Research Article

Virulence Genes among Enterococcus faecalis and Enterococcus faecium Isolated from Coastal Beaches and Human and Nonhuman Sources in Southern California and Puerto Rico

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Most Enterococcus faecalis and E. faecium are harmless to humans; however, strains harboring virulence genes, including esp, gelE, cylA, asa1, and hyl, have been associated with human infections. E. faecalis and E. faecium are present in beach waters worldwide, yet little is known about their virulence potential. Here, multiplex PCR was used to compare the distribution of virulence genes among E. faecalis and E. faecium isolated from beaches in Southern California and Puerto Rico to isolates from potential sources including humans, animals, birds, and plants. All five virulence genes were found in E. faecalis and E. faecium from beach water, mostly among E. faecalis. gelE was the most common among isolates from all source types. There was a lower incidence of asa1, esp, cylA, and hyl genes among isolates from beach water, sewage, seaport, urban runoff, sea wrack, and eelgrass as compared to human isolates, indicating that virulent strains of E. faecalis and E. faecium may not be widely disseminated at beaches. A higher frequency of asa1 and esp among E. faecalis from dogs and of asa1 among birds (mostly seagull) suggests that further studies on the distribution and virulence potential of strains carrying these genes may be warranted.

1. Introduction

Enterococcus faecalis and Enterococcus faecium are commonly found in the intestinal tracts of humans and animals and also ubiquitous in the environment [1]. While generally considered to be harmless, certain strains of E. faecalis and E. faecium are among the leading causes of nosocomial infections including urinary tract infections, abdominal and wound infections, endocarditis, and bacteremia [2–5]. E. faecalis and E. faecium isolated from patients in hospital settings have been shown to harbor a higher frequency of gelE (gelatinase), asa1 (aggregation substance), esp (enterococcal surface protein), cylA (cytolysin activator), and hyl (glycoside-hydrolase) as compared to strains found in nonhospitalized individuals, animals, and food [6–12]. Commensal, that is, harmless, E. faecalis and E. faecium can become opportunistic pathogens by acquiring antibiotic resistant and putative virulent genes from other bacteria via horizontal gene transfer [2, 4, 13–17].

E. faecalis and E. faecium are among the most common species of enterococci found in the beach environment [18–21]. Enterococci found in the beach environment can include naturalized populations existing in soil and vegetation as well as strains from humans, sewage, animals, birds, reptiles, and insects [1]. Presumably, potentially pathogenic E. faecalis and E. faecium in human fecal waste would harbor higher numbers of virulence genes as compared to strains from animal and environmental sources.
In Puerto Rico, beaches receive storm flows containing contaminated septage and agricultural runoff potentially carrying enterococci derived from human and animal fecal waste. In Southern California, urban runoff, beach sand, and sea wrack (macroalgae on beach sand) have been identified as important sources of enterococci to beach water [22–25].

Previous studies showed that *E. faecalis* and *E. faecium* from the beach water and sand harbor antibiotic resistant genes suggesting a potential health risk for beach goers [26–30]; however, the frequency of other virulence factors was not determined. Here, we compared the frequency of putative enterococcal virulence genes (*esp*, *gelE*, *cylA*, *asa1*, and *hyl*) among *E. faecalis* and *E. faecium* from beaches in Southern California and Puerto Rico impacted by different enterococci source inputs to assess beaches as an environmental reservoir of potentially virulent enterococci.

### 2. Materials and Methods

#### 2.1. Sources of *E. faecalis* and *E. faecium* Isolates

**2.1.1. Southern California.** A total of 170 *Enterococcus* (91 *E. faecalis* and 79 *E. faecium*) isolates were screened for putative enterococcal virulence genes (*esp*, *gelE*, *cylA*, *asa1*, and *hyl*) among *E. faecalis* and *E. faecium* from beaches in Southern California and Puerto Rico impacted by different enterococci source inputs to assess beaches as an environmental reservoir of potentially virulent enterococci.

#### 2.1.2. Puerto Rico.** A total of 247 *Enterococcus* (174 *E. faecalis* and 73 *E. faecium*) isolates from Puerto Rico were analyzed (Table 2). Enterococcal isolates from beach water were obtained from two beaches in Puerto Rico. Human (nonclinical) enterococcal strains were isolated from fresh fecal samples from nine healthy individuals from Mayaguez, Puerto Rico. Clinical enterococcal strains were isolated from urine specimens and identified to species level by a local hospital in Mayaguez. Six septage samples were obtained from individual houses or from septic tank trucks after emptying individual family tanks.

#### 2.2. Isolation and Identification of Enterococci

**2.2.1. Southern California.** Enterococcal isolates from all samples (except for clinical specimens) were obtained using mEI agar and identified to species level using the Vitek II (bioMérieux) plus additional biochemical tests and pigment and motility as per Ferguson et al. [32]. Clinical strains were isolated by OCPHL using TSA with 5% sheep’s blood; presumptive enterococcal colonies were gram-stained and identified using MicroScan (Siemens Healthcare) and/or API Strep 20 (bioMérieux). Up to 3 isolates per sample identified as *E. faecalis* and *E. faecium* were randomly selected for

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**Table 1: Sources of *E. faecalis* and *E. faecium* isolated from Southern California.**

| Source | Number of samples | Number of sites | Number of isolates | Total number of isolates |
|--------|------------------|----------------|-------------------|-------------------------|
| Environmental |                 |                |                   |                         |
| Beach water | 5               | 5              | 8                 | 10                      | 18                       |
| Urban runoff | 10              | 5              | 20                | 8                       | 28                       |
| Sand | 5              | 5              | 0                 | 5                       | 5                       |
| Sea wrack | 5              | 1              | 5                 | 5                       | 10                       |
| Eelgrass | 7              | 1              | 5                 | 3                       | 8                       |
| Wastewater influent | 4              | 4              | 6                 | 3                       | 9                       |
| Wastewater effluent | 5           | 2              | 6                 | 11                      | 17                       |
| Human |                 |                |                   |                         |
| Human, healthy | 18             | NA             | 15                | 8                       | 23                       |
| Human, clinical | 10           | Unk            | 10                | 5                       | 15                       |
| Vancomycin resistant enterococci | Unk | Unk | 0 | 10 | 10 |
| Animal |                 |                |                   |                         |
| Dogs | 7               | 7              | 7                 | 3                       | 10                       |
| Birds | 16              | 2              | 9                 | 8                       | 17                       |
| Total | 92              | 91             | 79                | 170                     |

NA = not applicable.
Unk = unknown.
virulence gene analysis. Species identification of 8 different isolates obtained using biochemical methods was also confirmed by 16S rRNA sequencing conducted at GenoSeq, University of California, Los Angeles.

2.2.2. Puerto Rico. Enterococcal isolates from all samples (except for clinical specimens) were obtained using mE agar. All isolates were divided into four groups based on pigmentation and motility. The isolates were identified to the genus level based on growth in BHI with 6.5% NaCl, growth pigmentation and motility. The isolates were identified to the species level based on growth in BHI with 6.5% NaCl, growth pigmentation and motility. The isolates were identified to the species level based on growth in BHI with 6.5% NaCl, growth pigmentation and motility.

Clinical strains obtained from Puerto Rico were analyzed for enterococcal virulence genes gelE, asal, esp, cylA, and hyl by PCR analysis. The PCR products were visualized using the FlashGel system. 2 μL of extracted DNA was diluted in 2 μL FlashGel loading dye and inserted into 12 + 1-cassette wells. A 50 bp–1.5 kb DNA ladder (Lonza) was used as a molecular size marker. FlashGels were run at 150 V for up to 13 minutes. Each PCR run included a no-template control; the positive control strain used for gelE, esp, asal, and cylA was E. faecalis MMH594 kindly donated by N. Shankar, Department of Medicinal Chemistry and Pharmaceutics, University of Oklahoma Health Sciences Center, Oklahoma City [14].

3. Results

A total of 170 E. faecalis and E. faecium isolates from Southern California (SC) and 247 isolates from Puerto Rico (PR) from beach water and potential sources of enterococci to beaches were analyzed for enterococcal virulence genes gelE, asal, esp, cylA, and hyl.

Eighty-seven (80.6%) E. faecalis isolates from PR beach water harbored one or more of the following genes: gelE (98.1%), asal (44.4%), esp (11.1%), and cylA (3.3%) (Table 3). The frequency of enterococcal virulence genes differed between E. faecalis and E. faecium. Virulence genes were absent among the majority of E. faecalis beach water isolates from SC and PR. esp was the most common virulence gene found among E. faecium from humans (12.5% to 83.3%). Ten clinical isolates

| Source          | Number of samples | Number of sites | Number of isolates | Total number of isolates |
|-----------------|-------------------|----------------|--------------------|--------------------------|
| Environmental   |                   |                |                    |                          |
| Beach water     | 9                 | 2              | 108                | 140                      |
| Septage         | 6                 | 6              | 19                 | 45                       |
| Human           |                   |                |                    |                          |
| Human, healthy  | 9                 | 1              | 5                  | 9                        |
| Human, clinical | 53                | 1              | 42                | 53                       |
| Total           | 77                |                | 174                | 247                      |

*Table 2: Sources of E. faecalis and E. faecium isolated from Puerto Rico.*
E. faecium isolates from Southern California (SC) that were identified as vancomycin resistant strains by OCPHL were positive for the esp (80%) and hyl (10%) genes. In SC, none of the 5 virulence genes were detected among E. faecium isolates obtained from dog stools, wrack, and beach sand. gelE was the only virulence gene found among E. faecium from eelgrass.

Of the five virulence genes overall (Figure 1), gelE was the most abundant virulence gene found among E. faecalis isolates from human, animal, and environmental sources (59.6% to 95%), followed by asaI (15.4% to 78.4%). cylA was found among 19.0% to 41.7% of E. faecalis human isolates and 4.9% to 19.0% environmental isolates and not detected in animal isolates.

esp was the most commonly found virulence gene detected among E. faecium isolates (0% to 47.9%), followed by gelE (0% to 18.8%), asaI (0% to 12.5%), and hyl (0% to 1.3%) (Figure 2). At both study sites, human derived E. faecium isolates had the highest frequency of esp (36.4% to 47.9%).

4. Discussion

E. faecalis and E. faecium obtained from multiple sources, including the beach environment, humans (clinical and nonclinical), animals, and birds in Southern California and Puerto Rico, harbored putative enterococcal virulence genes...
that differed in frequency depending on source. At both study locations, there was a higher prevalence of virulence genes among \textit{E. faecalis} as compared to \textit{E. faecium}. Among both species groups, virulence genes were less abundant among beach strains overall compared to human isolates, which was also consistent with a similar study conducted in Australia [35].

Enterococcal virulence genes \textit{asa1} (aggregation substance) and \textit{cylA} (cytolysin activator) were found among \textit{E. faecalis} isolates from beach water, humans, dogs, and birds, indicative of strains with enhanced virulence potential. \textit{asa1} and \textit{cylA} were first identified in the genome of multidrug resistant \textit{E. faecalis} strain MMH594 and have also been associated with \textit{E. faecalis} pathogenicity islands [16, 36]. Aggregation substance is encoded on a sex pheromone plasmid and mediates aggregation between bacteria, enabling the transfer of plasmids [37]. Cytolysins are toxins secreted by bacteria that damage cell membranes, facilitating the infection process. \textit{cylA} can be carried on a plasmid or occur on the bacterial chromosome [38].

The distribution of \textit{asa1} and \textit{cylA} among \textit{E. faecalis} from human clinical specimens was 90% and 70%, respectively, of \textit{E. faecalis} from SC as compared to 50% and 19%, respectively, of isolates from PR. These differences likely reflect variability in the types of clinical specimens analyzed from each study location; clinical isolates of \textit{E. faecalis} from SC were obtained from rectal swabs, urine, blood, abscess, ascites, vagina, and joints; those from PR were obtained primarily from urine specimens.

\textit{asa1} and \textit{esp} were also found among \textit{E. faecalis} strains in dogs and birds (mostly seagull), suggesting that they may be important reservoirs of strains that could potentially be transferred to humans. \textit{esp} is thought to aid enterococci in evading the immune system and also form biofilm [36, 39], which facilitates colonization of \textit{E. faecalis} in acute urinary tract infections [14]. Animals and birds have been suggested as potential sources of virulent strains to humans; \textit{gelE}, \textit{asa1}, \textit{esp}, and \textit{cylA} were detected in fecal \textit{E. faecalis} isolated from dogs at veterinary hospitals [40, 41], poultry [42], and ducks and wild geese [43]. The presence of these genes among \textit{E. faecalis} strains from dogs and birds warrants further studies to assess potential human health risks.

Among the virulence genes analyzed, \textit{gelE} was the most frequently detected and widely distributed among \textit{E. faecalis} strains from multiple sources, including the environment which is consistent with previous studies [9, 44, 45]. \textit{gelE} is thought to enhance survivability of enterococci in extraintestinal environments [46].

In the beach environment, \textit{E. faecium} was rare among enterococci identified from eelgrass, sewage influent, and dog samples, thus limiting the number of isolates that could be analyzed for virulence genes. \textit{E. faecium} and \textit{E. faecalis} were also rare or not detected in composite fecal samples from horses, goats, and pigs from PR, which is consistent with studies showing the low prevalence of these species in livestock [43, 47, 48]. Birds were rarely observed at the study beaches in PR, which precluded efforts to obtain enterococci isolates.

It is important to note that the presence of virulent strains among \textit{E. faecalis} and \textit{E. faecium} alone is not predictive of infection as there may be other mediators of pathogenicity that have yet to be elucidated [49]. It has been suggested that pathogenicity is also related to the ability of virulent strains to grow in high densities in the intestinal tract and
spread to other sites in the body [50]. Host factors, such as predisposing medical conditions, immune status, and exposure to antibiotics, are also thought to play a role in the ability of enterococci to establish infection [51].

5. Conclusion

The low incidence of asal, esp, and cyA among E. faecalis and E. faecium from the PR and SC beaches indicates that these virulence genes were not widely disseminated among strains found here, suggesting low potential health risks to humans. Still, the presence of E. faecalis and E. faecium harboring asal, esp, and cyA suggests humans, birds, and dogs as potential sources of enterococci to beach water. Future surveys of enterococcal virulence genes at beaches should include those with different source inputs and populations of enterococci.

Competing Interests

The authors declare that they have no competing interests.

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