50). The fact that the prototype VanB strain, V583, possesses a naturally insertionally disrupted cytolsin operon (unpublished observations) may also contribute to the limited spread of this isolate (95).

**Gelatinase**

Comparatively less is known about the contributions of other traits of enterococci to virulence (excluding the antibiotic resistances). Isogenic strains of \textit{E. faecalis} differing in gelatinase production appear to modestly affect acute toxicity in the bolus LD\textsubscript{50} murine model (99).

**Extracellular Superoxide**

Extracellular superoxide production is another trait associated with enterococcal virulence in bacteremia (51, 52, 97) and appears to vary among isolates. Most \textit{E. faecalis} and some \textit{E. faecium} strains generate substantial extracellular superoxide, with significantly greater production by invasive strains than by commensal isolates. Superoxide production was observed to enhance in vivo survival of \textit{E. faecalis} in mixed infection with \textit{Bacteroides fragilis} in a subcutaneous infection model (52).

**Extracellular Surface Protein**

Another variable trait that appears to be associated with enterococcal virulence is extracellular surface protein (Esp), initially derived from the original vanB \textit{E. faecalis} clinical isolate (95). The \textit{esp} gene encodes a large bacterial surface protein with an interesting structure. The central core of the protein consists of repetitions of distinct tandem repeating units, with a slightly divergent C-terminal cell wall anchor domain and an apparently globular N-terminal domain. It is currently hypothesized that the central repeat region serves as a retractive arm, extending the N-terminal globular domain through the cell wall to the surface. The number of central repeats was found to vary between 3 and 11 in various \textit{E. faecalis} isolates, supporting this hypothesis. It is plausible, under adverse conditions such as immune deficiency, that the ability to retract the surface protein may facilitate immune evasion (97). PCR amplification detected \textit{esp} in only 3% of \textit{E. faecalis} stool isolates but 41% of \textit{E. faecalis} blood isolates and 42% of \textit{E. faecalis} endocarditis isolates. The gene was not detected in isolates of \textit{E. faecium}, \textit{E. avium}, \textit{E. gallinarum}, \textit{E. casseliflavus}, or \textit{E. raffinosus} (97).

**CONTROL AFTER EMERGENCE OF RESISTANCE**

There are recognized tensions and controversies surrounding the current recommendations to prevent and control the spread of vancomycin resistance (47). Such recommendations are not only easy to endorse than to enforce, but also require resources beyond those often available to health care delivery systems today. In addition, the recommendations are somewhat conservative, given the current gaps in our existing knowledge of enterococcal virulence—a nearly complete void with respect to \textit{E. faecium}—and the uncertainty of the eventual emergence of vancomycin-resistant \textit{S. aureus}. This threat is further substantiated by the recognition of identical transposons in enterococci and \textit{S. aureus} and our limited ability in nosocomial settings to control the spread of either VRE or MRSA (48). In preparation for the emergence of vancomycin-resistant \textit{S. aureus}, the Centers for Disease Control and Prevention has outlined an extensive plan that can be readily adopted or modified by health care institutions across the country (20).

As noted above, given the current gaps in our understanding of enterococcal virulence and the eventual emergence of VRSA, efforts are under way to prevent and control the spread of vancomycin resistance, VRE, and MRSA. Are such resource-intensive efforts realistic, given the limited research and financial allocations directed towards this goal? Numerous basic science, applied research, and epidemiological studies have together provided a framework for the Hospital Infection Control Practice Advisory Committee recommendations for prevention of the spread of vancomycin resistance genes (47). These recommendations include surveillance, applied research, prevention and control measures, and development or expansion of infrastructure. Each component is described in detail below.

**Surveillance**

In infection control, surveillance strategies can be either active or passive, depending upon the purpose and available resources. Active surveillance includes the prospective collection of specimens for baseline and follow-up evaluation of disease burden. Passive surveillance occurs in most health care settings when specimens routinely collected for clinical care can be further assessed for infection control purposes. For VRE surveillance, some hospitals routinely screen enteric \textit{Clostridium difficile} specimens (L. Mundy, P. Traynor, and D. Sahm, Abstr. 35th Ann. Meet. Infect. Dis. Soc. Am., abstr. 343, 1997). In a recent study evaluating VRE detection in stool specimens submitted for \textit{C. difficile} toxin production, there was a 19% detection rate for VRE compared to 13% for \textit{C. difficile} toxin (33).

**Applied Research**

Molecular methods can provide supportive evidence for epidemiological findings (107, 108). These tools should be carefully employed after the hypothesis for their use is well formulated. Factors to consider for the use of PCR and pulsed-field gel electrophoretic tests include introduction of a new strain(s), dissemination of vancomycin-resistant genetic elements, outbreaks due to the spread of a single clone, and confirmation of initial clonal spread followed by establishment and maintenance of an endemic state. Coordinated investigations of clinical outcomes, linked with enterococcal virulence assessment, would further clarify the current enigmas regarding the significant risks for death associated with enterococcal bacteremia (25, 34, 70).

**Prevention and Control**

In determining appropriate infection control strategies to prevent the spread of multidrug-resistant organisms, it is imperative that the distinction between outbreak and endemic control be established. The literature clearly indicates that certain interventions, and hence resource utilization, are more appropriate for one type of control than another. In addition,
the recommendations for acute care are more rigorous than those for long-term care (5, 47, 83, 103). Recommendations for ambulatory care settings and home health environments are in the early phases of development and remain controversial (13, 45). Regardless of the clinical setting, central to prevention and control strategies is the practice of hand washing. Numerous studies have evaluated health care worker behaviors and noted major and minor violations in hand-washing techniques (2, 6, 12, 37, 38, 69, 88, 91); (J. Hernandez, T. McClellan, and J. Forsyth, Abstr. Eighth Annu. Meet. Soc. Healthcare Epidemiol. Am., 1998). Perhaps most important in the prevention and control of the spread of VRE is the recognition of the role that fecal carriage has in colonization pressure (15, 61). Among the known independent risk factors for VRE acquisition are extended length of stay, higher severity-of-illness scores, colonization pressure, and prolonged antimicrobial exposure, and thus measures to reduce or enhance these risks should be incorporated into routine clinical care.

Development or Expansion of Infrastructure

Over the past two decades there has been growing recognition of the economic impact of nosocomial infections across the continuum of care, which compound the substantial costs of health care in general (116, 117). Despite the on-going development and expansion of technological advances within health care delivery systems, pressures to reduce costs focus on downsizing programs, reducing waste, and limiting resource utilization. Ideally, a core infrastructure is needed in infection control programs that are linked to microbiology, pharmacy, and an informatics system.

FUTURE DIRECTIONS

We have focused on the emergence of enterococcal virulence and antimicrobial resistance, the former of which has been best characterized in E. faecalis and the latter of which is detected most often in E. faecium. More research is needed to characterize the molecular and cellular interactions between the host and enterococci which lead to colonization and subsequent infection, the interactions between different bacterial proteins, inter- and intra-species genetic transfer, virulence factors in species beyond E. faecalis, and current infection control guidelines. In addition, clinical investigations are needed to clarify the current strategies to prevent and control the spread of vancomycin resistance, inclusive of cost-benefit or cost-effectiveness analyses that can substantiate such recommendations.

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