# Vancomycin-Resistant *Enterococcus* sp. (VRE) and Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Marine Sand and Water

Katie M. Fellows, David No, and Marilyn C. Roberts, PhD

Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, WA.

## Abstract

*Enterococcus* sp. and *Staphylococcus aureus* are part of the normally human bacterial flora, but are able to cause disease. Over the last 60 years, bacteria have developed resistance to antibiotic therapy and today vancomycin resistant *Enterococcus* (VRE) and methicillin resistant *Staphylococcus aureus* (MRSA) are both of major health concern because disease by these resistant bacteria have limited number of treatments, are more costly to treat and often require longer recovery times. No environmental VRE has been identified in North America; however, MRSA has in the last few years been associated with skin contact in gyms, jails, and hospitals. The aim of this study was to isolate VRE and MRSA from marine sand and water. Sand samples were collected from two Southern California beaches (7 samples from the first beach, and 6 from the second) and one Washington beach (8 samples). Eight water samples were also collected from the Washington beach. Selective media allowed isolation of potential VRE and MRSA while the resistance genes were verified using PCR assays and confirmed by hybridization of PCR products. No *S. aureus* was isolated from either California beach, but both CA beaches were positive for *Enterococcus*; only one sample from one beach was positive for VRE. At the Washington beach, both VRE and MRSA were isolated from both sand and water samples. This is the first report of VRE in the environment in North America and only the second time VRE has been isolated in marine beaches and the first report of MRSA isolation from marine beaches.

## Introduction

Several studies have now found MRSA in marine sand and waters in the United States but no study has found VRE in the environment in North America, though VRE was found in one Brazilian study. MRSA and VRE cause disease which is more costly to treat, increases time for recovery, and have limited number of therapies available. MRSA has been associated with skin contact in gyms, jails, and hospitals. VRE is a resilient bacterium that can survive in the environment for long periods of time. It has been isolated from wastewater, hospital sewage, swine feeding operations, and hospital environments.

**The aim of this study was to isolate VRE and MRSA from marine sand and water samples from WA and CA public beaches.**

## Materials and Methods

- **Environmental samples**: 5 isolates found from two Southern California (So. CA) beaches and one Washington beach.
  - Seven sand samples from So. CA 1
  - Six sand samples from So. CA 2
  - Eight sand and seven water samples from one Washington beach
- **Phenotypic identification of bacterial strains**: Isolation using selective media.
  - *Enterococcus* sp. selection: mE media; VRE selection: mE van6 and van18
  - *S. aureus* selection: staph110 media; MRSA selection: staph110, meth10 and supplements
- **Genotypic identification of bacterial strains**: Polymerase chain reaction assays (PCR) using 1 µl proteinase K treated bacteria as template, using denaturation temperature of 96 ºC and elongation temperature of 72 ºC
  - *vanA* gene detection in VRE using annealing temperature of 45 ºC
  - *vanB* gene detection in VRE using annealing temperature of 53 ºC
  - mecA gene detection in MRSA using annealing temperature of 53 ºC
  - SCC *mecA* typing to determine MRSA strains using annealing temperature of 55 ºC

  PCR products were verified using internal labeled probes

Table 1: Number of positive samples for each location

| Beach | Sample Type | # Samples | *Enterococcus* sp. | VRE | *S. aureus* | MRSA |
|---|---|---|---|---|---|---|
| So CA 1 | Sand | 7 | 2 | - | - | - |
| So CA 2 | Sand | 6 | 5 | 1 | - | - |
| WA 3 | Sand | 8 | 4 | 3 | 2 | 1 |
| | Water | 7 | 1 | 1 | 1 | 1 |

Table 2: Number of sample isolates positive for VRE and MRSA at each location

| | # Isolates Analyzed | VRE Positive | # Isolates Analyzed | MRSA Positive |
|---|---|---|---|---|
| **So CA 2 Sand** | | | | |
| 1 | 12 | 5 | - | - |
| **WA 3 Sand** | | | | |
| 2 | 20 | Pending | - | - |
| 3 | 20 | Pending | - | - |
| 8 | 20 | 3 | 6 | 6 |
| **WA 3 Water** | | | | |
| 7 | 5 | 1 | 6 | 6 |

## Results

- VRE was isolated from two of the three [67%] beaches sampled
- MRSA was isolated from one of the three [33%] beaches sampled
- At So. CA beach # 1, *Enterococcus* sp. was isolated, but no VRE or *S. aureus* was found
- At So. CA beach 2, *Enterococcus* sp. was isolated, as was VRE but no *S. aureus* was found
- At WA beach 3, both *Enterococcus* sp., *S. aureus*, MRSA and VRE were found in sand and water samples

## Conclusion

- **First report of VRE in the environment in North America**
- **Second report world wide of VRE in the marine environment**
- **VRE and MRSA were both isolated in 1 of 3 beaches tested**
- **There is need for surveillance of antibiotic resistance in bacteria from the environment**

## Acknowledgements

This research project was made possible by the Environmental Health Research Experience Program (EH-REP) funded by the National Institute of Environmental Health Sciences (NIEHS) grant number 1R25ES016150 - 01; the Department of Environmental and Occupational Health Sciences at the University of Washington; and Dr. Marilyn Roberts' lab – Dr. Olusegun O. Soge, Teresa White and Mike Giardino.