OCCURRENCE OF ENTEROCOCCI IN OCEAN WATER, SEDIMENTS, STORM DRAINS, SOIL AND SEAGULLS AT BABY BEACH, DANA POINT HARBOR, CALIFORNIA

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Abstract

Signs warning the public not to swim at Baby Beach are frequently posted due to levels of enterococci bacteria that exceed California's ocean water quality standards. Enterococci are usually present in human and animal feces and are used to indicate fecal contaminated water that may harbor pathogenic species. However, since enterococci can also be found in the environment, their presence in water may give false indications of risks for human illness. Certain species of enterococci are more commonly associated with plants, while others are more predominant in feces and are considered opportunistic pathogens. Thus, the distribution of enterococci species and numbers of these organisms in marine water, sediments, storm drain, soil (from lawns), seagulls and sewage was compared. This was also done for isolates from beach water collected when enterococci levels exceeded standards. Enterococci isolates (N=794) were identified to species level using API 20 STREP with supplemental biochemical testing. E. faecalis and E. faecium were the predominant species found in marine water and sewage. E. casseliflavus, commonly associated with plants, was frequently found in soil and in storm drain water.

Introduction

As a result of previous epidemiological studies performed by the U.S. EPA indicating a direct correlation between swimming-associated gastroenteritis and *Enterococcus* levels in surface waters, these bacteria are currently being used as indicators for the presence of other fecal pathogens. However, there are a number of concerns regarding the public health significance of these organisms in recreational water. While some species of enterococci have been associated with human infections, they are also ubiquitous in the environment. Of the 23 distinct species, *E. faecalis* is one of the leading causes of nosocomial (hospital-acquired) infections such as bacteremia, endocarditis and urinary tract infections. Yet little is known about the virulence potential of enterococci present in storm drains, soil, plants, and animal feces. Furthermore, there is uncertainty as to whether the presence of enterococci found in water are indicative of fecal pollution. The levels found in surface water may reflect the persistence and possible growth of these organisms in the underlying sediments or in soil that is transported during runoff. Another concern is the occurrence of vancomycin resistant enterococci (VRE) in the environment, as the incidence of VRE in hospitals is on the rise. This study characterizes the distribution of enterococci in a marine environment, a critical step to conducting more specific studies to determine the likelihood of sources as being fecal or environmental in nature, or more likely, both. Future studies should include targeting the predominant species identified in this study to help identify the sources of these organisms, develop rapid indicator test methods, and to discriminate specific strains using molecular typing methods.

Study Objectives

- Determine *Enterococcus* species occurrence in the marine environment.
- Compare species distribution in marine water above and below water quality standards for enterococci.
- Compare enterococci levels in sediments to those in overlying marine water.
- Assess persistence and possible growth of enterococci in sediment.
- Determine the occurrence of vancomycin resistant enterococci (VRE) in the marine environment.

Materials and Methods

Distribution of species

Up to 5 bacterial isolates per sample (N=794) were isolated using m-EI media. Isolates were identified to enterococci species level or as "non-enterococci" using API 20 STREP® and additional biochemical testing. The species distribution was determined by counting each species only once per sample (N=498).

Enterococci levels in sediments and water

The mean levels of enterococci were determined for sediments and overlying water collected daily at the beach and near a storm drain outlet over a 4-day period (N=20).

Growth study

On the first day of the study, "day 0", two 5g aliquots of sediment obtained from a tidally influenced storm drain were sonicated and then filtered to obtain bacterial counts. The volume of water filtered was replaced with either sterile DI water (representing freshwater) or filter-sterilized marine water. The tubes were incubated in a covered outdoor tank as water from the estuary was continuously pumped into the tank, maintaining the temperature at 12 - 16°C. Bacterial counts were determined on daily basis for 9 days and 1 – 2 times/week for up to 58 days.

Vancomycin Resistance Testing

E. faecium and *E. faecalis* isolates from mEI media were inoculated onto brain heart infusion media containing 6 µg/ml of vancomycin (Remel) and assessed for bacterial growth.

Results

E. faecalis and *E. faecium*, both considered pathogens, were predominant in marine water, sediment, sewage, seagull stool and less common in storm drain water and soil. Up to 23 % of the isolates found in marine water were identified as species commonly associated with the environment. Up to 13% of marine water isolates were non-*Enterococcus* species, higher than the 6% false-positive rate reported for mEI media (**Table 1**).

A common phenotype of *E. faecalis* (API 20 SREP biotype code 7143711) was found in marine water, storm drains, sewage, and seagull stool.

There was no significant difference in the distribution of enterococci species in water above or below standards (**Figure 1**).

Mean concentrations of enterococci were higher in sediment than overlying water, particularly near the storm drain as compared to the beach area (**Figure 2**).

The similarity in species distribution of enterococci in sediment and marine water indicate that sediments may be a significant source of enterococci to water. "Environmental" species were predominant in soil and storm drain water (**Figure 3**).

Enterococci may grow in storm drain sediments and persist for up to 58 days, particularly in fresh water (**Figure 4**).

None of the *E. faecalis* (N=48) or *E. faecium* isolates (N=25) from marine water, sediments, storm drain water, seagull stool, soil and sewage were found to be resistant to vancomycin (6 µg/ml).

Table 1. Occurrence of Pathogenic vs. "Environmental" Species of *Enterococcus* (ENT) and ENT-like organisms in the Marine Environment

		Pathogens		Non-pathogens			"Environmental"			Other	Non-ENT	
Source	N (%)	*E. faecalis*	*E. faecium*	*E. hirae*	*E. durans*	*E. avium*	*E. casseliflavus*	*E. mundtii*	*E. gallinarum*	Unidentified ENT	*S. bovis*	Other
Marine Water	246 (49%)	29.3%	22.4%	9.8%	0.4%	0.4%	11.4%	10.6%	1.2%	1.2%	3.7%	9.8%
Sediment	108 (22%)	14.8%	35.2%	11.1%	0.9%	1.9%	9.3%	10.2%	1.9%	0.9%	3.7%	10.2%
Sewage	27 (5%)	37.0%	37.0%	11.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	14.8%	0.0%
Soil	23 (5%)	13.0%	13.0%	4.3%	0.0%	0.0%	52.2%	8.7%	4.3%	0.0%	0.0%	4.3%
Storm Drain Water	40 (8%)	22.5%	12.5%	5.0%	2.5%	0.0%	37.5%	5.0%	5.0%	0.0%	0.0%	10.0%
Gull Stool	54 (11%)	53.7%	9.3%	1.9%	1.9%	0.0%	1.9%	3.7%	0.0%	0.0%	22.2%	5.6%

Methods

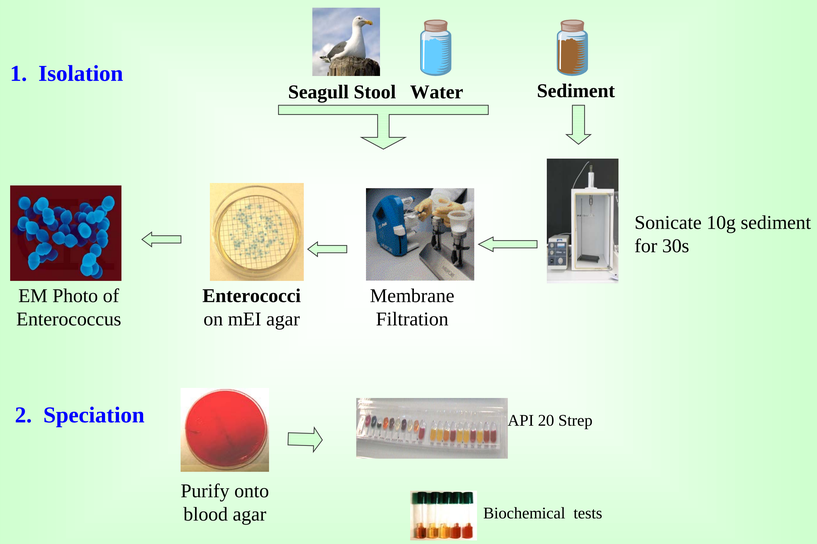

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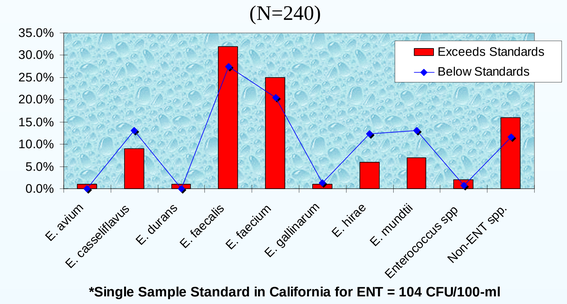
Figure 1. Species Distribution in Water Above and Below Enterococci Water Quality Standards* (N=240)



***Single Sample Standard in California for ENT = 104 CFU/100-ml**

Figure 2. Enterococci Levels in Marine Water and Sediments at Beach and Storm Drain Sites

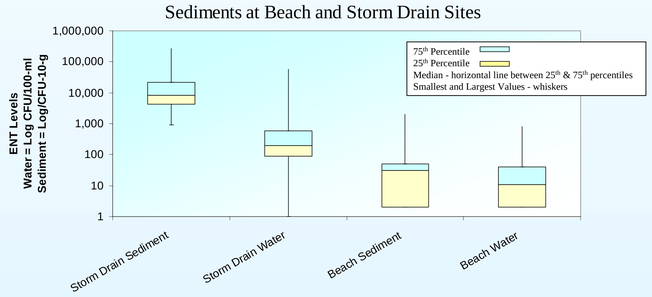
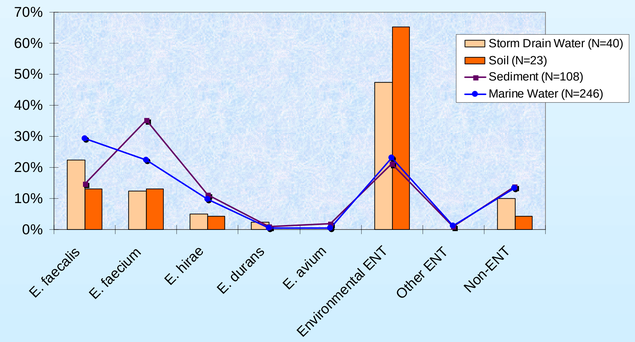
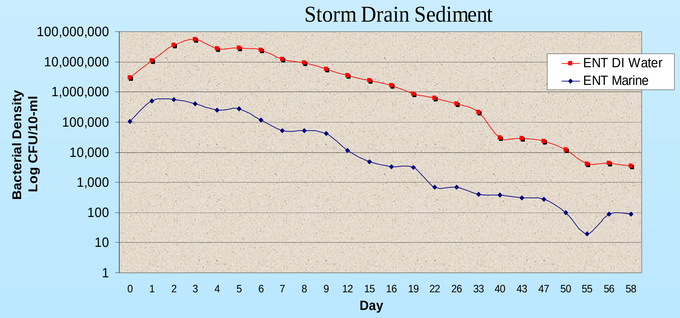


Figure 3. *Enterococcus* * Species Occurence (N=417)



***Includes enterococci-like and non-enterococci species obtained using mEI media.**

Figure 4. *Enterococcus* spp. Survival in Storm Drain Sediment



Conclusion

- The levels and species distribution pattern of enterococci found in water and sediments indicate that sediments may be an important source of these organisms in marine waters.
- *Enterococcus* species commonly found in human and animal feces were also found in environmental sources such as sediments, soil and plants.
- Further discrimination to the subspecies (strain) level* is needed to determine the human health risk of environmental strains.
- Enterococci may persist in storm drain sediment for up to 60 days, depending on growth conditions.
- Vancomycin resistant strains were not detected in this study, indicating low risk for human infections due to VRE.

*** Further studies**: We are currently characterizing enterococci associated with the environment at the strain level using PFGE.