Inside the Burch Lab: E. Coli and Triclosan Resistance

By: Pamela Lammonds
Purpose and Goals of Research

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From *The New York Times*

**JUNE 13, 2000**

**Antibiotic Misuse Turns Treatable to Incurable**

**DECEMBER 28, 2000.**

**Drug-Resistant Bacteria Still on the Rise**
Antimicrobial Resistance among Pathogens Causing Hospital-Onset Infections

Methicillin (oxacillin)-resistant Staphylococcus aureus

Vancomycin-resistant enterococci

Source: National Nosocomial Infections Surveillance System (CDC)
Purpose and Goals of Research

- Concerns over infectious disease have risen in the past few years.
- In response to this concern, many antibacterial products have been flooding the market.
A common ingredient found in antibacterial products is the biocide triclosan.

Triclosan kills bacteria by disrupting the enzyme FabI, which is involved in fatty acid biosynthesis.

As a result, the bacteria can develop resistance to triclosan through mutations in FabI or through active efflux.
Resistance through active efflux:

- Antibiotics
- Organic solvents
  - Triclosan
- Pine oils
- Bile salts
The resistance mechanisms, such as active efflux, can cause cross-resistance to other antibiotics. This can lead to the evolution of “super bacteria” that is unresponsive to many different antibiotics.
If resistance occurs through active efflux: Resistance to commercial antibacterials may give cross resistance to medical antibiotics.
One of the main ways to combat the antibiotic resistance rise is to minimize the commercial use of antibiotics. In this case, the use of triclosan should be reduced commercially.
The effect of triclosan at its MIC (minimum inhibitory growth) on E. coli was examined.

Triclosan resistant mutants were generated in the lab and were assayed to detect cross-resistance to other commonly used antibiotics.
Obtaining Triclosan Mutants

- Mutants were obtained by inoculating 10 mL of LB broth with one E. coli colony. After being incubated overnight at 37°C, 100 μL of the culture was plated on petri-plates containing triclosan.

- Wild type E. coli cannot grow on these plates. Only resistant mutants could grow.

- The cells were in a growth period of 48 hours to form colonies. A triclosan resistant mutant was isolated from each plate and frozen for later use. This process was repeated until 100 mutants were acquired.
Antibiotic Cross-resistance

- E. Coli does not grow at the MICs for triclosan and other antibiotics.
- In order to assay for cross-resistance of mutants, mutant strains were plated on LB plates containing different antibiotics at the E. coli MICs.
- 15 mutants were tested for cross-resistance to the antibiotics ampicillin, chloramphenicol, kanamycin, nalidixic acid.
X’s indicate cross-resistance, meaning colonies grew on the plates in question.
My Focus

- By sequencing a candidate gene for triclosan resistance in mutants with different cross-resistance profiles, you may be able to determine if the gene is most likely to confer cross-resistance.
- The gene focused on in this research was the soxS gene. SoxS is described as a regulatory gene.
PCR (polymerase chain reaction)

- In order to analyze the molecular structure of a gene, many copies have to be obtained. The polymerase chain reaction is the method used to obtain these copies.
- PCR is a DNA synthesis reaction in which a certain section of DNA is repeatedly copied to amplify the number of copies of that particular sequence.
1. Start with a solution containing template DNA, synthesized primers, and an abundant supply of the four dNTPs.

2. Denaturation
Heating leads to denaturation of the double-stranded DNA.

3. Primer binding
At cooler temperatures, the primers anneal to the template DNA by complementary base pairing.

4. Extension
During incubation, DNA polymerase synthesizes complementary DNA strand starting at the primer.

5. Repeat cycle of three steps (2–4) again, doubling the copies of DNA.

6. Repeat cycle again, up to 20–30 times, to produce millions of copies of template DNA.
Once many copies of the gene were made by PCR we could sequence the gene.
Results

- The sequences of the triclosan mutants can tell us if resistance is occurring through active efflux.

- Active efflux is dangerous because in addition to removing triclosan from the cell, active efflux also removes other helpful chemicals like antibiotics.
The End