Survey of *Clostridium difficile* and Methicillin-resistant *Staphylococcus aureus* in Swimming Pools

Laura Suppes[1], Sasha Showsh[2], Zachary Zirnhelt[1], Jared Kiander[1], Zoe Kremsreiter[1]
[1]UWEC Watershed Institute for Collaborative Environmental Studies – Environmental Public Health
[2]UWEC Biology Department

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Introduction

- Limited research in the U.S. exists exploring Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Clostridium difficile* in pool water
- Previous studies in Europe and Africa detected MRSA and *C. difficile* in 7.5% and 50% of pool water samples, respectively [(1),(2)]
- No research has explored the presence or quantity of either bacteria on pool surfaces (pool walls, ladders and decks), handrails or shared swim equipment (kickboards, chairs and physical therapy weights)
- *C. difficile* is a spore-forming, fecal-oral pathogen that spreads rapidly and can survive harsh environments. The bacterium is capable of causing severe damage to the colon and can be fatal [(3)]
- MRSA is an antibiotic resistant pathogen transmitted through direct contact with an infected individual or through contact with contaminated surfaces or media

Objectives

1. Quantify MRSA and *C. difficile* in U.S. pool water
2. Quantify MRSA and *C. difficile* on pool surfaces, handrails and swim equipment

Methods

Sites

- Eleven swimming pool facilities recruited in Western Wisconsin and Eastern Minnesota
- 17 sampling dates from 2017-2019
- Three categories at each facility selected for swab sampling: pool surfaces (pool walls, ladders and decks), handrails, and swim equipment (kickboards, chairs, and physical therapy weights)
- Pool water collected at each site visit

Swab Sampling and Processing

C. difficile

On Site

- Whirl-Pak bags individually labeled with site name and sample locations
- Sterile sponge swab immersed in 0.01% peptone saline solution
- 4 square inch surface area swabbed on sample surface
- Swab placed in Whirl-Pak with 5 mL 0.01% peptone saline solution
- Before placing in cooler, swab is agitated to dislodge bacteria

In laboratory

- *C. difficile* plates labeled in duplicate for each swab sample
- 1 mL peptone solution pipetted and spread onto plate (Fig. 1)
- Plates incubated at 35°C for 72 hours in a conventional anaerobic gas jar

MRSA

On Site

- Mannitol Salt Agar with oxacillin supplement used for samples collected spring 2017 – fall 2018. Oxacillin Resistance Screening Agar Base (ORSAB) used fall 2018 - present
- Sterile sponge swab immersed in 0.01% peptone saline solution
- 4 square inch surface area swabbed on sample surface
- One half of MSA/ORSAB plate swabbed by all parts of the cotton tip applicator
- Swabbing repeated on the same surface, but different location of the surface

In laboratory

- Plates incubated at 35°C for 48 hours

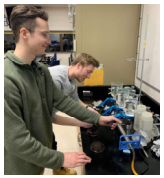
Figure 1: Peptone solution pipetted and spread onto *C. difficile* media

Water Sampling and Processing

- Pool water chemistry tested for free chlorine/bromine, combined chlorine, pH, temperature, and alkalinity (Fig. 2)
- 80 mg of sodium thiosulfate added to sterilized 1 L bottles (dechlorinate the sample once it has entered the bottle)
- Two pool water samples collected away from inlets by dipping 1L bottle in water and sweeping away from sampler in a continuous motion
- Samples placed in cooler with ice packs and immediately transported to laboratory

Figure 2: Pool water chemistry testing

Membrane filtration applied to pool water samples (Fig. 3)



- 500 mL sample shaken and poured into membrane filtration apparatus with 0.45 micrometer filter
- Filter placed on MSA/ORSAB or *C. difficile* media after filtration
- *C. difficile* plates incubated at 35°C for 72 hours in a conventional anaerobic gas chamber
- MSA/ORSAB plates incubated at 35°C for 48 hours

Figure 3: Membrane filtration

Quality Controls

Swab Samples

C. difficile

- 5 mL of 0.1% peptone solution pipetted into sterile Whirl-Pak, sterile sponge swab immersed in solution and agitated to dislodge bacteria
- 1 mL peptone pipetted and spread onto *C. difficile* plate
- Incubated at 35°C for 72 hours in a conventional anaerobic gas jar

MRSA

- Sterile cotton swab immersed in vile containing 0.1% peptone solution
- MSA/ORSAB plate swabbed with cotton applicator
- Incubated at 35°C for 48 hours

Water Samples

***C. difficile* and MRSA**

- Sterilized 1 L bottle filled with deionized water and 80 mg sodium thiosulfate
- Membrane filtration applied to DI water quality control
- Filters placed on MSA/ORSAB and *C. difficile* plates and incubated

Sample Analysis

MRSA

- Perform colony isolation for presumptive MRSA colonies (Fig. 4)
- Analyze isolated colonies by gram stain, catalase test, coagulase tube test and Polymerase Chain Reaction (PCR)

***C. difficile*:**

- Perform *C. difficile* rapid agglutination test (Fig. 5) on presumptive *C. difficile* colonies

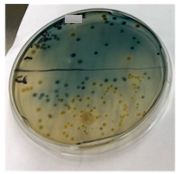


Figure 4: Presumptive MRSA colonies (blue) on ORSAB

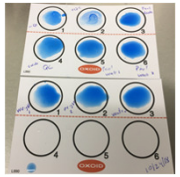


Figure 5: *C. difficile* agglutination test. Top cell second from the left shows a positive test for *C. difficile*. The remaining cells show negative tests

Preliminary Results

- Samples have been collected on 17 dates between February, 2017 to April, 2019 from 11 different swimming pools
- 7/17 samples collected from the same pool facility
- Table 1 illustrates sample locations within pool facilities and sample quantities (collected in duplicate)

Table 1: Sample locations and quantities within pool facilities

Sample location	Number of samples
Pool water	17
Handrail	13
Swim equipment	14
Pool Surfaces	17

- No MRSA or *C. difficile* has been detected in any water or swab samples
- Bacteria growth on MSA/ORSAB and *C difficile* media has been observed
- Standard deviation and mean Colony Forming Units (CFU) observed on MSA/ORSAB and *C. difficile* media by sample location are illustrated in Fig. 6. Bacteria quantities are measured in CFU/L for pool water samples and CFU/in² for swab samples

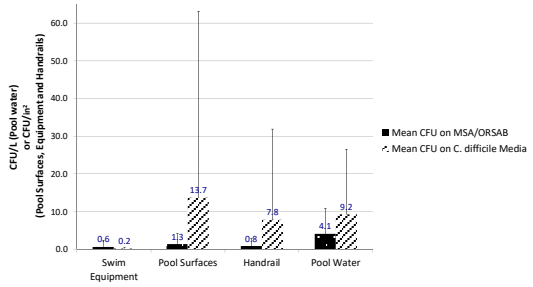


Figure 6: Bacteria Growth on *C. difficile* and MSA/ORSAB Media

Preliminary Discussion/Next Steps

- Although neither MRSA or *C. difficile* has been detected in any samples, bacteria growth on MSA/ORSAB and *C. difficile* media indicate presence of *Staphylococcus* and anaerobic bacteria species in pool water and on pool surfaces, swim equipment and handrails
- Pool surfaces (decks, ladders and pool walls) appear to have the most *Staphylococcus* and anaerobic bacteria growth, likely due to biofilm accumulation
- Handrails and swim equipment likely have the least amount of *Staphylococcus* and anaerobic bacteria growth because each are air dried. Handrails are made of stainless steel which has antimicrobial properties
- Sampling will continue into Spring 2020 to increase the sample size and reliability of results

References

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