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Comparative Analysis of Triclosan Resistance in *E. coli*, *S. aureus*, and *S. cerevisiae*

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ABSTRACT

The introduction of antibiotics into everyday life has led to untreatable infections because some bacteria are resistant to nearly all forms of antibiotics. One antimicrobial, triclosan, has been used for over 30 years in an attempt to control microbe growth on commercial products. In this study, *Escherichia coli*, *Staphylococcus aureus*, and *Saccharomyces cerevisiae* were used as model organisms to demonstrate the effects of triclosan on gram-negative, gram-positive, and eukaryotic organisms, respectively. The organisms were grown in a 96-well plate that contained serial dilutions of triclosan. This study propagated triclosan resistance over several generations, which could provide insight into which group of organisms are more susceptible or resistant to triclosan. The results of this study showed that *E. coli* is sensitive to triclosan, whereas *S. aureus* and *S. cerevisiae* demonstrated higher levels of resistance.

1 INTRODUCTION

The rampant use of antibiotics has led to the development of antibiotic resistant strains of bacteria, which are more difficult to treat and to control the spread of infections. With the market flooded with antibiotics and a public fear of "superbugs", the usage of broad-spectrum antimicrobials are on the rise (Alanis, 2005). Triclosan is a broad spectrum antimicrobial that has been incorporated in health care and other consumer products, including toys, textiles, carpets, and hospital linens (Schweizer, 2001). It has proven to be a powerful tool in fighting microbes, and triclosan is the active ingredient in some hand soaps. However, in recent years, it has been discovered that *S. aureus* can become resistant to the effects of triclosan (DiPetrillo et al., 2004).

The mechanism by which triclosan kills is understood and provides insight into its broad-spectrum activity. Triclosan is a synthetic, nonionic bisphenol, which impairs the formation of fatty acids (Bhargava & Leonard, 1996). FabI is a protein involved in the fatty acid biosynthesis cycle, and triclosan is able to bind to FabI resulting in a stable structure (Heath et al., 1999). The triclosan-FabI complex can no longer participate in fatty acid biosynthesis. A recent study has shown a missense mutation in the FabI gene will grant resistance to triclosan (Moore et al., 2014).

The purpose of this study was to evaluate whether a gram-negative, gram-positive, or eukaryote would demonstrate resistance to triclosan. These organisms were selected due to differences in their cell membranes. Due to the presence of an outer membrane in its cell wall, it was hypothesized that *E. coli* would have the least resistance.

2 METHODS AND MATERIALS

The broth-dilution assays were performed in a round bottom 96-well plate. BACDOWN antimicrobial hand soap (Fisher Scientific) was chosen for this experiment because its active ingredient is triclosan. The soap was diluted to 1/4 concentration in DI water and mixed to ensure a homogenous solution. Wells A-F: 2-12, had 100 µL of Mueller-Hinton (MH) broth added, and then 200 µL of the triclosan dilution was added to wells A-F. Then, 100 µL of solution from well 1 was transferred to well 2. Then 100 µL of solution from well 2 was transferred to well 3. A new pipette tip was used every time to prevent cross contamination. This process was repeated for every well, with 100 µL of solution removed from well 12 and decanted. The dilution series resulted in a row with a step-wise dilution of 50% decrease in concentration.

All of the following cultures came from an overnight stock and were not diluted. 100 µL of *E. coli* was pipetted into every well in rows A and B, 100 µL of *S. aureus* was pipetted into every well in rows C and D, and 100 µL of *S. cerevisiae* was pipetted into every well in rows E and F. Wells G1 and H1 had 100 µL of MH broth added with 100 µL of *E. coli* as a control condition to evaluate growth in the absence of triclosan. Similarly, wells G2 and H2 had 100 µL of MH broth added with 100 µL of *S. aureus* to create a control, and wells G3 and H3 had 100 µL of MH broth added with 100 µL of *S. cerevisiae* to create a control. The plate was incubated at 37 °C for 24 hours.

Growth was assessed by visual analysis of turbidity and for the presence of precipitated cells, or a spot, at the bottom of each well. When cell growth occurred, the organisms were removed from the well to be placed into the next plate to start a new cycle.

The second plate had E. coli transferred into 2 mL of MH broth, mixed, and then put into a new plate with the initial triclosan concentration at 1/6, and then the subsequent 50% reduction for rows A and B. *S. aureus* and *S. cerevisiae* was repeated again, so another series dilution was performed again. All the former steps remained the same, except the overnight stock was diluted 1/10 concentration in MH broth and mixed. The organisms were then pipetted into their respective wells and incubated for 24 hours at 37 °C.

The third plate was set up exactly like the first one, except a new overnight culture for all three organisms was created and then diluted to 1/10 concentration before being pipetted onto the plate and incubated for 24 hours at 37 °C.

The third plate was analyzed, and a Kirby-Bauer disk diffusion assay was created to determine if the biomass in the highest concentration wells were viable for cell growth. The *E. coli* plates had a 1/64 concentration triclosan disk created by diluting triclosan in DI water. The *S. aureus* and *S. cerevisiae* plates had a 1/4 concentration disk created by diluting triclosan in DI water. Each organism was plated three times, with the triclosan disk and an oxacillin as a control. The plates were incubated 24 hours at 37 °C, and the zones of inhibition were measured.

Table 1. Results from the first plate of triclosan dilution series represents *S. aureus* and *S. cerevisiae* grew in every well, and *E. coli* grew in 1/1024 dilution. This supports that *E. coli* is less resistant to triclosan, and *S. aureus* and *S. cerevisiae* are resistant.

		Dilutions											
Organism	**Column**	1 1/4	2 1/8	3 1/16	4 1/32	5 1/64	6 1/128	7 1/256	8 1/512	9 1/1024	10 1/2048	11 1/4096	12 1/8192
E. coli	A	O	O	O	O	O	O	O	O	X	X	X	X
E. coli	B	O	O	O	O	O	O	O	O	X	X	X	X
S. aureus	C	X	X	X	X	X	X	X	X	X	X	X	X
S. aureus	D	X	X	X	X	X	X	X	X	X	X	X	X
S. cerevisiae	E	X	X	X	X	X	X	X	X	X	X	X	X
S. cerevisiae	F	X	X	X	X	X	X	X	X	X	X	X	X
Control	G	X[1]	X[2]	X[3]									
Control	H	X[1]	X[2]	X[3]									

[1] *E. coli* control [2] *S. aureus* control [3] *S. cerevisiae* control

Table 2. Results from the second plate of triclosan dilution series. *E. coli* from the 1/1024 dilution from plate one was transferred to plate two, and experienced an increase in triclosan resistance. *S. aureus* and *S. cerevisiae* are resistant to triclosan, even at high concentrations.

		Dilutions											
Organism	**Column**	1 1/6	2 1/12	3 1/24	4 1/48	5 1/96	6 1/192	7 1/384	8 1/1536	9	10	11	12
E. coli	A	O	X	X	X	X	X	X	X				
E. coli	B	O	X	X	X	X	X	X	X				
		1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096	1/8192
S. aureus	C	O	O	X	X	X	X	X	X	X	X	X	X
S. aureus	D	O	O	X	X	X	X	X	X	X	X	X	X
S. cerevisiae	E	X	X	X	X	X	X	X	X	X	X	X	X
S. cerevisiae	F	X	X	X	X	X	X	X	X	X	X	X	X
Control	G	X[1]	X[2]	X[3]									
Control	H	X[1]	X[2]	X[3]									

[1] *E. coli* control [2] *S. aureus* control [3] *S. cerevisiae* control

Table 3. The protocol for plate one was repeated, except all the dilutions for the organism culture was diluted 1/10 to ensure initial inoculation was causing false growth. Results are similar to the first plate. Again, *E. coli* is less resistant to triclosan.

		Dilutions											
Organism	**Column**	1 1/4	2 1/8	3 1/16	4 1/32	5 1/64	6 1/128	7 1/256	8 1/512	9 1/1024	10 1/2048	11 1/4096	12 1/8192
E. coli	A	O	O	O	O	X	X	X	X	X	X	X	X
E. coli	B	O	O	O	O	X	X	X	X	X	X	X	X
S. aureus	C	X	X	X	X	X	X	X	X	X	X	X	X
S. aureus	D	X	X	X	X	X	X	X	X	X	X	X	X
S. cerevisiae	E	X	X	X	X	X	X	X	X	X	X	X	X
S. cerevisiae	F	X	X	X	X	X	X	X	X	X	X	X	X
Control	G	X[1]	X[2]	X[3]									
Control	H	X[1]	X[2]	X[3]									

[1] *E. coli* control [2] *S. aureus* control [3] *S. cerevisiae* control

3 RESULTS

In all tables, an O represents no observed growth and X represents observed growth. In the first plate, we observed that *E. coli* had growth in the 1/1024 concentration of triclosan and the subsequent lower concentrations. Surprisingly, *S. aureus* and *S. cerevisiae* had growth at every concentration of triclosan (Table 1).

The results from the second plate are shown in Table 2. *E. coli* taken from the 1/1024 concentration of triclosan in plate 1 showed substantial growth in 1/12 concentration of triclosan, and the subsequent lower concentrations. *S. aureus* demonstrated growth at 1/16 concentration of triclosan, and the subsequent lower concentrations. *S. cerevisiae* grew at every concentration of triclosan. Table 3 shows *E. coli* had growth at 1/64 concentration of triclosan, and the subsequent lower concentrations. *S. aureus* and *S. cerevisiae* exhibited growth at every concentration of triclosan.

The disk diffusion assay had no growth on all 9 plates, except for an unidentified contaminant colony on one of the plates.

4 DISCUSSION

The purpose of this experiment was to cultivate resistance over subsequent generations in the model organisms *E. coli*, *S. aureus*, and *S. cerevisiae*. However, the results do show that *E. coli* is more susceptible to triclosan than *S. aureus*, and *S. cerevisiae*.

E. coli did not grow at the highest concentrations because it is susceptible to triclosan. The gram-negative wall, which does not have a thick peptidoglycan layer, may have been unable to protect the cell from the soap mixture. Then, with FabI unable to function, the cell cannot make a plasma membrane, which would lead to lysis of the cell.

For *S. aureus*, and *S. cerevisiae*, the data suggests that both organisms are resistant to triclosan. This conclusion is supported by the 10-fold stock dilution for the second and third plates, which was done to decrease the presence of cellular debris, and resulted in a spot in every well.

Although the broth-dilution assays did not indicate susceptibility of *S. aureus* or *S. cerevisiae* to triclosan, the disk diffusion assay did not show any growth. These contradictory results may be due to inhibitory properties of the full strength triclosan (0.5%) placed on the disks, whereas the broth dilution assays tested 0.06% as the highest concentration. Higher concentrations of triclosan in the disk diffusion assay may have led to neutralization of FabI (Heath et al., 1999). It is also possible that other ingredients in the soap, such as surfactants, limited growth in the disk-diffusion assay. Once the organisms were spread onto the agar, the higher concentration of triclosan and soap mixture killed the cells, resulting in no growth.

Our results are consistent with the recent finding that *S. aureus* can become resistant to the effects of triclosan (DiPetrillo et al., 2004). Furthermore, our study indicates that fungal cells can also be less susceptible to the effects of triclosan than a gram-negative. Overuse of products containing triclosan may lead to an increase in resistant bacteria in the environment and limit the efficacy of antimicrobial products.

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