Could a Phosphotransferase System Provide the Means to Control Outbreaks of *Enterococcus faecium* Infection?

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(See the major article by Zhang et al on pages 1780–6.)

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The epidemiology of enterococcal infections has changed dramatically over the past 100 years [1]. Until relatively recently, the vast majority were caused by *Enterococcus faecalis* and were acquired in the community; then, during the 1970s–1980s, the percentage of nosocomial infections due to *E. faecalis* tripled [2, 3], an increase temporally associated with increasing use of third-generation cephalosporins. In the United States, an increase in the proportion of nosocomial infections caused by *Enterococcus faecium* was observed in the 1980s [4, 5], and by 2006–2007, a Centers for Disease Control survey of healthcare-associated infections found that approximately 38% of clinical enterococcal isolates identified to the species level were *E. faecium* [6]. This increase was blamed on the acquisition of vancomycin resistance by *E. faecium* (which seldom occurred in *E. faecalis)* and the increasing use of vancomycin in hospitalized patients. However, the epidemiology of vancomycin-resistant *E. faecium* (VREfm) presented a quandary: at the time, VREfm was already a common cause of US healthcare-associated infections, such organisms were not found in the community [7]. Yet, concurrently, VREfm was rare as a cause of infection in the European Union but were commonly found in feces of food animals in Europe, in products derived from these animals, in feces of individuals handling them, and even in feces of individuals in the general public [8]. These European community-associated VREfm strains were linked to use of a glycopeptide, avoparcin, in animal feeds, a practice that was not legal in the United States and has since been banned in the European Union.

What were the reasons for the rapid increase in VREfm infections in US hospitals, and how did they remain very uncommon in the European Union until considerably later, despite the large reservoir of community-based VREfm in Europe [9, 10]? It is now clear that there are many differences between *E. faecium* isolates recovered from healthcare-associated infections and outbreaks and those that are predominantly community-associated fecal commensals, such as the early vancomycin-resistant enterococci in the European Union community. First, healthcare-associated isolates usually belong to a small number of prominent clonal clusters, each composed of related multilocus sequence typing (MLST) types distinct from the diverse MLST types typically found, with a few notable exceptions, in community-associated isolates [11, 12]. Other traits noted to be common among healthcare-associated *E. faecium* but uncommon among community-associated commensals include the presence of *esp*, IS*16*, a "*hyl*"-like gene (now annotated as family 84 glycosyltransferase), and genes predicting MSCRAMM adhesins [12, 13]. A high minimum inhibitory concentration (MIC) of ampicillin is another characteristic of healthcare-associated *E. faecium* and is due to a version of PBP5 with markedly reduced affinity for penicillin [11, 14, 15]. Since higher ampicillin MICs predict higher MICs of antipseudomonal penicillins and cephalosporins, which are frequently used in hospitalized patients (some of which reach high gut concentrations), this trait should provide an important selective advantage for gastrointestinal colonization in the hospital setting. *E. faecium* strains with high-level resistance to ampicillin were first reported in the United States in the 1970s–1980s,

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including several nosocomial outbreaks, prior to the emergence of VREfm [4, 14, 16]; a similar increase in ampicillin-resistant *E. faecium* has more recently preceded the emergence of VREfm in [some European Union nosocomial outbreaks [9]. An important observation that we now know reflects fundamental differences among *E. faecium* strains was made by Hallgren et al, who found a bimodal distribution of ampicillin MICs among *E. faecium* isolates (although not among *E. faecalis*) [17]. In retrospect, we can conclude that, in the United States, it was the ampicillin-resistant *E. faecium* subgroup found in hospitals that first acquired vancomycin-resistance, while in the European Union, the community-associated subgroup with greater ampicillin susceptibility was the first to acquire vancomycin resistance. Thus, in the United States, ampicillin-resistant VREfm was likely promoted and perpetuated in hospitals by both vancomycin and β-lactam use, while ampicillin-susceptible VREfm strains common in the European Union community, albeit promoted by the use of avoparcin in animals, were much less resistant to β-lactams and, thus, not as likely to survive in the hospital milieu.

But is the increase in *E. faecium* as a cause of infection due solely to increased levels of resistance to ampicillin and vancomycin? It is now clear that the differences noted above in the accessory genome (eg, *esp, hyl*-like, and IS*16*) and in ampicillin resistance between the healthcare-associated and the community-associated *E. faecium* subgroups correlate with significant differences in the sequences of these subgroups' shared core genes. This correlation was clearly illustrated by the finding of a difference in approximately 5% of nucleotides through out the entire *pbp5* gene (not just those that cause increased ampicillin resistance) [15] and of a difference in 3%–10% of nucleotides between >100 other core genes of strains in the healthcare-associated subgroup versus the community-associated subgroup [15, 18]. Furthermore, an analysis of synonymous SNPs predicted that the divergence between the healthcare-associated and community-associated subgroups (now referred to as "clades") of *E. faecium* likely occurred hundreds of millennia ago or longer [18]. These analyses are consistent with a report of differences between "clade A" (healthcare associated) and "clade B" (community associated) strains [19]. Interestingly, many *E. faecium* strains appear to be "hybrids," with a mix of core genes from each clade or from different MLST types within the same clade [18, 20].

Given the extensive differences in the core and accessory genomes of the 2 *E. faecium* clades, how does one begin to tackle the question of which factors are responsible for the increase and predominance of the healthcare-associated clade in nosocomial infections? In this issue of the *Journal*, Van Schaik et al have approached this complex question from the point of view of the antibiotic-altered gastrointestinal tract, the site where VREfm is first established and from which most, if not all, VREfm infections are thought to originate [21, 22]. The effect of antibiotics on gastrointestinal VREfm is profound, and virtually all patients with VREfm infections are receiving, or have recently received, antibiotics. Although it is often assumed that antibiotic elimination of the other gut microflora facilitates colonization of VREfm through increased access to nutrients and adherence sites, a direct inhibitory effect of the obligate anaerobic flora on growth of VREfm has also been observed [23]. Another recently reported consequence of loss of the gram-negative microflora from the murine gut is a reduction in innate immune defenses, specifically, a reduction in RegIIIγ, a C-type lectin inhibitory to VREfm; this reduction resulted in a marked increase in the density of VREfm CFUs in the gastrointestinal tract [22, 24]. RegIIIγ was restored, and CFUs of VREfm were reduced by administration of lipopolysaccharide and flagellin derived from gram-negative bacteria [24].

But what about the organism itself? Very little is known about bacterial factors that might influence gastrointestinal colonization by *E. faecium* [13], and the study by Van Schaik et al is a valuable addition on 2 levels: it expands our current knowledge of the distinctive genetic characteristics of clinical versus commensal *E. faecium* isolates, and it shows the importance of one of the genes unique to the *E. faecium* clinical/healthcare-associated clade for murine intestinal colonization during antibiotic treatment. The investigators first compared 30 *E. faecium* genome sequences to identify genes unique to the healthcare-associated clade (clade A). From these, the authors then further analyzed a 4-gene cluster that collectively encodes a putative mannose-family phosphotransferase system. Phosphotransferase systems (PTSs) in bacteria are evolutionarily complex and quite intriguing: they function not only in carbohydrate transport, but also in influencing other bacterial processes, such as transcriptional regulation of multiple genes, including some associated with virulence and colonization [25]. To examine the role of this PTS gene cluster in intestinal colonization by *E. faecium*, the investigators constructed a deletion mutant of one of the PTS genes and then orogastrically inoculated mice depleted of their gut microbiota by antibiotics with a mixed population of the *E. faecium* wild type and its *ptsD* deletion mutant. They then collected fecal samples over 10 days while antibiotics were continually present in the drinking water. The important finding was that the *pts* mutant displayed a colonization defect, compared with the wild-type strain. This indicates that the mannose PTS of a clinical *E. faecium* strain plays a role in promoting colonization of the mouse gastrointestinal tract after antibiotic depletion of the gut flora and identifies *pts* as the first gene known to affect gastrointestinal colonization by VREfm. One caveat when interpreting these results is that inclusion of a *pts*-complemented mutant strain, to restore PTS

activity and resolve any concern about a possible unrelated mutation elsewhere, was not performed, because of the well-known plasmid vector instability when the host *E. faecium* strain is used in vivo. However, the likelihood of such an additional mutation is very low and, thus, unlikely to have influenced the results.

By identifying a specific gene that is important for gastrointestinal colonization by the healthcare-associated clade, this work represents a major advance in our knowledge relating to this important source of subsequent *E. faecium* infection. A number of additional interesting questions follow, including whether the decrease in colonization ability is due to altered carbohydrate uptake and/or use or to a regulatory effect, via interaction with non-PTS proteins, on other systems. It is still not known whether this gene cluster or other genes unique to this clade are sufficient to allow healthcare-associated *E. faecium* isolates, once they gain access to the gastrointestinal tract, to outcompete resident commensal *E. faecium*, or whether antibiotics are needed for healthcare-associated strains to achieve dominance. It is also important to acknowledge that this PTS is not present in all outbreak strains and, thus, that it surely is not the only factor that contributes to the success of the healthcare-associate clade. Whether outbreak strains of this clade that lack the mannose PTS are less able to colonize the gastrointestinal tract relative to the strain studied here, or whether they have evolved their own mechanism to enhance colonization, is another interesting question.

Can knowledge about factors that facilitate gastrointestinal colonization help decrease the VREfm problem? Perhaps. A high density of VREfm in the gastrointestinal tract is a recognized risk factor both for infection and for environmental contamination [22, 24]. It follows then that decreasing their density in the gut should help decrease acquisition of VREfm by noncolonized patients, as well as decrease VREfm infections in those who are already colonized. It is known that mannose PTS acts as a receptor of class II bacteriocins in many bacterial species [26], and it is intriguing to consider the possibility that PTS could be targeted by a bacteriocin or an engineered analog to try to decrease gut colonization by clinical *E. faecium* isolates expressing this PTS. Alternatively, it might be possible to build on the observation of λ bacteriophage–mediated targeting of mannose PTS to develop phage-derived mechanisms to control mannose PTS–positive isolates; the potential for use of phage against VREfm is supported in concept by their success in decreasing VRE in cattle compost and in salvaging moribund mice from mortality mediated by vancomycin-resistant enterococci [27–29]. Moreover, targeting a property unique to the troublesome healthcare-associated clade offers the attractive possibility of sparing true commensal strains, which very rarely cause infection, preserving this component of the normal gastrointestinal microflora. Thus, the new information provided by this important work not only adds to our knowledge of the differences between the 2 *E. faecium* clades and of factors involved in successful gastrointestinal colonization, but also opens up new avenues for future pursuits that may help generate strategies or applications for the control of this important cause of healthcare-associated infections.

Notes

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