

Prevalence and Distribution of methicillin-resistant *Staphylococcus aureus* (MRSA) on Three Seattle-Metro Beaches



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Abstract

Objective: Methicillin-resistant *Staphylococcus aureus* (MRSA) has become a major community acquired pathogen. The aim of this study was to spatially and temporally survey two marine and one fresh water public beach, in the Seattle-Metro area for the presence of MRSA. Multiple sand [50 gm] and water [1 L] samples were collected from each beach multiple weeks during the summer of 2010. The sites sampled included multiple low and high tide water sand, ankle deep and one meter deep water, and fresh water streams that flow through the beaches. The same sites were sampled during each sampling event during the summer. **Methods:** The samples were enrichment in Difco™ mStaphylococcus Broth 75 µg/ml polymyxin and 0.01% potassium tellurite. Positive samples that turned black were 10-fold diluted and plated onto Mannitol Salt Agar and Bacto® Staphylococcus Medium 110 agar supplemented with 0.01% potassium tellurite and 10 µg/ml methicillin. Colonies were placed on 5% sheep blood agar plates and those that were β-hemolytic were biochemically verified as *S. aureus*. The presence of the *mecA* gene and the type of SCC_{mec} chromosomal element carried was determined by PCR assays. **Results:** Each beach was sampled 2-3 different weeks and a total of 196 samples collected. Twenty-nine (14.8%) different samples were MRSA positive from all three beaches. Nineteen (27.5%) of the 69 stream samples were MRSA positive, 5 (5.8%) of 87 sand [3/87 (3.5%) marine and 2/87 (2.3%) fresh] and 5 (8.9%) of 56 water [1/56(1.8%) marine and 4/56 (7.1%) fresh]. **Conclusion:** Sand, water and stream samples from both the marine and fresh water beaches were MRSA positive with highest % coming from the streams suggesting that urban run-off might be a major source of MRSA found at the marine/fresh water beaches.

Enrichment & Isolation

- ❖ 10 g of sand into Difco™ mStaphylococcus Broth with 75 µg/ml polymyxin + 0.01% potassium tellurite [Staph+ broth] eluted for 2 min
- ❖ 25 ml water samples enriched in 12.5-50 ml Staph+ broth
- ❖ All samples Incubated until growth + black at 36.5 °C
- ❖ Positive sample 10-fold diluted and plated onto Mannitol Salt Agar and Bacto® Staphylococcus Medium 110 agar +0.01% potassium tellurite + 10 µg/ml methicillin

Yellow colonies from MS →BA verify β-hemolytic
Black colonies from Staph →BA verify β-hemolytic

- ❖ β-hemolytic verified biochemically as *S. aureus*
- ❖ Biochemical verification of *S. aureus*
- ❖ PCR verification of presence of *mecA* gene and the type of SCC_{mec} element present

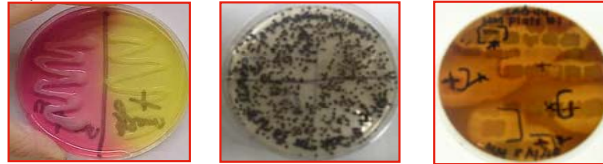


Fig 5-7 Mannitol Salt Agar Plate; Bacto® Staphylococcus Medium 110 agar +0.01% potassium tellurite + 10 µg/ml methicillin; B-hemolytic colonies on 5% Sheep Blood Agar Plate.

Results

Key

Each row for each table represents one sampling event.

Red	MRSA
Orange	MRCoNS
Yellow	CoNS
Green	Negative
Grey	Not Sampled

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a type *S. aureus* which carries the *mecA* gene coding for altered penicillin binding protein which confers resistance to all useable beta-lactam antibiotics, the drug of choice for therapy. Community acquired MRSA infections are commonly found on the skin and soft tissue. The potential risk of MRSA in recreational marine and fresh waters to the public is unknown.



Fig 1-3 L-R: Electron Micrograph of MRSA; Staph infection, located on back, caused by MRSA; Cutaneous, abscess located on arm, caused by MRSA.

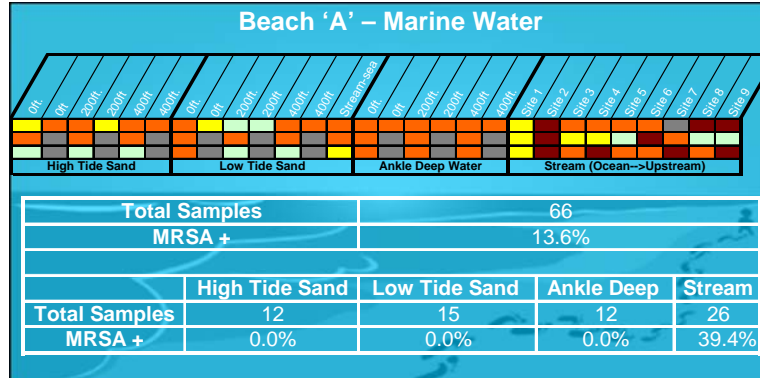
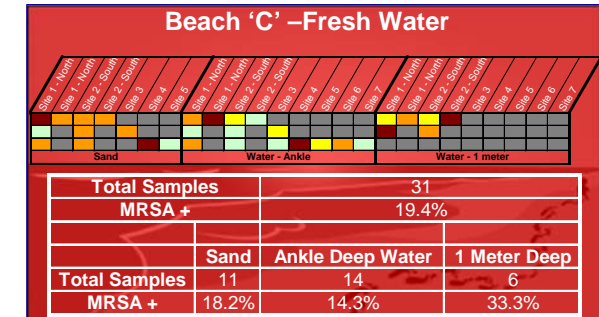
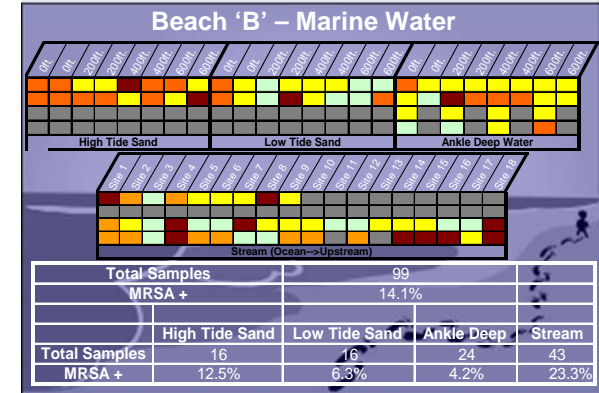
Materials & Methods

Beaches Sampled

- ❖ 2 urban marine public beaches
- ❖ 1 urban fresh water beach
- ❖ Beach samples were collected 2-3 different weeks
- ❖ Samples included 50 g sand samples at high & low tide line; ankle and 1L water samples



Fig 4: Ankle deep water sample collection at marine water beach.



- ❖ A total of 196 samples were collected from the three beaches.
- ❖ 29 MRSA+ samples were found (14.8%)
- ❖ 5 MRSA+ from 87 sand samples (5.8%); 3/87 (3.5%) marine and 2/87 (2.3%) fresh
- ❖ 5 MRSA+ from 56 water samples (8.9%); 1/56(1.8%) marine and 4/56 (7.1%) fresh
- ❖ 19 MRSA+ from 69 stream samples (27.5%)
- ❖ **65.5% of the MRSA+ samples came from the streams.**
- ❖ 17.2% of the MRSA+ samples came from the sand
33.3% from marine water beaches; 66.7% from the fresh water beach
- ❖ 17.2% of the MRSA+ samples came from the water; 75% from fresh water & 25% from marine water

Conclusions

- ❖ 14.8% of sand, water and stream samples from marine and fresh water beaches were MRSA positive
- ❖ Highest % coming from the streams
- ❖ Urban run-off might be a source of MRSA at beaches
- ❖ **First report of MRSA from freshwater beach sand/water**

Acknowledgments

A. Stiffarm funded by UW Stipends for Training Aspiring Researchers (UW STAR) - Funding Source: National Heart, Lung, and Blood Institute (NHLBI) - # 1 R25 HL103180-01