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

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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, ficolocal 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
• 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 the name and sample location
• Sterile sponge swab immersed in 0.01% peptone saline solution
• 4 square inch surface area swabbed on sample surface
• Swabs 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
• MacConkey Sat Agar with colnical supplement used for samples collected spring 2017 – fall 2018. Deoxyribonucleic Acid (DNA) test used fall 2018 - present
• Sterile sponge swab immersed in 0.01% peptone saline solution
• 4 square inch surface area swabbed on sample surface
• Sterile sponge swab immersed in 0.01% peptone saline solution

In laboratory
• Plates incubated at 35°C for 48 hours

WATER SAMPLING AND PROCESSING

• Pool water 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 tiles by dropping 3L bottle in water and stirring away from sample in a continuous motion
• Samples placed in cooler with ice packs and immediately transported to laboratory

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/ORSA or C. difficile media after filtration
• C. difficile plates incubated at 35°C for 72 hours in a conventional anaerobic gas chamber
• MSA/ORSA plates incubated at 35°C for 48 hours

QUALITY CONTROLS

• No MRSA or C. difficile has been detected in any water or swab samples
• Bacteria growth on MSA/ORSA and C. difficile media has been observed
• Standard deviation and mean Colony Forming Units (CFU) observed on MSA/ORSA and C. difficile plates are illustrated in Fig. 4. Bacteria quantities are measured in CFU/L for pool water samples and CFU/cm² for swab samples

SWAB SAMPLES

C. difficile

• 5 mL of 0.1% peptone solution pipetted into sterile MSA-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

C. difficile

• Sterile cotton swabs immersed in sterile 0.1% peptone saline solution
• One half of MSA/ORSA plate swabbed by all parts of the cotton tip applicator
• Swabbing repeated on the same surface, but different location of the surface
• Plates incubated at 35°C for 48 hours

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

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>
<thead>
<tr>
<th>Sample location</th>
<th>Number of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pool water</td>
<td>17</td>
</tr>
<tr>
<td>Handrail</td>
<td>13</td>
</tr>
<tr>
<td>Swim equipment</td>
<td>14</td>
</tr>
<tr>
<td>Pool Surfaces</td>
<td>17</td>
</tr>
</tbody>
</table>

PRELIMINARY DISCUSSION/Next Steps

• Although neither MRSA or C. difficile has been detected in any samples, bacteria growth on MSA/ORSA and C. difficile media indicate the presence of Staphylococcus aureus 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 because any are air dried. Handrails are made of stainless steel which have antimicrobial properties
• Sampling will continue into Spring 2020 to increase the sample size and reliability of results

REFERENCES


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