



Q-432: Species Distribution and Antibiotic Resistance of Enterococci Isolated from Surface and Ocean Water

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Introduction

- Enterococcus* is one of the indicator bacteria used in California to monitor marine waters, and in many sites, is the primary cause of water quality failures.
- In this study, speciation and susceptibility testing was performed on *Enterococcus* isolates recovered during water quality testing of ocean, bay (harbors, bays and wetlands), urban runoff, and sewage in two contrasting study locations.
- A total of 1413 isolates were speciated from 373 samples collected from 36 sites along the coastal areas of Orange County and Avalon, California.
- Determination of the *Enterococcus* species isolated from receiving waters and in potential pollution sources may assist in understanding the sources of pollution.



Southern California Study Sites

Orange County, CA

Area 2455 km²
Population ~ 3,100,000
Population density 1515/km²

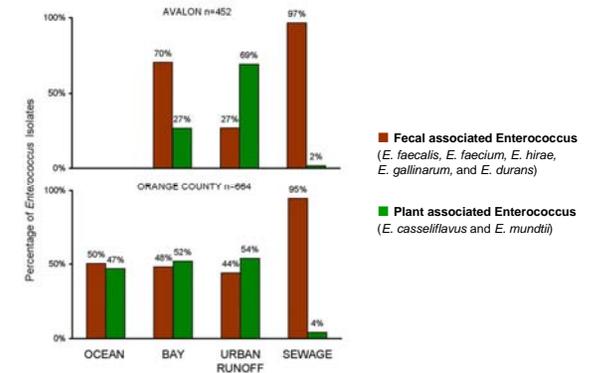


Avalon, Santa Catalina Island, CA

Area 8.15 km²
Population ~ 3,500
Population density 481/km²



OCEAN Site
BAY Site
URBAN RUNOFF Site
SEWAGE Site



Antibiotic Resistance Profiles Among *Enterococcus faecalis* and *Enterococcus faecium*

Antibiotic	Number (%) Resistant			
	<i>E. faecalis</i>		<i>E. faecium</i>	
	Environmental Water ^a	Sewage	Environmental Water ^a	Sewage
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) ^b
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) ^b
High Level Streptomycin	1 (0.4)	1 (5)	1 (0.5)	1 (1)
Tetracycline	32 (14)	7 (33)	50 (27)	11 (12) ^b
Vancomycin	0 (0)	0 (0)	0 (0)	0 (0)

^a Environmental Water (Urban Runoff, Bay, and Ocean samples)

^b Statistically different percentage of resistance between Sewage and Environmental Water (p < .01)

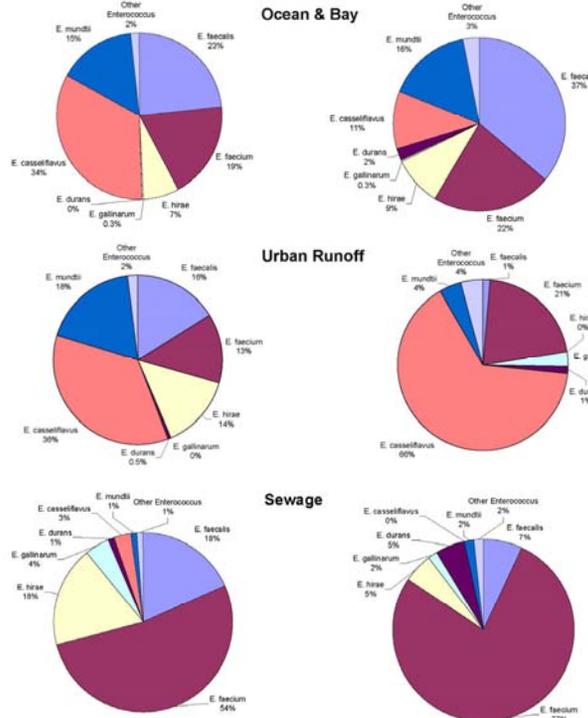
Materials and Methods

- 373 water samples were collected from two study location: Orange County, a large coastal urban area in Southern California, and Avalon, an isolated harbor town on offshore Santa Catalina Island located 45 km west of Orange County.
- 104 ocean, 178 bay, 58 urban runoff, and 33 sewage samples were tested. Samples were collected between February 2006 to October 2007.
- Sample collection, transport, and enumeration of Enterococci from water samples was performed following EPA Method 1600: Enterococci in Water by Membrane Filtration Using membrane-Enterococcus Indoxyl-β-D-Glucoside Agar (mEI).
- 1413 presumptive Enterococcus colonies were subcultured from mEI and purified, then identified to species level using the Microscan® Walk Away with Positive Combo 12 cards (Dade Behring, Sacramento, CA) and additional testing (pigment, motility, and biochemicals).
- Species identity for 14 *Aerococcus viridans* isolates was confirmed using 16S DNA sequencing with the MicroSeq database (MIDI Labs, Newark, DE).
- Susceptibility testing was performed on 258 *E. faecalis* and 278 *E. faecium* isolates on the Microscan® Walk Away with Positive Combo 12 cards.

Description of Study Sites & Enterococcus Concentrations

	No. Sites	No. Samples	Concentration (CFU/100ml)	
			Geomean	Maximum
Orange County				
Ocean	6	104	17	172
Bay	10	58	36	21000
Urban Runoff	5	43	106	1600
Sewage	2	20	2475909 [*]	12300000
Avalon				
Bay	4	120	78	2700
Urban Runoff	4	15	98745	1360000
Sewage	5	13	378070	4800000
Total	36	373		

^{*}Treated and chlorinated sewage samples removed from this calculation



Discussion / Conclusions

- Overall the five most frequently isolated *Enterococcus* species were *E. faecalis* (18%), *E. faecium* (20%), *E. hirae* (7%), *E. casseliflavus* (20%), and *E. mundtii* (11%).
- 1116 isolates confirmed as Enterococcus, while 297 isolates were not Enterococcus – 216 *Aerococcus* viridans, 18 *Streptococcus* bovis, 63 Other not-Enterococcus.
- Receiving waters at both study locations contained similar distribution of these five species with a frequency of 7-37% of *Enterococcus* isolated.
- In urban runoff, *E. casseliflavus* was the single most frequently isolated species at both study locations making up 36% of *Enterococcus* isolates in Orange County and 66% of isolates in Avalon.
- The Orange County urban runoff species distribution was similar to the receiving water distribution, supporting the theory that the source of pollution in receiving waters is urban runoff.
- Avalon urban runoff species distribution was dominated by *E. casseliflavus* (66%) and *E. faecium* (21%), which was very different than the species distribution found in receiving waters.
- In both study locations sewage samples were dominated by *E. faecium* (54-77%) with *E. faecalis* and *E. hirae* (5-18%) also present.
- E. casseliflavus* and *E. mundtii* are considered epiphytes and are rarely associated with human or animal fecal material.
- The frequency of *E. casseliflavus* and *E. mundtii* in receiving waters in Orange County and Avalon suggest that 49% and 27% respectively, of Enterococcus isolated is from plants.
- No vancomycin or high level gentamycin resistance was detected in *E. faecalis* and *E. faecium* isolates from all the environmental water samples or sewage. Four isolates (0.7%) were resistant to high level streptomycin and four (0.7%) were resistant to penicillin.

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