

Isolation of Vancomycin-Resistant Enterococcus Species (VRE) and Methicillin-Resistant Staphylococcus aureus (MRSA) in Washington State fresh and marine recreational waters Luke Swart, Olusegun O. Soge, PhD, Marilyn C. Roberts, PhD



Abstract

Aim: The aim of this study was to determine whether vancomycin-resistant enterococci (VRE) and/or methicillin-resistant Staphylococcus aureus (MRSA), both opportunistic pathogens, were detectable in marine or freshwater recreational public parks and fountain. Methods: Water and sand samples were collected and processed within 24 h of collection. The water samples were processed by filtering 100 ml through a sterile membrane filter with one filter placed on Staphylococcus Medium 110 supplemented with 75 mg/L polymyxin B and 0.01% potassium tellurite and another filter on m Enterococcus media supplemented with 6 mg/ml vancomycin. Sand samples were enriched for enterococci using Brain Heart Infusion broth supplemented with 5% NaCl and enrichment for staphylococci used m Staphylococcus broth supplemented with 25 mg/L polymyxin B and 0.01% potassium tellurite. All enterococci media was incubated at 42 °C and staphylococci at 36.5 °C. Presumptive VRE samples were determined by growth on media supplemented with 18 mg/L vancomycin. Presumptive MRSA was given to isolates that grew on media supplemented with 10 mg/ml methicillin, produced b-hemolysin on blood plates and were coagulase positive. PCR assays are in progress to verify the vancomycin resistance genes (vanA and/or vanB) and presence of the methicillin-resistance gene (mecA). Results: All of the five freshwater sites were presumptive positive for VRE, while one site was confirmed positive for MRSA by PCR. In contrast, none of the six marine water sites were presumptive positive for presumptive VRE and one was presumptive positive for MRSA. Conclusion: This represents the first time that VRE and MRSA have been identified in local recreational fresh water beaches and only the second time that VRE and MRSA has been found in recreational waters in North America. The presence of these potential pathogens may pose a risk to the public.

Introduction

Methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant enterococci (VRE) are opportunistic pathogens which are able to cause serious and life-threatening infections. It is not clear how common MRSA and/or VRE are in the environments outside hospital-impacted wastewater and the routes of community transmission have not been well characterized for these pathogens. Both MRSA and VRE isolates have been identified once in North American marine waters and have not been identified in North American recreational fresh waters. The presence of VRE and MRSA in recreational marine and fresh waters would suggest that these sites are potential reservoirs for community transmission, and a potential risk to the public.

Materials & Methods

□ 52 VRE and 13 MRSA isolates from 9 water and 32 sand samples from 5 freshwater and 6 marine recreational sites

- 100 ml of water were filtered
- 5 gm of sand enriched prior to isolation of VRE and MRSA on selective selective media
- Uverification of mecA (MRSA) and vanA/vanB (VRE) using specific PCR assavs

Results

Table 1: Results for VRE, S. aureus, and MRSA from 11 sites

	VRE*	S. aureus	MRSA*
Freshwater Site 1	+		-
2	+	+	+**
3	+	-	-
4	+	-	
5	+		-
Marine Beaches			

6	-		-
7	-	-	-
8	-	-	-
9	-	+	+
10	-	-	-
11	-	-	-

*Verification by PCR and hybridization is in progress ** Confirmed by PCR

Table 2: Number of VRE and MRSA isolated from 11 sites

	# of VRE Isolates*	# of MRSA Isolates*
Freshwater Site		
1	8	0
2	17	4**
3	19	0
4	4	0
5 Marine Beaches	4	0
9	0	9

*Verification by PCR and hybridization is in progress ** 4 of 9 isolates confirmed by PCR

1 2 3 4 5 - + Ld
400bp
200bp 75bp
Figure 1: Gel showing site 2 samples for <i>mecA</i> PCR of freshwater MRSA.
Lanes 1-4 positive, - is negative control, + is positive control, Ld is ladder
Conclusions
This represents the first time that VRE and MRSA have been identified in local recreational fresh water beaches
Only the second time that VRE and MRSA has been found in recreational waters in North America
The presence of VRE and MRSA may indicate such sites as potential reservoirs

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