Prevalence of the fsr Locus in Enterococcus faecalis Infections

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The fsr locus of Enterococcus faecalis confers virulence in animal models. A retrospective analysis of fsr prevalence in diverse Enterococcus faecalis clinical isolates demonstrated fsr in all endocarditis isolates versus 53% of stool isolates (P = 0.005). This supports a role for fsr-mediated virulence in the pathogenesis of enterococcal infections in humans.

In contrast to their role as normal commensals of the gastrointestinal and female genital tracts, enterococci have emerged as important pathogens that cause endocarditis and nosocomial infections (12). While the search for virulence factors that might account for the dichotomy between their roles as normal flora and pathogens has implicated certain phenotypic markers, including hemolysins, aggregation factors, and gelatinase, to date there is no unifying paradigm (2, 4, 16).

Reportedly present in approximately 70% of Enterococcus faecalis clinical isolates, the recently described fsr locus is comprised of three genes (fsrA, fsrB, and fsrC) and has been shown to regulate the transcription of at least two genes: those for a gelatinase (gelE) and serine protease (sprE). Moreover, suggestive of a role for fsr in bacterial virulence, E. faecalis strains that contain fsr gene knockouts have diminished virulence in a mouse peritonitis model (16). Similarly, in a Caenorhabditis elegans model developed for identifying gram-positive virulence factors, fsrB-deficient mutants displayed diminished nematode killing (7).

Homology between the fsr locus and the accessory gene regulator (agr) locus of staphylococci has been noted (16). The agr locus encodes a two-component regulatory system that controls synthesis of virulence factors in a cell density-dependent manner (1, 9–11). Furthermore, it has been demonstrated that a secreted peptide, encoded by the terminal portion of fsrB, is the autoinducer of the regulatory system and is similar in structure to the autoinducer protein of agr (13). Collectively, the above data suggest that the function of fsr in E. faecalis may be analogous to that of agr in Staphylococcus aureus; namely, that of a global regulator of virulence factors. Therefore, this study was undertaken to examine whether the presence of the fsr locus correlates with the virulence of E. faecalis clinical isolates, as reflected by a higher prevalence of fsr in isolates derived from a pathogenic setting (i.e., endocarditis) compared with those from a commensal milieu (i.e., from stool).

In order to assess for these and potential historical variations in fsr prevalence, 31 clinical isolates of E. faecalis collected in our laboratory over the past 30 years were studied. Twelve isolates were from patients catalogued as having had endocarditis. Nineteen fecal isolates were studied; eight isolates were obtained in the 1960s from antibiotic-inexperienced subjects from the Solomon Islands (6, 8). The remaining eleven fecal isolates were obtained from collections generated from previously reported studies of hospitalized patients in the United States (5, 17).

Single colonies were inoculated in 3 ml of brain heart infusion broth (Becton Dickinson, Sparks, Md.) and incubated overnight with shaking at 37°C. Extraction of genomic DNA with guanidinium thiocyanate was performed as previously described (14). Primers that amplified the entire fsr locus were designed from previously published data and GenBank (accession no. D85393). The primer nucleotide sequences were 5′-AACCAGATCGACCAATGAAT-3′ (upstream primer) (15) and 5′-GCCCTCATAACTCAATACC-3′ (downstream primer, corresponding to bases 18 to 37 in GenBank accession no. D85393). The PCR conditions consisted of 5 min of lysis and denaturation at 95°C, 30 cycles of denaturing, annealing, and extension at 94°C (30 s), 60°C (30 s), and 72°C (2.5 min), respectively; and a 10-min final extension step at 72°C. PCR product was separated by 1% agarose gel electrophoresis, gel extracted and purified (kit provided by Qiagen, Valencia, Calif.), labeled with digoxigenin DNA labeling mix (Roche Diagnostics, Indianapolis, Ind.), and subsequently used for dot blot analyses. Two-microliter samples of genomic DNA were denatured by boiling for 10 min and then bound to a nylon membrane (Osmonics, Westborough, Mass.) by baking for 2 h in a vacuum oven at 80°C. The membrane was prehybridized overnight and then hybridized overnight with the fsr probe. Immunological detection was then performed (anti-digoxigenin-AP Fab fragments, blocking reagents, and the NBT and BCIP colorizing reagents provided by Roche Diagnostics). Statistical analysis was done by two-tailed Fisher’s exact test.

The fsr dot blot analyses were positive for all 12 (100%) isolates from enterococcal endocarditis patients, compared to 10 of 19 fecal isolates (53%). There was a statistically significant difference between the prevalence of the fsr locus in the endocarditis versus the fecal isolates (P = 0.005). No significant correlation was found between the presence of the fsr locus and the temporal origin of the clinical isolates (P = 1.0 for fsr prevalence in study isolates from prior to 1975 versus those from after 1975). Endocarditis isolates were collected...
from 1971 to 2001. Three isolates were from 1971 to 1973, while the remainder were from 1986 to 2001. Similarly, fecal isolates were collected from the 1960s to the present. Four of eight isolates from the antibiotic-naïve Solomon Island population from the 1960s probed positive for fsr. Of the eleven contemporary hospitalized patient isolates, six were fsr positive.

Efforts have been made to correlate E. faecalis phenotypic markers and virulence. The recent identification of the fsr locus, the finding of diminished virulence with fsr knockouts in an animal model, the homology of fsr with staphylococcal agr, and the recent descriptions of agr as a regulator of virulence factors, and possibly possessing a global regulatory function (3, 9, 15, 16), implicate fsr as possibly providing an analogous role in E. faecalis. Qin et al. (16) noted that 69% of their clinical isolates were fsrB positive. All endocarditis-derived isolates in our study were positive, compared to only 53% of stool-derived isolates. This represents a statistically significant association between the presence of the fsr locus and the derivation of isolates from clinical infection, compared to derivation from intestinal colonization. Isolates positive for fsr therefore may have an advantage in establishing infection by the use of a regulatory system to up-regulate the production of virulence factors.

Prospective studies that determine the prevalence of fsr among clinical isolates of E. faecalis (e.g., from native and prosthetic valve endocarditis, central venous catheter infections, wound infections, and urinary tract infections) versus colonizing isolates would be helpful in further defining the association of fsr and clinically relevant virulence. Also, ascertaining the presence or absence of fsr may be a useful tool in discriminating between true infection and colonization and thereby decrease the unnecessary use of antibiotics. Finally, transcriptional profiling may help determine whether fsr has a more global metabolic regulatory function, similar to that recently described for agr (3, 18). Although enterococci are generally viewed as bacteria with relatively low pathogenic potential, those with the fsr locus may represent a virulent subpopulation capable of causing invasive disease in humans.

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REFERENCES