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Species distribution and antimicrobial resistance of enterococci isolated from surface and ocean water

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Abstract

Aims: The species identification and antimicrobial resistance profiles were determined for enterococci isolated from Southern California surface and ocean waters.

Methods and Results: Species identification was determined for 1413 presumptive *Enterococcus* isolates from urban runoff, bay, ocean and sewage water samples. The most frequently isolated species were *Enterococcus faecalis, Enterococcus faecium, Enterococcus hirae, Enterococcus casseliflavus* and *Enterococcus mundtii.* All five of these species were isolated from ocean and bay water with a frequency ranging from 7% to 36%. *Enterococcus casseliflavus* was the most frequently isolated species in urban runoff making up 36–65% of isolates while *E. faecium* was the most frequently isolated species in sewage making up 53–78% of isolates. The similar distribution of species in urban runoff and receiving water suggests that urban runoff may be the source of *Enterococcus*. No vancomycin or high level gentamycin resistance was detected in *E. faecalis* and *E. faecium* isolates.

Conclusions: *Enterococcus faecalis, E. faecium, E. casseliflavus* and *E. mundtii* are the most commonly isolated *Enterococcus* species from urban runoff and receiving waters in Southern California.

Significance and Impact of the Study: Determination of the *Enterococcus* species isolated from receiving waters and potential pollution sources may assist in determining the sources of pollution.

Introduction

The presence of large concentrations of enterococci in the human intestinal tract and faeces has been long known and is the basis for utilizing *Enterococcus* as a water quality indicator organism (Prescott *et al.* 1946). The current USEPA *Enterococcus* recreational water quality standard was developed based on the correlation of swimming-associated illnesses rates and *Enterococcus* concentration demonstrated in several large epidemiological studies performed in water bodies impacted with sewage (Cabelli *et al.* 1979; USEPA 1983, 1986). The methods utilized for measuring *Enterococcus* concentrations for these studies and newer methods that are in current use do not differentiate between species of the genus. Besides containing species that are present in human and animal intestinal tracts and faeces (*Enterococcus faecalis, Enterococcus faecium, Enterococcus durans, Enterococcus gallinarum,* and *Enterococcus hirae*), the *Enterococcus* genera contains species that are considered epiphytes and associated primarily with plants (*Enterococcus casseliflavus, Enterococcus mundtii* and *Enterococcus sulfureus*) and others that are associated with insects, specific hosts or other undefined sources (Mundt and Graham 1968; Martinez-Murcia and Collins 1991; Devriese *et al.* 1993; Sinton *et al.* 1993; Leclerc *et al.* 1996; Ott *et al.* 2001; Aarestrup *et al.* 2002). As the *Enterococcus* water quality testing techniques can detect many of these species (Rhodes and Kator 1997; Ferguson *et al.* 2005), selection and identification of the species isolated could potentially assist in determining the source. Identification of the *Enterococcus* species enumerated in routine monitoring is not a standard procedure in testing laboratories but several studies have identified the predominate species in environmental water. *Enterococcus faecium* and *E. faecalis* were identified as the prominent species in several studies (Stern *et al.* 1994; Pinto *et al.* 1999; Švec and Sedláček 1999, Grammenou *et al.* 2006), however, one study identified *Enterococcus avium*, *Enterococcus raffinosus* and *E. faecium* as the dominate species (Arvanitidou *et al.* 2001). Two other studies have identified the plant-associated species *E. casseliflavus* as a prevalent species in streams, urban runoff and receiving water not impacted by sewage (Bayne *et al.* 1983; Ferguson *et al.* 2005). These studies were performed at different sites and often in different countries. As species prevalence in receiving water will depend on environmental factors including the geographical area, potential sources and the sewage and storm drain infrastructure, detailed studies of individual locations will be necessary to understand the *Enterococcus* species distribution and provide useful information to help determine sources.

While enterococci are useful as indicator bacteria, *E. faecalis* and *E. faecium* are also opportunistic human pathogens. Antimicrobial resistant strains including vancomycin resistant enterococci (VRE) and high level aminoglycoside resistance (HLAR) have become more prevalent in US and some European hospitals and are very difficult to treat and are the cause of excess morbidity and mortality (Murray 1990; Huycke *et al.* 1998; Fridkin *et al.* 2001; Low *et al.* 2001; Carmeli *et al.* 2002; NNIS System 2003, Sofianou *et al.* 2004). The spread of these organisms from the hospital environment or other sources to environmental waters through discharged sewage or other means could increase the prevalence of these strains in the human population and become a potential risk to human health (Rice *et al.* 1995; Iversen *et al.* 2002). Several studies have detected VRE and HLAR strains in the environment. VRE can be isolated from hospital-impacted sewage (Harwood *et al.* 2001; Iversen *et al.* 2002; Novais *et al.* 2005), sewage (Poole *et al.* 2005) and in receiving waters (Novais *et al.* 2005). HLAR strains have been isolated from sewage and recreational water (Rice *et al.* 1995).

In this study, we determined the species of *Enterococcus* present in urban runoff, bays, ocean water and sewage from two different and contrasting Southern California locations, coastal Orange County, a large urbanized area, and Avalon, a small isolated town on an offshore island. The initial isolation of strains was from the mEI membrane filtration test to accurately gauge the distribution of species measured by standard water quality testing protocols. To determine if VRE and HLAR strains are common in environmental water and sewage, the antimicrobial resistance patterns of *E. faecalis* and *E. faecium* isolates from environmental sites and sewage were determined.

Materials and methods

The two study locations were Orange County, a large coastal urban area in Southern California, 2455 km^2 with a population of 3098 121 and a population density of 1515 km^{-2}, and Avalon, an 8·15 km^2 isolated harbour town on offshore Santa Catalina Island located 45 km west of Orange County with a population of 3500 and population density of 481 km^{-2}. Both locations are semi-arid with a Mediterranean climate and have separate sewer and storm drain systems. Ocean, bay, urban runoff and sewage sample sites were selected to represent the variability seen within the two study locations. Urban runoff was fresh water from a storm drain system collected directly either from a pipe or channel or at the point of flow into bay or ocean receiving water. Bay samples were brackish to salt water samples from wetlands, harbours and bays. Orange County samples were collected from February 2006 to July 2007 and Avalon samples from July to October 2007. All samples were collected in sterile bottles maintained at 1–4°C and analysed for *Enterococcus* levels within 6 h of collection using the membrane filtration method as per Standard Methods (APHA 2005). Enterococci were enumerated using EPA method 1600 (USEPA 2000, 2002), with mEI agar (Northeast Laboratory, Waterville, ME, USA) incubated for 24 h at 41°C and reported as colony forming units (CFU) per 100 ml of water. Colonies with blue halos were considered presumptive for *Enterococcus* species and up to five colonies per sample were picked for further study. Isolates were subcultured and purified on Trypticase™ soy agar with 5% sheep blood (BBL, Bethesda, MD, USA) and incubated at 35°C for 24 h. Isolates were identified to species level using the MicroScan® Walk Away with Positive Combo 12 cards (Dade Behring, West Sacramento, CA, USA) and additional biochemical testing including carbohydrate fermentation in Purple broth with 1% Sucrose (BBL), Motility Test Medium with TTC (Hardy Diagnostics, Santa Maria, CA, USA), and pigment production (Moore *et al.* 2006). The biochemical test results were interpreted using published standard biochemical identification charts (Facklam and Collins 1989; Manero and Blanch 1999; Teixeira *et al.* 2007). The species identity was confirmed for 14 *Aerococcus viridans* isolates utilizing 16S DNA sequencing with the MicroSeq database (MIDI Labs, Newark, DE, USA). Susceptibility testing was performed on the MicroScan® Walk Away with Positive Combo 12 cards to determine minimal inhibitory concentration (MIC). The antibiotics tested and the resistant breakpoints in μg ml^{-1} were; Ampicillin ≥ 8, Ciprofloxacin ≥ 2, Erythromycin ≥ 4, high level Gentamycin ≥ 500, Penicillin ≥8; Rifampin ≥ 2, high level Streptomycin ≥ 1000, Tetracycline ≥ 8 and Vancomycin ≥ 16. Statistical significance was calculated using the Pearson chi-squared test.

Results

Enterococcus species distribution

The species distribution of isolates presumptively identified as *Enterococcus* was determined for Southern California water samples taken from the ocean, bays, urban runoff and sewage. The two study sites, coastal Orange County, a large urban area, and Avalon, a small isolated harbour town on offshore Santa Catalina Island, differed in population, geography, ecology and sewage and storm drain infrastructure. The number of sampling sites, samples and the concentration of *Enterococcus* in the specimen types are summarized in Table 1. In both study locations, the concentration of *Enterococcus* was overall the highest in sewage, lower in urban runoff and still lower in receiving waters. At the Avalon study location, the geomean of the *Enterococcus* concentration was over two logs higher for urban runoff and one log lower for untreated sewage than that of the Orange County sites. A total of 1413 presumptive *Enterococcus* isolates were identified from the two study locations. The number and percentage of each species isolated by study location and sample type is presented in Table 2. *Enterococcus faecalis*, *E. faecium*, *E. casseliflavus*, *E. mundtii* and *E. hirae* were the most common *Enterococcus* species isolated with *E. gallinarum*, *E. durans* and unidentified *Enterococcus* species together making up 3%

Table 1 Description of study sites and *Enterococcus* concentrations

	No. sites	No. samples	Concentration (CFU 100 ml^{-1}) Geomean	Maximum
Orange County				
Ocean	6	104	17	172
Bay	10	58	36	21000
Urban runoff	5	43	106	1600
Sewage	2	20	2475 909*	12 300 000
Avalon				
Bay	4	120	78	2700
Urban runoff	4	15	98 745	1360 000
Sewage	5	13	378 070	4800 000
Total	36	373		

*Treated and chlorinated sewage samples removed from this calculation.

Table 2 Species distribution of presumptive *Enterococcus* isolates

	No. isolates	Number (%) of isolates *Enterococcus faecalis*	*Enterococcus faecium*	*Enterococcus hirae*	*Enterococcus gallinarum*	*Enterococcus durans*	*Enterococcus casseliflavus*	*Enterococcus mundtii*	Other *Enterococcus* sp.	Total *Enterococcus* sp.	*Aerococcus viridans*	*Streptococcus bovis*	Other, not *Enterococcus* sp.
Orange County													
Ocean	262	62 (24)	37 (14)	11 (4)	1 (0·4)	0 (0)	80 (31)	23 (9)	6 (2)	220 (84)	3 (1)	13 (5)	26 (10)
Bay	176	26 (15)	35 (20)	15 (9)	0 (0)	0 (0)	47 (27)	35 (20)	0 (0)	158 (90)	6 (3)	0 (0)	12 (7)
Urban runoff	202	31 (15)	26 (13)	27 (13)	0 (0)	1 (0·5)	69 (34)	35 (17)	4 (2)	193 (96)	1 (0·5)	0 (0)	8 (4)
Sewage	98	17 (17)	49 (50)	17 (17)	4 (4)	1 (1)	3 (3)	1 (1)	1 (1)	93 (95)	2 (2)	1 (1)	2 (2)
Avalon													
Bay	539	117 (22)	72 (13)	30 (6)	1 (0·2)	7 (1)	35 (6)	51 (9)	10 (2)	323 (60)	202 (37)	2 (0·4)	12 (2)
Urban runoff	75	1 (1)	15 (20)	0 (0)	2 (3)	1 (1)	46 (61)	3 (4)	3 (4)	71 (95)	2 (3)	0 (0)	2 (3)
Sewage	61	4 (7)	45 (74)	3 (5)	1 (2)	3 (5)	0 (0)	1 (2)	1 (2)	58 (95)	0 (0)	2 (3)	1 (2)
Total	1413	258 (18)	279 (20)	103 (7)	9 (1)	13 (1)	280 (20)	149 (11)	25 (2)	1116 (79)	216 (15)	18 (1)	63 (4)

(47/1413) of isolates. The overall percentage of isolates confirmed as *Enterococcus* was 79% and varied between 84% and 96% for all sample types except for the bay samples from Avalon (Table 1). Thirty-seven per cent of all isolates of this sample type proved to be presumptive false positives because of *Aerococcus viridans.* While this result was not seen overall in any other sample type, it was also seen in one of the 10 Orange County bay sites, where *A. viridans* made up 33% (5/15) of all isolates.

To compare the distribution of *Enterococcus* species by sample type and study location, the frequency of isolation of each *Enterococcus* species was calculated utilizing the 1116 isolates confirmed as *Enterococcus* species as the denominator. Figure 1 compares the species distribution in receiving waters (ocean and bay sites), urban runoff and sewage for the two study locations for the five most frequently isolated *Enterococcus* species. Ocean and bay receiving waters at both study locations had similar frequency distributions of species. *Enterococcus casseliflavus* was the most frequently isolated species in Orange County and was isolated three times more frequently than at Avalon sites. *Enterococcus faecalis* was the species isolated most frequently at the Avalon sites. The other three species (*E. faecium, E. mundtii* and *E. hirae)* were isolated with very similar frequencies between sites and were present at frequencies between 7% and 22%. In urban runoff, *E. casseliflavus* was the single most frequently isolated species at both study locations making up 36% of isolates in Orange County and 65% of isolates in Avalon. However, the overall frequency distribution of species was dissimilar. The Orange County urban runoff species distribution was similar to the receiving water distribution with similar frequencies of isolation of each species while the Avalon urban runoff species distribution was dominated by *E. casseliflavus* (65%) and E. *faecium* (21%) with other species present at a frequency ≤4%. Sewage samples in both study locations were dominated by *E. faecium* (53–78%) with *E. faecalis* and *E. hirae* present at frequencies of 5–18%.

Figure 2 compares the frequency distribution of *Enterococcus* species associated with human and animal faecal sources (*E. faecalis, E. faecium, E. hirae, E. gallinarum* and *E. durans),* with two species considered epiphytes (*E. casseliflavus* and *E. mundtii*), for the different sample types at the two study locations. In Orange County, all of urban runoff, bay and ocean samples contain 44–50% faecal associated species and 47–54% plant associated species. In Avalon, plant associated species were most frequently isolated from urban runoff (69%) while faecal-associated species were most frequently isolated from the bay water samples (70%). In both study locations, faecal associated species make up 95–97% of isolates from sewage samples.

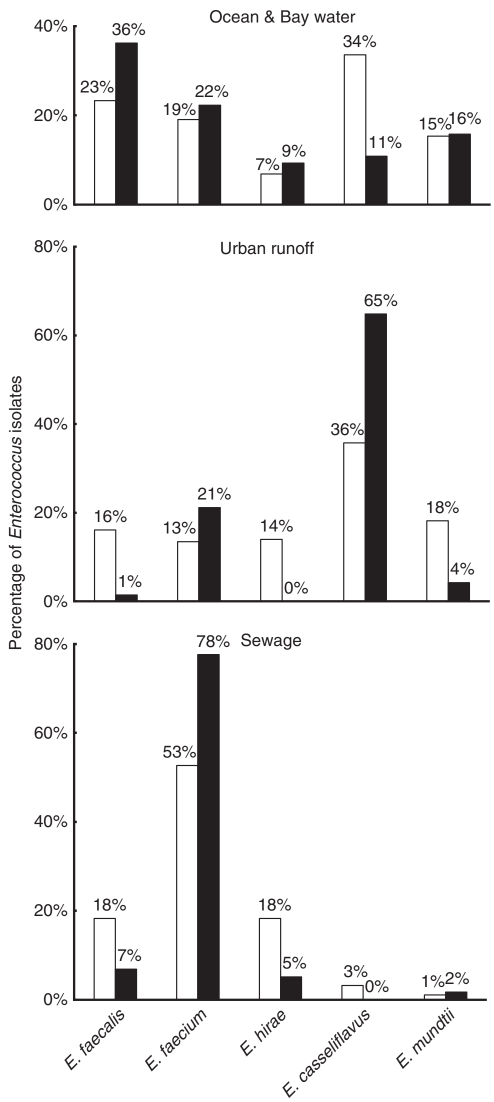

Figure 1 Frequency of isolation of *Enterococcus* species from (□) Orange County and (■) Avalon study locations.

Antimicrobial resistant patterns of *E. faecalis* and *E. faecium*

Antimicrobial resistant patterns were determined for 258 *E. faecalis* and 278 *E. faecium* isolates. Table 3 contains the percentage of resistance to nine antimicrobial agents used for treating human infections. Overall, 55% of *E. faecalis* and 21% of *E. faecium* isolates were susceptible to all antimicrobial agents tested. There was no resistance detected to vancomycin, ampicillin or high level gentamycin. Four isolates (0·7%) were resistant to high level

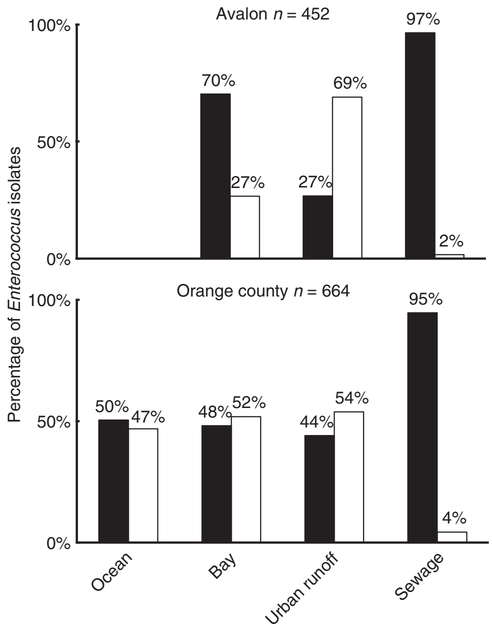

Figure 2 Distibution of (■) Faecal associated (*Enterococcus faecalis*, *Enterococcus faecium*, *Enterococcus hirae*, *Enterococcus gallinarum*, and *Enterococcus durans*) and (□) Plant associated (*Enterococcus casseliflavus* and *Enterococcus mundtii*) *Enterococcus* by sample type.

streptomycin and four (0·7%) were resistant to penicillin. Resistance to erythromycin, rifampin and tetracycline was common for both *E. faecalis* and *E. faecium* with *E. faecium* having a higher frequency of resistance to erythromycin and tetracycline. Sewage isolates of *E. faecium* had a higher rate of resistance to erythromycin and rifampin and a lower rate of resistance to tetracycline than that of environmental isolates ($P < 0{\cdot}01$). Multi-drug resistance was present in 22% (119/536) of isolates tested. Double resistant patterns of erythromycin–rifampin, erythromycin–tetracycline and rifampin–tetracycline were seen in 74 (14%) and a triple resistant pattern of erythromycin–tetracycline–rifampin was seen in 27 (5%) of the isolates tested.

Discussion

This study determined the species of *Enterococcus* present in coastal receiving waters and two potential sources of pollution, sewage and urban runoff, at two study locations chosen to be as dissimilar as possible in coastal Southern California. This was to allow us to detect the difference in *Enterococcus* species distributions at separate locations, both of which report regular water quality standard failures without known point sources (Noble *et al.* 2003). In this study, presumptive *Enterococcus* colonies were picked directly from the mEI media utilized for routine water quality monitoring and the data was pooled by the sample type. The result is an overall species distribution for each sample type that is representative of the *Enterococcus* species that are quantified during routine water quality monitoring. The species distribution in the receiving waters at both study locations was similar to that seen previously in a stream above an outfall in Scotland (Bayne *et al.* 1983), streams and lakes in the US (Rhodes and Kator 1997) and brackish water in Italy (Pinto *et al.* 1999) with *E. casseliflavus* isolated at frequencies greater than or similar to either *E. faecium* or *E. faecalis*. Several studies have shown the presence of the same *Enterococcus* species but with a higher percentage of *E. faecalis* and *E. faecium* and a lower percentage of E. *casseliflavus* (Stern *et al.* 1994; Švec and Sedláček 1999;

Table 3 Antibiotic resistance profiles among *Enterococcus faecalis* and *Enterococcus faecium*

	Number (%) resistant			
	Enterococcus faecalis		*Enterococcus faecium*	
Antibiotic	Env. water (*n* = 237)	Sewage (*n* = 21)	Env. water (*n* = 184)	Sewage (*n* = 94)
Ampicillin	0 (0)	0 (0)	0 (0)	0 (0)
Ciprofloxacin	2 (1)	0 (0)	10 (5)	1 (1)
Erythromycin	44 (19)	3 (14)	82 (45)	62 (66)*
High level gentamycin	0 (0)	0 (0)	0 (0)	0 (0)
Penicillin	1 (0·4)	0 (0)	2 (1)	1 (1)
Rifampin	72 (30)	7 (33)	61 (33)	47 (50)*
High level streptomycin	1 (0·4)	1 (5)	1 (0·5)	1 (1)
Tetracycline	32 (14)	7 (33)	50 (27)	11 (12)*
Vancomycin	0 (0)	0 (0)	0 (0)	0 (0)

Env. water, environmental water (Urban Runoff, Bay, and Ocean samples).
*Statistically different percentage of resistance between sewage and environmental water ($P < 0{\cdot}01$).

Ferguson *et al.* 2005). Two studies have shown species distributions without *E. casseliflavus* or *E. mundtii* (Grammenou *et al.* 2006) or with *E. avium* and *E. raffinosus* as the dominant species (Arvanitidou *et al.* 2001). It would be expected that the frequency of *Enterococcus* species in receiving water is dependent on potential sources. Two of the previous studies address the effect of different sources on receiving waters. Bayne *et al.* (1983) demonstrated that the frequency of *E. faecium* was increased and the percentage of *E. casseliflavus* was decreased immediately below a sewage outfall in a stream. At a site similar to this study, the distribution of species in an enclosed bay was similar to that of the urban runoff flowing into the site (Ferguson *et al.* 2005).

Both locations in this study contained storm drain systems designed to prevent flooding during rain storms by conveying runoff into drains, pipes and creeks and culverts which empty into bay and ocean receiving waters. These systems are completely separate from sewer systems. In dry weather, the low flow in these systems, termed urban runoff, flows directly into receiving waters unless diverted or treated. The urban runoff samples at both sites in this study were dominated by *E. casseliflavus* and also contained *E. mundtii*, both considered epiphytes, (Mundt and Graham 1968; Aarestrup *et al.* 2002). *Enterococcus casseliflavus* was also the most frequently isolated *Enterococcus* in stream water (Bayne *et al.* 1983) and made up 33% of isolates in urban runoff in a previous study in Southern California (Ferguson *et al.* 2005). At both study locations, the sewage species distribution was dominated by *E. faecium* with *E. faecalis* and *E. hirae* isolated at much lower frequencies. This result was slightly different from that of previous detailed studies of sewage isolates where *E. faecalis* and *E. faecium* were present in more equal frequencies (Pinto *et al.* 1999; Vilanova and Blanch 2005). The overall frequency of initial false positive mEI results was similar to that of earlier studies which reported a false positive rate of 6% (Messer and Dufour 1998). An exception to this was the unexplained high rate of *A. viridans* isolated in the Avalon bay samples and in one Orange County bay site. While this species was seen in a previous study in a similar location in Southern California, it made up only 1% of isolates (Ferguson *et al.* 2005). The source of this species in this study is unknown.

The similar distribution of *Enterococcus* species in urban runoff and receiving waters and the completely different species distribution between sewage and receiving waters at the Orange County location support the hypothesis that the source of *Enterococcus* in bays and oceans is urban runoff. Results are not as clear at the Avalon location where the distribution of species in the bay receiving water did not match either of the two potential sources studied. It is possible that the actual source could be both sewage and urban runoff, which combined, would contain all the *Enterococcus* species seen in the receiving water but at slightly different frequencies. As evidenced by the isolation of *A. viridans* in high frequencies only in Avalon bay water samples and not in sewage or urban runoff, it is also possible that there could be other potential sources that were not analysed in this study as suggested by an earlier study at this site (Boehm *et al.* 2003). Others researchers have previously suggested that determining the *Enterococcus* species present in environmental water may help determine the source (Sinton *et al.* 1993; Ott *et al.* 2001; Wheeler *et al.* 2002). This has proven difficult because the two species commonly found in high concentrations in humans faeces and sewage, *E. faecalis* and *E. faecium*, are also present in animal faecal material (Sinton *et al.* 1993; Aarestrup *et al.* 2002; Poeta *et al.* 2005). However, because *E. casseliflavus* and *E. mundtii* are epiphytes and rarely associated with human and animal faecal material (Mundt and Graham 1968; Aarestrup *et al.* 2002,), their frequent isolation, as seen here, may accurately indicate a plant source of *Enterococcus*. The frequency that these species were isolated from the receiving water in this study suggests that the source of *c.* 50% of the Orange County and 27% of the Avalon enterococci is plant material.

Besides being useful to determine the *Enterococcus* species present in receiving water in comparison to potential sources, species determination is also necessary as a first step in employing molecular fingerprinting methods such as PFGE to further characterize bacterial isolates for potential genetic relationships. *Enterococcus* species determination may also be useful in future water quality epidemiology studies and may help explain the variation in correlation between *Enterococcus* concentration and swimmer health seen in different studies (Pruss 1998; Colford *et al.* 2007).

The spread of VRE or HLAR, strains of *E. faecalis* or *E. faecium* from hospitals into surface waters through discharged sewage is a potential route for increased prevalence of these strains in the human population. The risk of this has been demonstrated by reports that have isolated VRE from both raw and treated sewage (Harwood *et al.* 2001; Iversen *et al.* 2002; Vilanova *et al.* 2004; Poole *et al.* 2005) and receiving waters (Iversen *et al.* 2002; Novais *et al.* 2005). In certain studies, VRE was associated with hospital impacted sewage only (Harwood *et al.* 2001). The study here found no VRE in sewage, urban runoff or receiving water. This is a comparable result to that of two other studies that did not isolate VRE from nonhospital impacted sewage sites (Harwood *et al.* 2001 and Novais *et al.* 2005). HLAR in the form of high level streptomycin resistance was detected at a low level in both environmental water and sewage at both study locations. This was also seen in previous studies that detected 6–12% high level streptomycin resistance in surface water and sewage (Knudtson and Hartman 1993; Rice *et al.* 1995). Similar lack of VRE and low level HLAR resistance rates were also seen in *Enterococcus* isolates from environmental water and wild animals (Arvanitidou *et al.* 2001; Poeta *et al.* 2005). In comparison to environmental isolates, VRE isolation rates in clinical enterococcal infections in US hospitals are much higher. A standardized survey demonstrated that VRE made up 12·7%, 11·5% and 4·65% of *Enterococcus* isolates from intensive care, inpatient, and outpatient clinics respectively (NNIS System 2003). Several studies have utilized enrichment or selection media containing vancomycin to increase the sensitivity of detection of resistant strains. The low levels of VRE that can be detected utilizing these techniques (0·01% of *Enterococcus* isolates) (Vilanova and Blanch 2005) could not be detected in this study. This indicates that while VRE was not detected in this study, and is not present in high concentrations in the samples examined, it may however, be present in a low frequency that will require further, more focused, studies to detect.

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References

Aarestrup, F.M., Butaye, P. and Witte, W. (2002) Nonhuman reservoirs of Enterococci. In *The Enterococci: Pathogenesis, Molecular Biology, and Antibiotic Resistance* ed. Gilmore, M.S., Clewell, D.B., Courvalin, P., Dunny, G.M., Murray, B.E. and Rice, L.B. p. 55. Washington, DC: ASM Press.

APHA (2005) *Standard Methods for the Examination of Water and Wastewater*, 21st edn. Washington, DC: American Public Health Association.

Arvanitidou, M., Katsouyannopoulos, V. and Tsakris, A. (2001) Antibiotic resistance patterns of enterococci isolated from coastal bathing waters. *J Med Microbiol* **50**, 1001–1005.

Bayne, S., Blankson, M. and Thirkell, D. (1983) Enumeration and speciation of group D streptococci from above and below a sewer outfall, their susceptibilities to six antibiotics and a comparison with clinical isolates. *Antonie Van Leeuwenhoek* **49**, 399–410.

Boehm, A.B., Fuhrman, J.A., Mrše, R.D. and Grant, S.B. (2003) Tiered approach for identification of a human fecal pollution source at a recreational beach: case study at Avalon Bay, Catalina Island, California. *Environ Sci Technol* **37**, 673–680.

Cabelli, V.J., Dufour, A.P., Levin, M.A., McCabe, L.J. and Haberman, P.W. (1979) Relationship of microbial indicators to health effects at marine bathing beaches. *AJPH* **69**, 690–696.

Carmeli, Y., Eliopoulos, G., Mozaffari, E. and Samore, M. (2002) Health and economic outcomes of vancomycin-resistant enterococci. *Arch Intern Med* **162**, 2223–2228.

Colford, J.M., Jr, Wade, T.J., Schiff, K.C., Wright, C.C., Griffith, J.F., Sandhu, S.K., Burns, S., Sobsey, M. *et al.* (2007) Water quality indicators and the risk of illness at beaches with nonpoint sources of fecal contamination. *Epidemiology* **18**, 27–35.

Devriese, L.A., Pot, B. and Collins, M.D. (1993) Phenotypic identification of the genus *Enterococcus* and differentiation of phylogenetically distinct enterococcal species and species groups. *J Appl Bacteriol* **75**, 399–408.

Facklam, R.R. and Collins, M.D. (1989) Identification of *Enterococcus* species isolated from human infections by a conventional test scheme. *J Clin Microbiol* **27**, 731–734.

Ferguson, D.M., Moore, D.F., Getrich, M.A. and Zhowandai, M.H. (2005) Enumeration and speciation of enterococci found in marine and intertidal sediments and coastal water in southern California. *J Appl Microbiol* **99**, 598–608.

Fridkin, S.K., Edwards, J.R., Courval, J.M., Hill, H., Tenover, F.C., Lawton, R., Gaynes, R.P. and McGowan, J.E., Jr (2001) The effect of vancomycin and third-generation cephalosporins on prevalence of vancomycin-resistant enterococci in 126 U.S. adult intensive care units. *Ann Intern Med* **135**, 175–183.

Grammenou, P., Spiliopoulou, I., Sazakli, E. and Papapetropoulou, M. (2006) PFGE analysis of enterococci isolates from recreational and drinking water in Greece. *J Water Health* **04**, 263–269.

Harwood, V.J., Brownell, M., Perusek, W. and Whitlock, J.E. (2001) Vancomycin-resistant *Enterococcus* spp. isolated from wastewater and chicken feces in the United States. *Appl Environ Microbiol* **67**, 4930–4933.

Huycke, M.M., Sahm, D.F. and Gilmore, M.S. (1998) Multiple-drug resistant enterococci: the nature of the problem and an agenda for the future. *Emerg Infect Dis* **4**, 239–249.

Iversen, A., Kühn, I., Franklin, A. and Möllby, R. (2002) High prevalence of vancomycin-resistant enterococci in Swedish sewage. *Appl Environ Microbiol* **68**, 2838–2842.

Knudtson, L.M. and Hartman, P.A. (1993) Antibiotic resistance among enterococcal isolates from environmental and clinical sources. *J Food Prot* **56**, 489–492.

Leclerc, H., Devriese, L.A. and Mossel, D.A.A. (1996) Taxonomical changes in intestinal (faecal) enterococci and streptococci: consequences on their use as indicators of faecal contamination in drinking water. *J Appl Bacteriol* **81**, 459–466.

Low, D.E., Keller, N., Barth, A. and Jones, R.N. (2001) Clinical prevalence, antimicrobial susceptibility, and geographic resistance patterns of enterococci: results from the SENTRY antimicrobial surveillance program, 1997–1999. *Clin Infect Dis* **32**, S133–S145.

Manero, A. and Blanch, A.R. (1999) Identification of *Enterococcus* spp. with a biochemical key. *Appl Environ Microbiol* **65**, 4425–4430.

Martinez-Murcia, A.J. and Collins, M.D. (1991) *Enterococcus sulfureus*, a new yellow-pigmented *Enterococcus* species. *FEMS Microb Lett* **80**, 69–74.

Messer, J.W. and Dufour, A.P. (1998) A rapid, specific membrane filtration procedure for enumeration of enterococci in recreational water. *Appl Environ Microbiol* **64**, 678–680.

Moore, D.F., Zhowandai, M.H., Ferguson, D.M., McGee, C., Mott, J.B. and Stewart, J.C. (2006) Comparison of 16S rRNA sequencing with conventional and commercial phenotypic techniques for identification of enterococci from the marine environment. *J Appl Microbiol* **100**, 1272–1281.

Mundt, J.O. and Graham, W.F. (1968) *Streptococcus faecium* var. *casseliflavus*, nov. var. *J Bacteriol* **95**, 2005–2009.

Murray, B.E. (1990) The life and times of the *Enterococcus*. *Clin Microbiol Rev* **3**, 46–65.

NNIS System (2003) National Nosocomial Infections Surveillance (NNIS) System Report, data summary from January 1992 through June 2003, issued August 2003. *Am J Infect Control* **31**, 481–498.

Noble, R.T., Moore, D.F., Leecaster, M.K., McGee, C.D. and Weisberg, S.B. (2003) Comparison of total coliform, fecal coliform, and *Enterococcus* bacterial indicator response for ocean recreational water quality testing. *Water Res* **37**, 1637–1643.

Novais, C., Coque, T.M., Ferreira, H., Sousa, J.C. and Peixe, L. (2005) Environmental contamination with vancomycin-resistant enterococci from hospital sewage in Portugal. *Appl Environ Microbiol* **71**, 3364–3368.

Ott, E.M., Müller, T., Müller, M., Franz, C.M.A.P., Ulrich, A., Gabel, M. and Seyfarth, W. (2001) Population dynamics and antagonistic potential of enterococci colonizing the phyllosphere of grasses. *J Appl Microbiol* **91**, 54–66.

Pinto, B., Pierotti, R., Canale, G. and Reali, D. (1999) Characterization of 'faecal streptococci' as indicators of faecal pollution and distribution in the environment. *Lett Appl Microbiol* **29**, 258–263.

Poeta, P., Costa, D., Sáenz, Y., Klibi, N., Ruiz-Larrea, F., Rodrigues, J. and Torres, C. (2005) Characterization of antibiotic resistance genes and virulence factors in faecal enterococci of wild animals in Portugal. *J Vet Med B* **52**, 396–402.

Poole, T.L., Hume, M.E., Campbell, L.D., Scott, H.M., Alali, W.Q. and Harvey, R.B. (2005) Vancomycin-resistant *Enterococcus faecium* strains isolated from community wastewater from a semiclosed agri-food system in Texas. *Antimicrob Agents Chemother* **49**, 4382–4385.

Prescott, S.C., Winslow, C.A. and McCrady, M.H., eds (1946) Other intestinal bacteria which have been used as indices of pollution. In *Water Bacteriology*, 6th edn. p. 207. New York: Wiley & Sons.

Prüss, A. (1998) Review of epidemiological studies on health effects from exposure to recreational water. *Int J Epidemiol* **27**, 1–9.

Rhodes, M.W. and Kator, H. (1997) Enumeration of *Enterococcus* sp. using a modified mE method. *J Appl Microbiol* **83**, 120–126.

Rice, E.W., Messer, J.W., Johnson, C.H. and Reasoner, D.J. (1995) Occurrence of high-level aminoglycoside resistance in environmental isolates of enterococci. *Appl Environ Microbiol* **61**, 374–376.

Sinton, L.W., Donnison, A.M. and Hastie, C.M. (1993) Faecal streptococci as faecal pollution indicators: a review. Part II: Sanitary significance, survival, and use. *NZ J Mar Freshwater Res* **27**, 117–137.

Sofianou, D., Pournaras, S., Giosi, M., Polyzou, A., Maniatis, A.N. and Tsakris, A. (2004) Substantially increased faecal carriage of vancomycin-resistant enterococci in a tertiary Greek hospital after a 4 year time interval. *J Antimicrob Chemother* **54**, 251–254.

Stern, C.S., Carvalho, M.G.S. and Teixeira, L.M. (1994) Characterization of enterococci isolated from human and nonhuman sources in Brazil. *Diagn Microbiol Infect Dis* **20**, 61–67.

Švec, P. and Sedláček, I. (1999) Occurrence of *Enterococcus* spp. in waters. *Folia Microbiol* **44**, 3–10.

Teixeira, L.M., Carvalho, M.G.S. and Facklam, R.R. (2007) Enterococcus. In *Manual of Clinical Microbiology*, 9th edn, ed. Murray, P.R., Baron, E.J., Jorgensen, J.H., Landry, M.L. and Pfaller, M.A. p. 430. Washington, DC: ASM Press.

USEPA (1983) *Health Effects Criteria for Marine Recreational Waters. EPA-600/1-80-031*. Washington, DC: United States Environmental Protection Agency.

USEPA (1986) *Ambient Water Quality Criteria for Bacteria – 1986. EPA440/5-84-002*. Washington, DC: United States Environmental Protection Agency.

USEPA (2000) *Improved Enumeration Methods for the Recreational Water Quality Indicators: Enterococci and Escherichia coli. EPA/821/R-97/004*. Washington, DC: United States Environmental Protection Agency.

USEPA (2002) *Method 1600: Enterococci in Water by Membrane Filtration Using membrane-Enterococcus Indoxyl-β-D-Glucoside Agar (mEI)*. EPA 821-R-02-022. Washington, DC: United States Environmental Protection Agency.

Vilanova, X. and Blanch, A.R. (2005) Distribution and persistence of fecal bacterial populations in liquid and dewatered sludge from a biological treatment plant. *J Gen Appl Microbiol* **51**, 361–368.

Vilanova, X., Manero, A., Cerdá-Cuéllar, M. and Blanch, A.R. (2004) The composition and persistence of feacal coliforms and enterococcal populations in sewage treatment plants. *J Appl Microbiol* **96**, 279–288.

Wheeler, A.L., Hartel, P.G., Godfrey, D.G., Hill, J.L. and Segars, W.I. (2002) Potential of *Enterococcus faecalis* as a human fecal indicator for microbial source tracking. *J Environ Qual* **31**, 1286–1293.

