Characterization of *Enterococcus* Species in Coastal Waters Exceeding Water Quality Standards

D. M. Ferguson, D. F. Moore, M. H. Zhowandai, E. Hernandez, M. A. Getrich, P. Hannah, J. Wolfe;
Orange County Public Health Laboratory, Newport Beach, CA, USA

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ABSTRACT

A section of Huntington Beach (Fig. 1), California, has frequently been posted or closed for swimming since 1999, primarily due to high levels of enterococci fecal indicator bacteria at the surf zone. Numerous multi-disciplinary investigations have not identified specific sources responsible for the beach water quality failures. A bacteriological study was conducted to characterize enterococci species distribution at 4 surf zone and 2 river mouth sites for samples that passed or failed water quality standards. Samples were collected 4-6 days/week for a 6-week period that included 3 spring tide cycles. Enterococci isolates (N=338) were obtained using mEI media (EPA Method 1600) and up to 5 isolates/sample were speciated using API 20 STREP (Biomérieux, France) with additional biochemical testing. Enterococci single sample standards (> 104 CFU/100 ml) were exceeded on 21 of 31 sampling days with the highest levels found at the surf zone sites during spring tides. During spring tide cycles, 33% of samples (N=58) exceeded standards as compared to 16% (N=55) during neap tides. The most common species found in coastal waters were *E. faecalis* (30%), *S. bovis* (16%), *E. faecium* (15%), *E. hirae* (12%), *E. casseliflavus* (7%) and other species identified as non-enterococcus (15%). *Enterococcus faecalis* and *Streptococcus bovis* were the predominant species at the surf zone sites when levels were both well above and below standards. *E hirae* and *E. casseliflavus* were more commonly detected at the river sites. There were no significant changes in species distribution overall, in samples either passing or failing standards at all of the sites. These results suggest that these species are generally present in coastal waters at these surf zone and river mouth sites and that increased levels may be related to tidal and current conditions.

INTRODUCTION

Enterococcus (ENT) is one group of fecal indicator bacteria (FIB) used by government agencies to determine whether beaches are safe for swimming. Contributing sources of ENT include humans, animals, birds, sewage, and natural and urban runoff. Some species of ENT, such as *E. faecalis* and *E. faecium* are commonly associated with fecal sources. However, *E. casseliflavus*, *E. mundtii* and *E. gallinarum* are more commonly found in plants and soil. ENT may also accumulate, persist and possibly replicate in marine sediments that, when resuspended, can be a source of ENT to overlying water. Thus, high levels of ENT that are environmental in origin may give a false indication of fecal contamination.

At Huntington Beach, high levels of ENT appear to correlate with spring tide conditions, particularly in summer. Spring tides occur over a period of a few consecutive days before and after a full moon (Fig 7). During this time, the gravitational forces from the moon and sun are at a maximum, resulting in the highest exchange of ocean water flowing in and out of coastal outlets. Contaminants in the Santa Ana River (SAR), including bacterial-laden sediments, are flushed out to the ocean during ebb tides, possibly causing beach water quality failures.

The aim of this study was to investigate the sources of ENT to Huntington Beach.

STUDY OBJECTIVES

1. **-Identify ENT species distribution in marine water and sediments to determine occurrence of environmental vs. fecal associated species and to identify predominant species for Pulsed-Field Gel Electrophoresis (PFGE) typing.**
2. **-Determine levels of FIB in sediments from locations suspected of impacting beach.**
3. **-Investigate relationship between beach failures and tidal conditions.**
4. **-Compare ENT species distribution in beach water meeting and failing single sample ENT standards (104 CFU/100 ml).**
5. **-Compare relatedness of ENT strains using PFGE to determine spatial distribution and growth, as indicated by clonal strains.**

MATERIALS & METHODS

Sample collection

Water samples were collected at the HB surfzone and SAR mouth 4-6 days/week for a 6-week period that included 3 spring tide cycles. Ocean bottom sediments were collected at depths from 10 to 71 m using a clamshell sampler. Surface sediments (top 3") in the SAR were collected into a sterile bottle. Sediments were collected from areas suspected of contributing bacteria to the beach (Fig. 5):

A. Santa Ana River (SAR), above Pacific Coast Highway (PCH)
B. SAR below PCH
C. Huntington Beach Coast, near the AES power plant
D. Huntington Beach Coast, 0.2 mi from shore at 10 m depth
E. Orange County Sanitation District (OCSD) sewage outfall
F. Newport Beach coast (control site)

Enterococci levels in sediments and water

Enterococci were extracted from sediment using sonication (30s at 30% output using a Branson Sonifier® 450) and enumerated using Membrane Filtration (EPA Method 1600).

Enterococci speciation and distribution

Up to 5 isolates per sample were isolated from m-EI media. Isolates were identified to *Enterococcus* species (biotype) 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.

Pulsed-field Gel Electrophoresis

E. faecalis strains were cultured in brain heart infusion broth for 18-24 h at 37°C and pelleted by centrifugation at 12,000g for 4 min. Bacterial cells were embedded in agarose plugs and lysed for 48 h at 37°C with EC lysis buffer and Mutanolysin stock. Lysed plugs were washed and digested with *Sma* I endonuclease for 4 h at 25°C. Electrophoresis was performed with a contour-clamped homogenous electric field device (CHEF-DRIII or CHEF MAPPER, Bio-Rad, CA) at 200V, 18h. Gels were stained with ethidium bromide (Bio-Rad, CA) and analyzed with BioNumerics program (Applied Maths, Kortrijk, Belgium). A dendrogram was derived from the unweighted pair group method.

1 *Enterococcus* species distribution in HB surfzone water, SAR sediments and offshore sediments

- Note: Although m-EI media is selective for enterococci, species other than ENT (false positives) were also isolated.

Fig 1. HB Surfzone Marine Water N=338 isolates

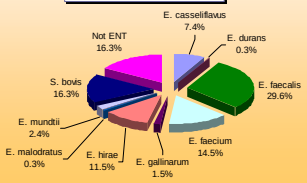


Fig 2. Santa Ana River Sediments N=112 isolates

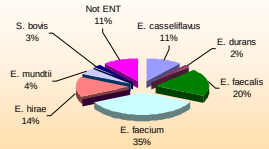
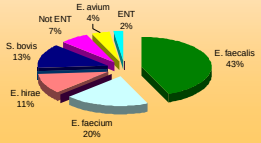


Fig 3. HB Offshore Sediments N=46 isolates



2 Levels of FIB in sediments from areas suspected of impacting beach area

- The highest levels of FIB were found in the river sediments as compared to the offshore sites. These levels suggest that FIB, particularly ENT, may be replicating in this environment.

Fig 4. Total Coliform (TC), Fecal Coliform (FC) and Enterococci (ENT) Levels Found in Sediments (N=116 samples)

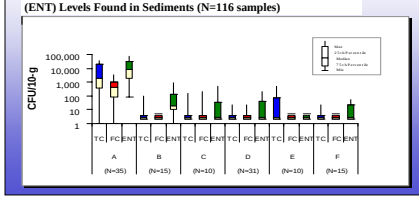
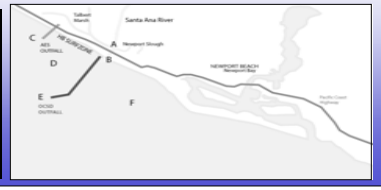


Fig 5. Map of Study Site



3 Relationship between beach failures and tidal conditions

- During the summer, episodic failures are directly related to spring tides; the greatest number of exceedances occurring within 3 days of a New or Full Moon (Apex).

Fig 6. Water Quality Failures at HB Associated w/Spring Tides (Apr – Oct, 2002-2004)

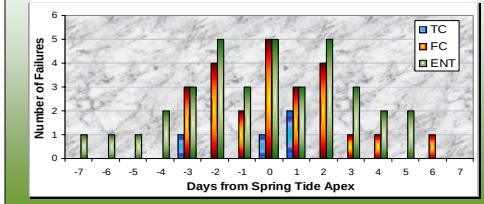
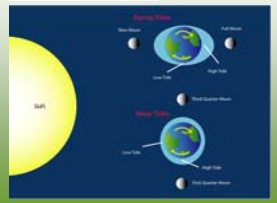


Fig 7. Tide Cycles



4 Comparison of ENT species distribution in beach water meeting and failing single sample standards (104 CFU/100 ml)

- There was no difference in species distribution between failures and ambient conditions.
- The 3 most common API biotype profiles were seen in samples both meeting and failing standards.

Fig 8. Distribution of ENT Species (N=338 isolates)

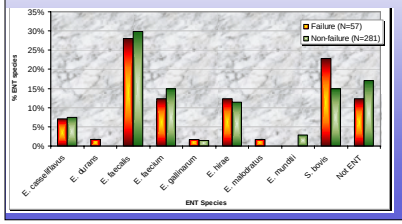
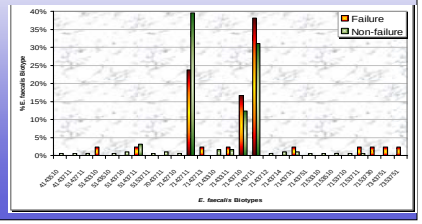


Fig 9. Distribution of *E. faecalis* Biotypes (N=229)



5 Relatedness of *E. faecalis* strains using PFGE

- Populations of *E. faecalis* that appear to be clonal were found in water, sediments and gull stools from Huntington Beach as well as Baby Beach, located 20 miles south. The majority of clones had the same PFGE type (I) and included isolates that were collected within a 2 month period.

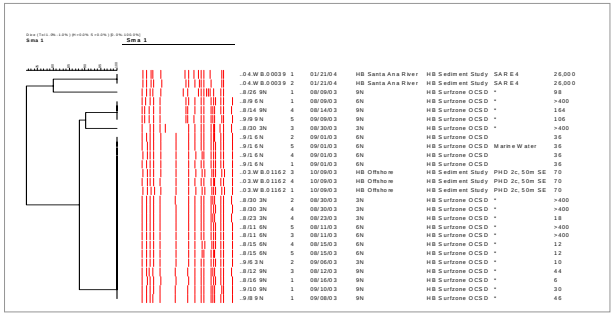
Fig 10. Genotypic Characterization of *E. faecalis* Isolates in Correlation to Geographic Location, Source and Biotype

PFGE Pattern	No. of isolates (samples)										
	HB Surfzone H2O	HB Coast Sediment	HB Coast H2O	NB Coast Sediment	SAR River Sediment	BB Gull Stool	BB Marine H2O	BB SD H2O	BB SD Biofilm	API 20 S Biotype(s)	Total
I	61 (42)	5 (3)		1 (1)		4 (3)	15 (7)			7142711	86 (54)
II	1 (1)	6 (2)								7143311 & 7143711	7 (3)
III					2 (2)					7142711	2 (2)
IV	9 (9)		1 (1)							7142711	10 (10)
V	1 (1)	3 (2)			1 (1)					7142711 & 7143711	5 (4)
a	5 (5)									7142711	5 (5)
b	2 (2)									7142711	2 (2)
c	2 (2)									7142711	2 (2)
d	2 (2)									7142711	2 (2)
e						2 (1)				7142711	2 (1)
f	2 (1)									7143711	2 (1)
g		2 (2)			1 (1)					7142711 & 7143711	3 (3)
h	1 (1)				1 (1)					7143711	2 (2)
j	1 (1)				1 (1)					7143711	2 (2)
k	2 (1)									7143711	2 (1)
l	3 (2)									7143711	3 (2)
m		1 (1)	1 (1)							7143711	2 (2)
n	1 (1)	1 (1)		1 (1)						7143711	3 (3)
o	1 (1)	1 (1)								7143711	2 (2)
p								2 (1)		7143711	2 (1)
q					2 (1)					7143711	2 (1)
r	2 (2)									7143711	2 (2)
s	2 (1)									7143711	2 (1)
t		1 (1)					3 (2)			7142711	4 (3)
u	1 (1)				1 (1)					7143711	2 (2)
w	2 (2)									7143711	2 (2)
x								2 (1)	3 (1)	7143711	5 (2)
y						3 (2)				7143711	3 (2)
z		2 (1)								4352710	2 (1)
aa		2 (2)								4352710	2 (2)
bb		2 (1)								4352710	2 (1)
No. Clonal types per site	19	11	2	2	7	2	3	2	1		
Total: Isolates (Samples)	101 (78)	26 (17)	2 (2)	2 (2)	9 (8)	6 (4)	21 (11)	4 (2)	3 (1)		174 (123)

LEGEND

HB – Huntington Beach
NB – Newport Beach
SAR – Santa Ana River
BB – Baby Beach
SD – Storm Drain

Fig 11. PFGE Dendogram of *E. faecalis* Isolates



DISCUSSION

Epidemiological studies have shown that swimming-related gastroenteritis is associated with ENT in fecal-contaminated coasts. At Huntington Beach, tidal events appear to correlate with increased levels of ENT, indicating bacterial input from a source that is tidally influenced. There was no significant change in species distribution when levels surpassed standards, suggesting persistent source(s) of ENT. We hypothesize that the SAR is one of these sources. Previous dye studies described how pollutants in the SAR could be transported to Huntington Beach. The high levels of ENT found in the SAR sediments indicate they may be replicating in this environment. The origin of ENT in sediments is still undetermined. The predominant species found in sediments are also common inhabitants of the intestinal microflora of humans and animals. The PFGE results suggest that there may be a clonal population of *E. faecalis* that is widely distributed in this coastal environment. It is possible that this "clonal" population is comprised of strains that are so closely related that additional restriction enzymes are needed for further discrimination. Future studies include using PFGE in conjunction with an additional typing technique for clonality assessment.

ACKNOWLEDGEMENTS

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Fig 12. Santa Ana River

Fig 13. Resuspended Sediments as a Result of Dredging Activities.

CONTACT INFORMATION
Dferguson@ochca.com
Mzhowandai@ochca.com