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Does Triclosan Exposure and Tolerance Lead to Chlorine Resistant Bacteria?

Sherry Blackmon

Triclosan is an antimicrobial agent added to a wide variety of medical and consumer care products such as soaps, deodorants, toothpastes, and cleaning supplies. Bacterial exposure to triclosan could lead to chlorine resistant bacteria. These bacteria may survive chlorination, the standard method used to disinfect our drinking water. Water samples were obtained downstream a wastewater treatment plant (WWTP) in Bridgewater, MA and reference virgin stream (VS) in Monroe, MA. Bacteria were isolated from water samples, exposed to triclosan (0.001 or 0.05mg/mL), and then exposed to chlorine (0.05g/mL). 49% of all bacterial strains increased chlorine resistance after at least one triclosan exposure. Bacteria from WWTP increased chlorine resistance 80% while VS only increased resistance 19%. However, the concentration of triclosan (0.001 and 0.05mg/mL) was not significant regarding whether bacteria gained chlorine resistance (38% and 35%, respectively).

Introduction

Triclosan is used as a synthetic broad-spectrum antimicrobial agent. It was introduced as a surgical scrub containing 0.3% triclosan in 1972 and used to prevent the spread of infection in health care settings (Serafini et al., 2009). Since the mid-1990s, triclosan has been added to many personal care products including toothpaste, body soap, hand soap, hand lotions and creams, mouthwashes, and underarm deodorants (Serafini et al., 2009). These personal care products typically contain 0.1% triclosan at bacteriostatic concentrations. At bacteriostatic concentrations triclosan interferes with bacterial growth and reproduction by binding to the active site of the enoyl-acyl carrier protein reductase (ENR) enzyme (Yazdankhah et al., 2006). This target enzyme is present in microbes but not in humans.

Optimal wastewater treatment can degrade and remove 95% of triclosan (Samsoe-Petersen 2003). However, the remaining 5% may pass through the treatment plants and be released into rivers. Triclosan was found in 58 percent of 85 streams across the U.S. (Kolpin et al., 2002), the likely result of its presence in wastewater effluent.

Triclosan (2,4,4’-trichloro-2’-hydroxydiphenyl ether) is a chlorinated aromatic compound. Its functional groups include both phenols and ethers (Figure 1).
Overuse of triclosan at bacteriostatic concentrations by otherwise healthy consumers could cause strains of resistant bacteria to develop. Triclosan resistant bacteria may develop chlorine resistance due to the reactivity of the three chlorine atoms that may become bioavailable. Chlorine tolerant bacteria may survive municipal water disinfection. The standard method used to disinfect our municipal water supply is to add chlorine at concentrations sufficient to ensure a 2.0 mg/L residual chlorine concentration. Bacteria resistant to chlorine could potentially survive standard disinfection, thereby threatening the safety of our drinking water and increasing the risk for human illness. The goal of this project is to identify if triclosan can lead to chlorine tolerance in environmental bacteria strains.

Methodology

Two water samples containing bacteria were collected in October and November, 2009. The source of these samples was a virgin stream (VS) in Monroe, MA located at 42°44'7.30"N, 73°0'26.44"W and from the Taunton River, downstream from the wastewater treatment plant (WWTP) in Bridgewater, MA located at 41°59'48.04"N, 70°58'3.30"W. The VS, a tributary of the Deerfield River, was used as a reference site due to its geographic isolation. The area consists of mountainous terrain without anthropogenic influence. Bacteria obtained from an environment with minimal human impact are not expected to have been previously exposed to chemicals such as triclosan as is typically found in municipal waste.

The WWTP bacteria collected in Bridgewater, MA were obtained from the Taunton River. The Taunton River Watershed includes all or part of 43 communities in southeastern Massachusetts, including Brockton, Fall River, Foxboro, Wrentham, Plymouth and Carver. Water from the river is first collected by an up-stream city or town and is disinfected by chlorination. Once disinfected, the water is pumped to homes and businesses as drinking water. Used water sewers is collected by the WWTP where it is filtered and pollutants are bacterially degraded for safe release back into the river for collection by the next town downstream where the disinfection process will repeat.

The WWTP bacteria obtained for this research were obtained from the effluent released by the Bridgewater wastewater treatment plant. Bacteria contained in the effluent have faced prior chemical exposure to substances such as triclosan and may have developed tolerance. VS bacteria would not be expected to have had previous triclosan exposure.

To obtain a chlorine dose-response inhibition assay, four different bacterial colonies were isolated and grown overnight at 37°C and 275 rpm and the next day transferred in a 1:100 dilution. The bacteria were then pipetted into LB nutrient broth containing 2.0 mg/mL, 0.67 mg/mL, 0.22 mg/mL, 0.07 mg/mL, 0.02 mg/mL, 0.008 mg/mL, 0.003 mg/mL or 0 mg/mL chlorine. The bacteria were left in solution and incubated overnight at 37°C and 275 rpm. The next day the sample was read on a spectrophotometer at 600nm and the absorbance measured. Statistical significance was determined using t-test if p ≤ 0.05.

After obtaining a chlorine dose-response inhibition assay, a similar procedure was followed to obtain a triclosan dose-response inhibition assay. This time three different strains of bacteria were diluted following the same procedure as above. The bacteria were then exposed to LB nutrient broth containing 4.0 mg/mL, 1.0 mg/mL, 0.25 mg/mL, 0.06 mg/mL, 0.02 mg/mL, 0.004 mg/mL, 0.001 mg/mL or 0 mg/mL triclosan. The bacteria were incubated overnight at 37°C and then read on a spectrophotometer at 600nm. Statistical significance was determined using t-test if p ≤ 0.05.

The next experiments isolated bacteria that increased chlorine tolerance after triclosan exposure. 84 WWTP bacterial strains were cultured in a 96 well-plate containing nutrient broth and grown overnight at 37°C and 275 rpm. The next day the bacteria were diluted in a 1:100 dilution and pipetted to a 96 well-plate containing plain nutrient broth, 0.001 mg/mL triclosan (triclosan low), 0.05 mg/mL triclosan (triclosan high), or 0.05 g/mL chlorine. The bacteria were incubated overnight at 37°C and 275 rpm and then read on a spectrophotometer at 600nm. The bacteria in the triclosan low and triclosan high microplates were diluted in a 1:100 dilution and then exposed to 0.05g/mL chlorine. The bacteria were incubated overnight at 37°C and 275 rpm and then read on a spectrophotometer at 600nm. The entire process was then repeated using 84 VS bacterial strains.

Results

A chlorine dose-response inhibition assay was obtained. Bacterial strains expressed a dose response inhibition to chlorine (Figure 2). The Lowest Observed Effect Concentration (LOEC) was 0.67 mg/mL chlorine. This value represents the lowest chlorine concentration resulting in bacterial growth.
distinguishable from the normal (control) bacterial growth under the same exposure conditions. The No Observed Effect Concentration (NOEC) was 0.22 mg/mL chlorine. This value represents the chlorine concentration that produced no detectable alteration in bacterial growth when compared to the normal (control) bacterial growth.

Bacterial strains also expressed a dose response inhibition to Triclosan (Figure 3). The Lowest Observed Effect Concentration (LOEC) was 0.004 mg/mL triclosan. The highest concentration of triclosan that did not effect bacterial growth (NOEC) was 0.0001mg/mL.

In both samples (VS & WWTP) the bacterial strains showed an increase in chlorine tolerance after exposure to both high and low concentrations of triclosan (Figure 4). 49% of all bacterial strains (82/168 strains) showed increased resistance to chlorine following one of the triclosan exposures. WWTP bacterial strains significantly increased chlorine resistance after triclosan exposure (80%) when compared to VS bacterial strains (19%). There was no significant difference in chlorine resistance regardless of triclosan concentration: 38% (65/168 bacterial strains) increased resistance to chlorine following exposure to low triclosan (0.001 mg/mL) whereas 35% (59/168 bacterial strains) increased resistance to chlorine following exposure to high triclosan (0.05 mg/mL). Significantly fewer strains (0% and 6%) decreased resistance to chlorine following low and high triclosan, respectively.

Table 1: Bacterial acclimation to chlorine after triclosan exposure, (+) indicates an increase in chlorine resistance after triclosan exposure. (-) indicates a decrease in chlorine resistance after triclosan exposure.

<table>
<thead>
<tr>
<th>Change in chlorine tolerance</th>
<th>Virgin Stream (VS)</th>
<th>Wastewater Treatment Plant (WWTP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(0.001 mg/mL triclosan)</td>
<td>7 (+)</td>
<td>58 (+)</td>
</tr>
<tr>
<td>(0.05 mg/mL triclosan)</td>
<td>12 (+)</td>
<td>47 (+)</td>
</tr>
</tbody>
</table>

**Discussion**

49% of all bacterial strains showed increased resistance to chlorine following exposure to triclosan. Bacteria resistant to triclosan could potentially survive chlorination, thereby threatening the safety of our drinking water and increasing the risk of human illness. While alternatives to chlorination do exist, they are more expensive and our infrastructure is not currently well suited to accommodate these alternative methods of water disinfection.

Furthermore, while half of all bacterial strains showed resistance to chlorine, perhaps of greater concern is the distribution of bacteria showing chlorine resistance. 80% of WWTP bacteria showed increased resistance to chlorine and these bacteria pose a potentially greater risk to human health than the 19% VS bacterial strains showing resistance to chlorine. The VS
bacterial strains have likely had no previous triclosan exposure as they were obtained from an area with minimal human impact, whereas the WWTP bacteria are more likely to have been previously exposed to chemical compounds such as triclosan. In addition to the potential threat to the safety of our municipal water supply, chlorine resistant bacteria in the 80% range could alter the ecology of the Taunton River over time.

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Works Cited

