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Survival of Shigella flexneri in swimming pool water

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Shigellosis is a disease caused by the pathogenic bacterium *Shigella flexneri*. The bacterium causes bloody diarrhea, fever, and abdominal pain. Infections can be and may cause fatal. The disease may also cause bloody diarrhea and intense intestinal crampings. In the United States, there have been many outbreaks of shigellosis have been traced back to associated with inadequately disinfected chlorinated public swimming pools and wading pools. These outbreaks of shigellosis are most commonly seen among small children who play in these pools. My goal is to determine how well *Shigella* survives in freshly chlorinated pool water and in pool water that has been stored outside in Las Vegas weather for fixed periods. It is well documented that water dechlorinates and loses its bacterial killing power with exposure to high temperatures and UV. In my experiment, pool water will be distributed into flasks and inoculated with two different strains of *Shigella*, 2457T (wild type) and BS103 (cured of its virulence plasmid). The pool water will not be chlorinated once the experiment has begun. Chlorine levels will be monitored over time and samples of water will be removed to determine the number of viable *Shigella* cells. I hypothesize that both strains of *Shigella* will not survive in pool water that is treated properly. However, once the levels of chlorine have dropped, the survival rate of wild type *Shigella* will increase.
Survival of Shigella flexneri in Swimming Pool Water
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Introduction

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 swimming 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 (2, 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 24577 (W135 Type) and BS103 (cured of its virulence plasmid) in Tryptic Soy Broth (TSB) (overnight). The cultures were washed by three repetitions of centrifuging and resuspending with filter-sterilized pool water and normalized to cell density.
- 50 ml 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 spread onto TCS plates and incubated at 37°C over night.
- Chlorine levels were monitored using Pool Time 6-way test strips.

Results

First inoculation
- S. flexneri strains did not survive after washing in inadequately chlorinated pool water (Fig. 2a)
- Only the wild type S. flexneri persisted after two days exposure (10 cells/50 mL, Table 1)

Second inoculation
- S. flexneri strains were washed with and inoculated into inadequately chlorinated pool water (Fig. 2b).
- Samples were retrieved and plated every 30 mins for 7 hours.
- Cell density was unquantifiable due to the plates containing colonies that were too numerous to count.

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

<table>
<thead>
<tr>
<th>Day</th>
<th>24577</th>
<th>BS103</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.2x10⁶ cells/mL</td>
<td>0.99x10⁶ cells/mL</td>
</tr>
<tr>
<td>2</td>
<td>10 cells/50 mL</td>
<td>0 cells/50 mL</td>
</tr>
</tbody>
</table>

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

Conclusion/Discussion

- 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 for an an exposure time of one hour.
- After three days only 24577 (wild type) cells persist (Table 1).
- Persistence of cell growth could be due to potential nutrients introduced by previous inoculation of cells.

References

2) Crane, L. (2001, July 13). Another Bacteria Sickening Drinkers. The Columbus Dispatch

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