Shigella flexneri is a gram negative bacterium that causes dysentery resulting in bloody diarrhea, fever, and abdominal pain. Complications caused by this infection can be fatal. A common source of Shigella infection is from the consumption of contaminated water. In the United States outbreaks of dysentery have been traced back, as recent as 2008, to inadequately chlorinated wading pools. These outbreaks are most commonly seen among small children who play in these pools (2, 3). It is well documented that water dechlorinates and loses its bactericidal capacity with exposure to high temperatures and UV (1, 4). My hypothesis is that Shigella flexneri will not survive in pool water that is properly chlorinated. In addition, the virulence plasmid carried by S. flexneri contains genes with potential to confer resistance to environmental stresses. Therefore, my second hypothesis is that the survival of wild type versus virulence plasmid cured S. flexneri will be greater once the levels of chlorine have dropped below adequate levels.

Objective

Determine how well S. flexneri survives in pool water that has been allowed to naturally dechlorinate after exposure to heat and UV in Las Vegas weather for fixed periods.

Materials and Methods

• Inoculum for pool microcosms was made by growing cultures of 2457T (Wild Type) and BS103 (cured of its virulence plasmid) in Tryptic Soy Broth (TSB) over night. The cultures were washed by three repetitions of centrifuging and resuspension with filter-sterilized pool water and normalized to cell density.
• Aliquots of 1 L of pool water were dispensed into acid-washed 2 L flasks and inoculated to a final cell concentration of a million cells/mL of washed cells.
• Samples were collected and plated every 30 mins for 7 hours.
• Cell density was unquantifiable due to the plates containing colonies that were too numerous to count.

First inoculation
• S. flexneri strains did not survive after washing in adequately chlorinated pool water (Fig. 2a).

Second inoculation
• S. flexneri strains were washed with and inoculated into inadequately chlorinated pool water (Fig. 2b).
• Only the wild type S. flexneri persisted after two days exposure (10 cells /50 mL, Table 1).

Third inoculation
• S. flexneri strains were washed with and inoculated into inadequately chlorinated pool water (Fig. 2b).

Results

Table 1. Survival of Shigella flexneri after second inoculation in inadequately chlorinated pool water.

<table>
<thead>
<tr>
<th>Day</th>
<th>2457T</th>
<th>BS103</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.2x10^6 cells/mL</td>
<td>0.99x10^6 cells/mL</td>
</tr>
<tr>
<td>2</td>
<td>10 cells/50mL</td>
<td>0 cells/50mL</td>
</tr>
</tbody>
</table>

• Adequately chlorinated pool water effectively kills Shigella flexneri after an exposure time of one hour.
• Inadequately chlorinated pool water allows for the survival of Shigella flexneri after an exposure time of one hour.
• After three days only 2457T (wild type) cells persist (Table 1).

References


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