High occurrence of esp among ampicillin-resistant and vancomycin-susceptible Enterococcus faecium clones from hospitalized patients

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The ability to colonize patients successfully may be essential for the emergence and spread of resistant nosocomial strains. We determined the presence of Esp, a surface protein involved in colonization ability in Enterococcus faecalis, in 96 Enterococcus faecium isolates from hospitalized patients (77 PFGE clones), 33 faecal isolates from healthy volunteers (32 clones) and 20 environmental isolates (20 clones). Esp was found significantly more often in E. faecium isolated from hospitalized patients than in isolates from the community setting (26% versus 6%, P < 0.01) and was significantly more common among ampicillin-resistant than among ampicillin-susceptible strains (37% versus 4%, P < 0.001), regardless of the isolation site. The frequency of the esp gene in the hospital clearly correlates with antibiotic-resistant E. faecium clones. This observation indicates that antibiotic-resistant variants may frequently arise under antibiotic selective pressure among esp-positive clones reaching ecological abundance in the nosocomial habitat.

Keywords: esp, Enterococcus faecium, ampicillin

Introduction

Nosocomial infections caused by Enterococcus faecium have been reported increasingly worldwide and have frequently been associated with vancomycin resistance. Willems et al.1 have described the presence of specific vancomycin-resistant E. faecium (VREF) genogroups among hospitalized patients from different geographical areas (UK, USA, Australia). Moreover, widely disseminated ampicillin-resistant (AmpR) E. faecium clones have been reported as the source for vancomycin resistance in hospitals where occurrence of VREF was low, which has led to the emergence of extended VREF outbreaks.2 Consequently, success in colonization ability in the nosocomial habitat may be an essential risk factor for the emergence and spread of resistant strains. However, unlike Staphylococcus aureus, little is known about the link between ecological abundance and virulence of this species.3

An enterococcal surface protein, Esp, has recently been identified as a marker of highly prevalent VREF clones among hospitalized patients.1 This protein contributes to colonization and persistence of Enterococcus faecalis in the urinary tract and the ability of this species to produce biofilms.4,5 Several recent European studies have also demonstrated the presence of the esp gene in vancomycin-sensitive E. faecium (VSEF) frequently found in the hospital environment and recovered from urine samples.1,6,7 However, few data are available on the prevalence of esp-positive isolates in bacteremia, or among VSEF strains.1,6,7 Moreover, data about the prevalence of esp in clones colonizing healthy people are still needed. The aim of this work was to provide such data and to investigate whether specific E. faecium subpopulations carrying a variant of the E. faecalis esp gene are associated with disease or epidemicity in this species in Spain, where the prevalence of VREF in hospitals remains very low (<3%).

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Materials and methods

Bacterial isolates

We screened 149 E. faecium isolates comprising 65 blood isolates (from 63 patients) recovered in our hospital between 1995 and 2000 (two were kindly provided by Dr Liñares from Hospital Bellvitge, Barcelona); 31 non-blood isolates from consecutive patients (11 isolated from organic fluids, 10 from soft-tissue infections, nine from urine and one from the respiratory tract) collected between 1999 and 2000; 33 faecal isolates from 33 healthy volunteers (HV) living in the same community and without exposure to a hospital environment or antibiotics in the previous 6 months, which were recovered in 2000, and 20 from environmental samples collected in the same area.

Species identification and antibiotic susceptibility

Presumptive species identification was performed by the semi-automated WIDER system (Fco. Soria Melguizo, Madrid, Spain) for clinical isolates, or by standard biochemical procedures for faecal isolates.8 Confirmation of species identification was performed by PCR using oligonucleotide primer sets to amplify genes coding for EfaA (derived from E. faecalis) and aminoglycoside 6′-N-acetyltransferase [AAC(6′)-Ii] (derived from E. faecium).8 Antimicrobial susceptibility was determined by the agar dilution method according to the 2000 NCCLS guidelines.

PFGE and analysis of the banding patterns

Chromosomal DNA was prepared as described previously.8 The SmaI-digested genomic DNA banding patterns were analysed by visual examination by two independent investigators. Following the standard criteria given by Tenover et al.9 to establish clonal relationships, isolates were considered to be related if they exhibited differences of up to six bands (if there was good epidemiological evidence to suggest the relatedness among isolates or if they had been isolated over extended periods of time).

Esp detection

The presence of esp was detected by PCR using primers to amplify an 800 bp gene sequence coding for esp from E. faecium (GenBank Accession number AF443999): 5′-GGAACGCCTTGGATG-3′ and 5′-CCGCTTTTGATGCCATT-3′. E. faecium strain E-774 was used as a positive control. Isolates positive for the esp gene were considered to express Esp on their surface.1

Results and discussion

Ninety-six clinical isolates were classified in 77 clonal types on the basis of PFGE, and the 33 faecal isolates from HV were considered as 32 clones. Resistance to vancomycin was detected in eight out of the 129 isolates from humans (6.2%): three from blood, two from urine and organic fluid and three from faeces of healthy volunteers, which were classified in seven clones. Resistance to ampicillin (Amp) was observed in 65/129 isolates (50%), which were considered to be 46 clonal types (Table 1). There were 38 blood isolates and 27 from other sites. Isolates from environmental samples were susceptible to Amp and vancomycin (Van).

The esp gene was detected in 27 (21%) of the 129 human isolates studied corresponding to 19/109 clonal types, and was more frequently found among clinical than among faecal isolates from HV (26% versus 6%, P < 0.01). esp was identified in 10/31 clones from clinical non-blood isolates (32%), in 7/46 clonal types recovered from blood (15%) and in only 2/32 clones in the HV group (6%). It should be noted that these differences are more attributable to the different rates of Amp resistance of isolates from those three sites of isolation than to the site of isolation itself (Table 1). Indeed esp was found much more frequently among AmpR clones than among ampicillin-susceptible strains (AmpS) (37% versus...
**esp occurrence in E. faecium clones**

**Figure 1.** Antibiotic resistance among *esp*-positive and *esp*-negative *E. faecium* clones from hospitalized patients and healthy volunteers in Spain. The y-axis represents the percentage of clones resistant to different antibiotics, and the x-axis represents resistance to the antibiotics mentioned. Amp, ampicillin; Cip, ciprofloxacin; Erm, erythromycin; HLRSm, high-level resistance to streptomycin; HLRGm, high-level resistance to gentamicin; Van, vancomycin.

4%, *P < 0.001*). Moreover, *esp*-positive *E. faecium* clones resistant to ampicillin (Amp<sup>r</sup>), erythromycin (Erm<sup>r</sup>) or ciprofloxacin (Cip<sup>r</sup>) were more prevalent than *esp*-negative Amp<sup>r</sup>, Erm<sup>r</sup> or Cip<sup>r</sup> *E. faecium* clones (*P < 0.001; Figure 1). All Amp<sup>r</sup> *E. faecium* clones containing *esp* were also resistant to at least three more antibiotics. Two VREF blood isolates (two different clones) from two patients were positive for *esp*. Interestingly, *esp*-positive VSEF isolates corresponding to the same clonal types as those of *esp*-positive VREF were recovered from both patients. Nevertheless, we should be aware that the size of the sample studied is small and additional samples would stress the conclusions reached.

Our data confirm that *esp* is frequently found in *E. faecium* strains from the hospital setting, although the occurrence of *esp*-positive isolates (21%) was lower than that reported by Woodford et al. for both VREF and VSEF nosocomial isolates from different UK hospitals (61% and 64%, respectively). This could be due to regional differences or over-representation of a number of clonal types in the English study and, since we focused on strains of unique chromosomal lineage, our data indicate that the trait has not deeply penetrated the species in our area. Regarding the origin of the *esp*-positive isolates, Woodford et al. found a significant relationship between *esp* and *E. faecium* urine isolates (23/27 from different hospitals), thus confirming previously published data that showed a role of Esp in the colonization and persistence of *E. faecalis* in the urinary tract. The occurrence of *esp* among the urine samples we studied was lower (3/9 clones, 33%), but the low number of strains studied does not permit us to reach any final conclusion. It is interesting to point out that occurrences of *esp* in *E. faecium* clones causing bloodstream infections or collected from healthy volunteers (15% and 6%, respectively) are lower than those reported in a recent study of *E. faecalis* strains from The Netherlands (45% and 40%), which could reflect either species differences or, again, regional differences in the epidemiology of enterococci.

The presence of *esp* in isolates susceptible and resistant to different antibiotics indicates that this trait probably emerged prior to the acquisition of resistance not only to Van<sup>6,7</sup> but also to other antibiotics commonly used in the hospital setting (this study). The significant association between resistance to Amp, Erm or Cip and *esp* in the *E. faecium* isolates studied suggests that antibiotic treatment selects particular clones among those that have reached ecological abundance in the nosocomial habitat due to the presence of *esp*. This may explain why in some hospitals, strains containing *esp* were frequent among VRE strains. The higher occurrence in our study of this trait in Amp<sup>r</sup> nosocomial hospital-associated versus community isolates supports this hypothesis. Willems et al. have shown the existence of *E. faecium* and *E. faecalis* ecovars (genogroups associated with particular hosts and environments) and have suggested the existence of a specific VREF genogroup possibly adapted to nosocomial transmission by the presence of *esp*.

In summary, our results show that *esp* clones are most prevalent among *E. faecium* isolates from hospitalized patients, and only rarely occur in the community setting. The frequency of the *esp* gene in hospitals correlates with the occurrence of antibiotic-resistant *E. faecium* clones. This observation suggests that antibiotic-resistant variants may arise frequently under antibiotic selective pressure among *esp*-positive clones reaching ecological abundance in the nosocomial habitat.

**References**


