NOTES

Bloodstream Isolates of *Enterococcus faecium* Enriched with the Enterococcal Surface Protein Gene, *esp*, Show Increased Adhesion to Eukaryotic Cells

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Received 21 April 2003/Returned for modification 24 June 2003/Accepted 22 August 2003

Infection-derived *Enterococcus faecium* strains enriched with *esp* had increased ability to adhere to Caco-2 cells ($P < 0.05$) and were less genetically diverse than *esp*-negative isolates. *esp*-negative *E. faecium* fecal isolates from healthy individuals adhered significantly better than *esp*-negative infection isolates ($P < 0.05$), indicating additional factors of importance to adhesion.

The enterococcal surface protein, Esp, was first described for *Enterococcus faecalis* (18) but was later detected in *Enterococcus faecium* of infectious origin (2, 6, 24; A. M. Hammerum and L. B. Jensen, Letter, J. Clin. Microbiol. **40**:4396, 2002; N. Woodford, M. Soltani, and K. J. Hardy, Letter, Lancet **358**: 584, 2001). In isolates of nonhuman origin, the *esp* gene has mostly been found in *E. faecalis* (6; Hammerum and Jensen, letter; Woodford et al., letter) but also sporadically in *E. faecium* (2, 8). Esp is believed to have a role in immune evasion (18) and biofilm formation (21). In a study by Shankar et al., Esp was found to promote maintenance and colonization of *E. faecalis* in acute urinary tract infection, thus suggesting an adhesive role for this protein (17). Later a DNA sequence analysis of a variant enterococcal surface protein, Esp_{fm}, in *E. faecium* revealed similarities with several other adhesion proteins in other gram-positive bacteria, further supporting the hypothesis that Esp contributes to adherence (7). Considering the oral-fecal route of infection, adherence to intestinal mucosal cells might be the first step in enterococcal infection.

The aim of the present study was to determine the presence of the *esp* gene by PCR in *E. faecium* isolates from different origins, i.e., bacteremia patients, fecal microflora of healthy individuals, and two probiotic products, and to investigate the in vitro adhesion of these strains to epithelial cells.

Thirty *E. faecium* isolates from a culture collection of isolates from different bacteremia patients obtained during the period 2000 to 2002 at the Clinical Bacteriology Laboratory, Huddinge University Hospital, Stockholm, Sweden, were included. Thirty fecal *E. faecium* strains collected pretreatment from 30 healthy donors participating in clinical trials conducted in 1998 (13, 16) and two *E. faecium* strains from probiotic products, the SF68 strain (VentruX, AB Cernelle, An-

gelholm, Sweden) and the Causido-derived *E. faecium* strain (Gaio; MD Foods, Aarhus, Denmark), were also included. All blood culture isolates were genotyped with pulsed-field gel electrophoresis (4, 11). To avoid repeated analyses of nosocomial clones, only one isolate of each unique banding pattern was included. A dendrogram was created as described elsewhere (12). The susceptibility to ampicillin, vancomycin, and gentamicin was investigated by the disk diffusion method (15). The presence of the *esp* gene was detected by PCR (18). A negative control, *E. faecalis* ATCC 29212, a positive control, *E. faecalis* MMH594 (10), and a contamination control were included in each set of PCR. Caco-2 human colon cancer cells (ATCC HTB-37) were used in the adhesion assay, performed as described by Hagggar et al. (9). The number of adhered enterococci was quantified by determination of viable counts and calculated as the mean result of at least duplicate assays. Differences in the occurrence of the *esp* gene between blood and normal microflora isolates were analyzed with the chi-square test. Differences in the number of adhered bacteria between the groups were calculated with the Mann-Whitney U test for unpaired observations.

The genotyping illustrated that *esp*-positive strains were less genetically diverse than *esp*-negative strains (data not shown). Eleven of the *esp*-positive strains could be divided into four groups, with a similarity of 80% or more within the groups. The sole vancomycin-resistant isolate showed a distant genetic relationship (57%) to the other *esp*-positive strains.

Twenty-five of the 30 blood isolates were resistant to ampicillin, 1 was resistant to vancomycin, and 2 were resistant to gentamicin, while none of the intestinal or probiotic strains expressed any resistance. The *esp* gene was detected in 16 of 30 (53.3%) of the blood isolates (64% of the ampicillin-resistant blood isolates [16 of 25]) and in 2 of 30 (6.7%) of the normal intestinal microflora isolates ($P < 0.0001$). All five ampicillin-susceptible blood isolates, 36% of the ampicillin-resistant blood isolates (9 of 25), and both probiotic strains were *esp* negative.

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FIG. 1. Chart showing the median numbers of adhered *E. faecium* per well after 2 h of incubation with Caco-2 cell cultures. *n*, number of strains in each group. Error bars indicate the 25th and 75th percentiles.

The median value of adhered bacteria was highest for the *esp*-positive blood isolates (Fig. 1). There was no difference in adhesion between the groups of isolates with respect to origin, i.e., all derived from blood compared to all derived from feces. However, among the blood culture isolates, adherence was significantly greater when *esp* was present than when it was absent ($P < 0.05$). The *esp*-negative normal microflora isolates adhered significantly better than the *esp*-negative blood isolates ($P < 0.05$). The difference in adherence between the minimum binders and the maximum binders was large in both population groups, ranging from less than 1 million to more than 10 million bacteria/well. The probiotic strain SF68 adhered 5.5 times better than the Causido-derived strain.

The enrichment of *esp* in *E. faecium* infectious isolates and the low occurrence in normal microflora isolates found in the present investigation correlate well with findings in other studies, which reported frequencies ranging from 26 (3) to 78% (6) for medical isolates and 6% for isolates from feces of healthy volunteers (3). The *esp*-positive strains could be divided in four groups with internal relatively high genotypic relationship. This fact indicates that the *esp*-positive strains within a group are related and may derive from a common ancestor and indirectly suggests that the presence of the *esp* gene gives these isolates an advantage for survival and dissemination in the hospital environment compared to the *esp*-negative isolates.

A majority of the ampicillin-resistant isolates were found to be *esp* positive, while all ampicillin-susceptible infectious strains lacked *esp*; similar results have previously been described (3). This is not unexpected since both ampicillin resistance and the occurrence of *Esp* have previously been separately correlated with infectious isolates. These characteristics are therefore not necessarily related to each other. In Sweden vancomycin-resistant enterococci are still rare (20, 23), and few outbreaks have been reported (14, 22); this is consistent with the low genotypic relationship between the vancomycin-resistant strain and the other *esp*-positive isolates.

The increased adherence to Caco-2 cells in blood isolates with *esp* compared to those without suggests that *esp* contributes to adherence to gut mucosal cells. The advantage of *esp* is

probably related to the infection situation, since *esp* is not selected for in fecal isolates. There might be other adhesion factors of importance to gut mucosal binding in enterococci besides *esp*, and these may be down-regulated when the bacteria enter the bloodstream. This hypothesis is supported by the observation that the *esp*-negative normal microflora isolates adhered significantly better to Caco-2 cells than the *esp*-negative blood isolates. In another study regarding *E. faecalis*, adherence to Int-407 and Giardia heart cell lines was not associated with the presence of *Esp* (1). Possession of *esp* might affect adhesion differently in different surroundings due to environmentally induced variations of regulation. In a study regarding virulence modulation in *E. faecalis* in different environments, a 10-fold increase in *esp* expression was found during log phase when bacteria were cultured in serum compared to that when they were cultured in laboratory medium (19). In a recent study by Dupré et al. (5), where a staining method was used for assaying adherence, no relation between *esp* and adhesion to Caco-2 cells was found.

In conclusion, infection-derived *E. faecium* isolates were enriched with *esp*. These *esp*-positive strains had greater ability to adhere to Caco-2 cells in vitro and were less genetically diverse than *esp*-negative blood isolates. Furthermore, adhesion of *esp*-negative *E. faecium* isolates to Caco-2 cells was significantly higher in isolates derived from feces than in those derived from blood, suggesting additional factors of importance for adhesion to gut mucosa.

This work was supported by grants from the Scandinavian Society for Antimicrobial Chemotherapy.

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