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

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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 the second time VRE has been isolated in marine beaches and the first report of MRSA isolation from marine 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 µ proteinase K treated bacteria as template, using denaturation temperature of 96 °C and elongation temperature of 72 °C
  - vanA gene detection in VRE using annealing temperature of 45 °C
  - vanB gene detection in VRE using annealing temperature of 53 °C
  - mecA gene detection in MRSA using annealing temperature of 53 °C
  - SCC mecA typing to determine MRSA strains using annealing temperature of 55 °C
  - PCR products were verified using internal labeled probes

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

Table 1: Number of positive samples for each location

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Beach Type</th>
<th># Samples</th>
<th>Enterococcus sp.</th>
<th>VRE</th>
<th>S. aureus</th>
<th>MRSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand</td>
<td>So CA 1</td>
<td>7</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sand</td>
<td>So CA 2</td>
<td>6</td>
<td>5</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sand</td>
<td>WA 3</td>
<td>8</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Water</td>
<td>WA 3</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>So CA 2 Sand</th>
<th># Isolates Analyzed</th>
<th>VRE Positive</th>
<th># Isolates Analyzed</th>
<th>MRSA Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>WA 3 Sand</td>
<td>2</td>
<td>20</td>
<td>Pending</td>
<td>-</td>
<td></td>
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<tr>
<td>3</td>
<td>20</td>
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<td>-</td>
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</tr>
<tr>
<td>8</td>
<td>20</td>
<td>3</td>
<td>6</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>WA 3 Water</td>
<td>7</td>
<td>5</td>
<td>1</td>
<td>6</td>
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</tr>
</tbody>
</table>

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.

Acknowledgements

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