Q-487 *Enterococcus* Species in WaterandSedimentsatBabyBeach,DanaPointHarbor,California

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ABSTRACT

Baby Beach, inside Dana Point Harbor, CA has had a history of impaired water quality resulting in beach postings and closures. Best Management Practices (BMPs) to reduce levels of fecal indicator bacteria have been implemented. Weekly bacteriologic al monitoring data indicate a general decline in concentrations of fecal and total coliforms from 1999-2002, suggesting some benefit of current BMPs such as storm drain plugs. However, enterococci concentrations have increased during the same period. Bacterial monitoring studies to identify potential sources of enterococci such as storm drains, birds and sediments were conducted from July through October 2002. Enterococci levels were determined in water, gull stool, and sediment samples. Sediments were collected at swash zone sites, up to 30 ft. from the waterline and near storm drains. Ten grams of sediment were resuspended in 1% sodium metaphosphate solution and sonicated for 30s at 30% output (Branson Sonifier 450). Resuspended sediment and water were analyzed for enterococci using the mEI membrane filtration method (EPA Method 1600). Enterococci concentrations ranged from 20 to 24,000 CFU/10 g in sediments and 10 to 40,000 CFU/100 ml in water. Up to 1.4 x 10^7 CFU/g were found in seagull stools. The distribution of enterococci species in marine water, sediment, and seagulls were compared. Isolates were speciated with API 20 STREP (Biomerieux, France). *E. faecalis* was the most common species in all sample types from the swash zone and storm drain areas. Notably, a number of marine water isolates from the mEI media were identified as *Streptococcus spp.* (22%) or could not be speciated (17%) using API 20 STREP. These results are higher than the 6% false positive rate reported for mEI. The marine recreational water quality standard for a single sample is 104 enterococci/100 ml, so a 20% or higher false positive rate could have significant implications with regards to beach posting and closures. Accurate species identification will contribute to determining the sources of enterococci.

INTRODUCTION

In 1999, the State of California mandated statewide monitoring of ocean waters for fecal indicator bacteria (FIB) at all public beaches with more than 50,000 annual visitors and established concentration standards for these organisms in recreational water. High levels of FIB in water indicate increased risk of illness from fecal contamination. The single sample standards for total coliforms (TC), fecal coliforms (FC) and *Enterococcus* (ENT) in recreational water, respectively, are 10,000, 400 and 104 colony-forming units (CFU) or most probable number (MPN) per 100 ml. When water adjacent to the beaches is in violation with any of these standards, local health officials post signs to restrict beach access. Prior to 1999, Baby Beach was frequently posted due to high levels of TC and FC. Several best management practices were implemented resulting in a general decline of these indicators. However, with the addition of monitoring for ENT, increased postings have been due to violation of enterococci standards as compared to TC and FC. Based on weekly monitoring data from 2002, there were 76 exceedances of ENT standards, 24 of which were also high for FC and 4 for TC. Since ENT are also present in soils and plants, their reliability as indicators of recent fecal contamination in marine water is questionable. On the other hand, persistence of ENT in the environment may enhance their usefulness for source tracking. Thus, speciation of ENT may be useful for determining sources of water pollution, given that there is host specificity or differences in the distribution of species among sources. The objective of this study was to evaluate the usefulness of speciation of ENT at Baby Beach to help identify sources of fecal pollution using an efficient and reliable speciation method. The spatial and speciation differences of ENT isolates obtained from marine water were compared to those from potential sources of ocean water pollution such as seagulls, storm drains and sediments. Isolates (N=150) from mEI culture media, typically used to recover ENT from water, were initially speciated using API 20 Strep. However, a number of isolates were identified as *Streptococcus* species or could not be identified (Table 1). Therefore, 263 additional isolates from water, sediments and seagull stools using mEI were also speciated using API 20 Strep, 28 of which were also tested with MicroScan Gram Positive PC-12 Panel and biochemical testing. The results were compared to determine the reliability and efficiency of these three methods for species identification.

Recently, several bacterial source tracking methods have been reported as being useful for discriminating human and nonhuman sources of fecal pollution in water. The ENT isolates obtained in this study are being archived for future studies using such molecular typing techniques for further strain discrimination for source identification.

MATERIALSANDMETHODS

Sample collection. Water (N=121) and sediment (N=55) samples were collected at nearshore sites (Figure 1) and west storm drain outfall area (Figure 2) in 100 ml sterile, disposable bottles (IDEXX). To characterize ENT species distribution at the west storm drain area and to compare speciation methods, 20 samples each of marine and storm drain water as well as the underlying sediments were collected at same time of day for 4 consecutive days. Approximately 10 g of sediments were collected up to 2 cm depths after collecting the overlying water. Seagull stools were collected during the same time period from a clean tarp placed on the beach.

Sample processing. 100 ml of 1% sodium metaphosphate was added to 10 g of sediment and sonicated for 30s @ 30% output with a Branson® Sonifier 450. 50ml MgPO_4 buffer was added to stool samples in centrifuge tubes and vortexed. Suspended sediments and stools were filtered using the membrane filtration method (Figure 3) (2). Membranes were placed onto mEI media as per EPA Method 1600 (9) and incubated @ 41.0 ? 0.5 ?C for 24h.

Speciation methods. Isolates from mEI including *S. bovis*, *E. faecium*, *E. faecalis*, unidentified species and ATCC control organisms [*E. faecalis* (#29212), *E. faecium* (# 35566?), *S. bovis* (# 49147), *A. viridans* (# 700406)] were speciated using API 20 Strep (Biomerieux), MicroScan PC-12 Panel (Dade Behring), and biochemical tests using various identification charts (3-8). Biochemical tests included: gram stain, hemolysis on blood agar plates (BAP), colonial pigmentation on BAP, motility, catalase (3%), bile esculin, growth @ 45?C in brain heart infusion broth, modified salt broth (6.5%), Moeller decarboxylase arginine, phenol red broth base mannitol, sorbitol, arabinose, raffinose, and sucrose. MicroScan panels were tested using the log-phase technique, as per manufacturer's instructions. Antibiotic susceptibility testing was also done using MicroScan. API 20 Strep was used as per manufacturer's instructions, but without anaerobic incubation.

RESULTS

Of 265 isolates from marine water, *E. faecium* was most commonly isolated, followed by *E. faecalis* and *E. gallinarum* (Figure 4). The species distribution in sediment was similar to marine water. Whereas most of the isolates from mEI were speciated using API 20 Strep alone, 80 isolates from the west storm drain area were speciated using API 20 Strep with supplemental biochemical testing (Figure 5). Therefore, speciation results for samples collected at the storm drain area may be slightly more accurate than isolates from the nearshore areas, since some species such as *E. casseliflavus* cannot be identified using API 20 Strep alone. *E. faecium* was also the most prevalent species in marine water and sediments below the storm drain. There was also a higher percentage of *S. bovis* in seagull stools collected in March (Figure 5) as compared to those collected in the fall of last year (Figure 4). In storm drain water samples, *E. casseliflavus* was most common, followed by *E. faecium* and *E. faecalis*.

Based on species identification of 28 isolates using API 20 Strep, MicroScan, and biochemical testing, API 20 Strep was found to be more reliable than MicroScan based on species identification using biochemical test reactions. The antibiotic resistance profiles obtained using MicroScan was not useful for discriminating sources of species, however this was based on the limited number of samples tested in this study. Interestingly, none of the ENT isolates were found to be vancomycin resistant.

Colonies of species other than ENT such as *Streptococcus*, *Leuconostoc*, and *Aerococcus* appear identical to ENT on mEI. Since mEI media is prepared in our laboratory, the ability to recover different species of *Streptococcus* and non-enterococcal species was verified using mEI prepared at another local laboratory using the same formulation. Identical isolates of *S. bovis* and other non-enterococcal species grew on both media and the colonies appeared larger on mEI prepared at the other laboratory.

Identification	No. Isolates (%)		
	Water (N=24)	Seagull Stool (N=18)	Sediment (N=23)
Enterococcus avium	2 (8%)	4 (7.4%)	2 (7.1%)
Enterococcus durans	6 (10.7%)	4 (7.4%)	4 (8.5%)
Enterococcus faecalis	12 (22.2%)	26 (48.1%)	18 (23.8%)
Enterococcus faecium	11 (20.4%)	11 (20.4%)	7 (16.7%)
Enterococcus gallinarum	3 (5.3%)	0 (0%)	4 (19.0%)
Enterococcus viridans	0 (0%)	0 (0%)	1 (2.4%)
Streptococcus bovis	11 (20.4%)	5 (9.3%)	3 (7.1%)
Streptococcus uberis	0 (0%)	0 (0%)	1 (2.4%)
Unable to identify	9 (16.7%)	4 (7.4%)	5 (13.5%)
Total	54 (100%)	54 (100%)	42 (100%)

Table1.Speciesidentificationsofisolatesfromwater,seagullstoolsandsedimentsamplesEE.

Figure1.Nearshoreseagullgroupies.

Figure2.Weststormdrain.

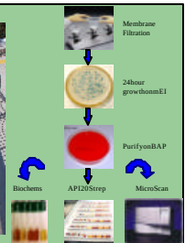


Figure3.FlowchartofmethodsusedforenterococciandidentifyIsolates.

Figure4a

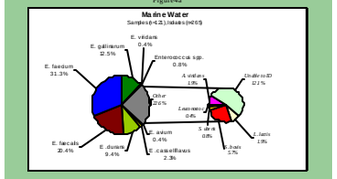


Figure4b

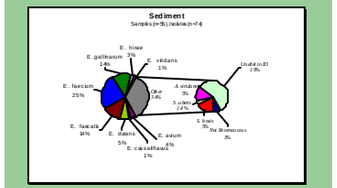
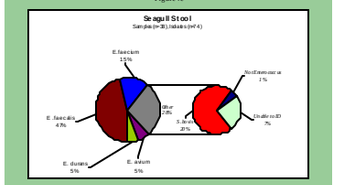


Figure4c



Figures4.a-cDistributionofspeciesatnearshore,weststormdrainsitesandinseagulls(June-October,2002).

Figure5a

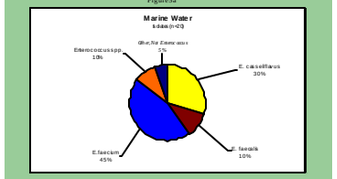


Figure5b

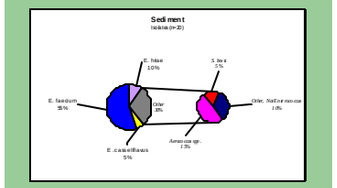


Figure5c

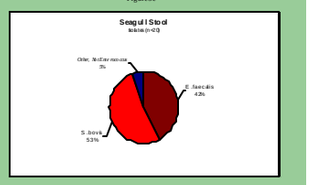
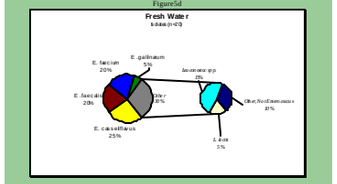


Figure5d



Figures5.a-dDistributionofspeciesatweststormdrainareaandinseagulls(March,2003).

DISCUSSION

To date, more than 20 species of *Enterococcus* have been identified. In this study, *E. faecium* was the most prevalent species in marine water and sediments, while *E. faecalis* was most common in seagull stools. *E. faecalis* and *E. faecium* are commonly found in human feces. *E. faecalis* is commonly found in cats and dogs, with *E. faecium* occurring less frequently. *E. faecium*, *E. faecalis*, *E. hirae* and *E. durans* are frequently found in farm animals. *E. faecalis*, *E. faecium* and *E. casseliflavus* are present in insects. *E. casseliflavus* is also associated with plants (1). Although the same species of ENT can be found in several different hosts, there is still potential for using species information to identify sources of fecal pollution in water. The species identified in sediments at Baby Beach may indicate species that are more resistant to environmental conditions. Future studies are needed to determine whether ENT occur in the water only in association with feces or whether they persist and grow in sediments and on plants, independent of a fecal contamination event. Clearly, additional studies on species distribution and host specificity will establish the reliability of ENT as indicators of fecal pollution in water. A single non-enterococcal CFU of non-fecal origin could have serious implications as to beach postings, especially if small volumes (100 ml or less) are used to determine ENT levels, since the single standard exceedance for ENT is 104 CFU/100 ml.

The mEI media used to isolate ENT contains indoxyl-4-D-glucoside to differentiate these organisms from fecal streptococci. However, up to 20% of the isolates were either *Streptococcus* spp., could not be identified or were non-enterococcal species commonly found in the environment that may or may not be clinically significant.

Most enterococcal isolates can be rapidly identified using API 20 Strep and MicroScan identification systems, although MicroScan had biochemical test discrepancies when compared to routine biochemical tests. For complete and accurate identifications, we recommend the following supplemental testing in addition to API 20 S: gram stain, pigment, motility, catalase, and growth studies at 45°C and 6.5% NaCl. Some isolates identified as *E. faecium* by API may be identified as *E. casseliflavus* using biochemical testing, however, *E. casseliflavus* may be a subspecies of *E. faecium*.

CONCLUSIONS

- *E. faecium* was the most prevalent enterococci species found in marine and sediment samples using mEI, whereas *E. casseliflavus* was the most prevalent species in storm drain water. *E. faecalis* was also the most common species in seagull stools, however there may be differences in species distribution due to seasonality. The species distribution in marine water, storm drain water, sediments and seagull stools was not found to be specific or unique to each of these sources.
- The distribution of ENT species in sediments was similar to that of the overlying water, suggesting that coastal sediments may be an important reservoir and perhaps source of these organisms in water.
- Further strain discrimination of *E. faecium* or *E. faecalis* using molecular techniques may be useful for identifying sources of ENT in water and sediments.
- The API 20 Strep and MicroScan identification systems were found to be generally reliable for identification of *E. faecalis* from environmental sources, although API 20 Strep had more comparable biochemical reactions when compared to routine biochemical testing.
- Up to 20% of the isolates from mEI may be species other than ENT that may or may not indicate fecal contamination.

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