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HONORS THESIS

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Introduction

Within a society concerned with the spread of infectious disease, many common cleaning products boast high germ mortality rates. Consumers have learned to trust marketed disinfectants to protect them from disease causing microbes. While "antibacterial" sells in the consumer pursuit of cleanliness, concerns regarding the overuse of antibacterial agents have recently arisen. Bacteria have the ability to mutate and become resistant to antibiotics. Resistant mutations can result from prolonged or repeated exposure of the bacteria to the antibiotic. Theoretically, the genes that code for resistance negatively affect the bacteria's fitness. It is expected that a change in a gene's normal function will alter the fitness of an organism. Therefore, when no longer exposed to the antibiotic, the bacteria may "evolve backward" and lose resistance in order to be a better competitor. But recent studies indicate that this is often not the case.[1] The bacterium *E. coli* demonstrates reduced mutation reversion, and in many cases the development of further compensatory mutations in the absence of antibiotics. When consumers reach to antibacterial products to kill bacteria, they may actually be encouraging its growth. Creating a bacteria-free home may be futile, or even counterproductive.

Triclosan (5-chloro-2-(2,4-dichlorophenoxy) phenol) is a common antibacterial agent present in many soaps, detergents, and cleaners. Triclosan blocks lipid synthesis in *E. coli* by specifically inhibiting the enzyme enoyl-acyl carrier protein reductase. A mutation in

[1] "Overuse of Triclosan may be creating resistant bacteria." Infectious Disease News. September 1998. Proquest. Online. 3 October 2000.

the gene *fab1*, which encodes enoyl reductase, prevents this blockage.[2] N-alkyl is another common household disinfectant present in bleach products and Lysol®. N-alkyl demonstrates *in vitro* antibacterial activity and is thought to be a protease inhibitor. N-alkyl is a toxin to humans as well as bacteria. It is therefore-not prescribed orally to treat bacterial infections. As a result, bacterial resistance to n-alkyl appears to be less common than to widely used antibiotic families.[3]

This experiment seeks to determine and compare the mortality rate of Wild Type *Escherichia coli* using cleaning products containing the known antibacterial agents triclosan and n-alkyl at 1X, .1X, and 10X manufacture recommended dilution. In addition, the mortality rate of *E. coli* using general cleaning products will be studied. Comparison of these target and non-target products will reveal the most significantly effective products on reducing *E. coli* viability.

Materials and Methods

Bacterial isolates of *Escherichia coli* (Young lab) were obtained and plated on agar plates containing LB Broth EZMix™ (Sigma Chemical Co.). Plates were incubated overnight at 37° C. An overnight liquid culture was then started from a single colony and a 10^{-6} dilution of this culture was used for subsequent experiments.

Household disinfectants were purchased from the shelf at a local grocery store. Two liquid dish soap detergents, Joy® (Proctor and Gamble, Cincinnati, OH) without triclosan and Clout® (Kirkland Signature, Seattle, WA) with triclosan were selected. Two liquid hand soaps Clean and Smooth® (Benckiser, Greenwich, CT) without triclosan and Softsoap® (Colgate-Palmolive Co., New York, NY) with triclosan were selected. Two laundry detergents, and Arm and Hammer® (Church and Dwight Co., Inc., Princeton, NJ) without n-alkyl and Tide with Bleach® (Proctor and Gamble, Cincinnati, OH)

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[2] McMurry, LM; Oethinger, M; Levy, SB. "Triclosan targets lipid synthesis". Nature. Volume 394; 1998; 831-832.

[3] Pagani, G; Pregnolato, M; Ubiali, D; Terreni, M; Piersimoni, C; Scaglione, F; Fraschini, F; Gascon, AR; Munoz, JLP. "Synthesis and in Vitro Anti-Mycobacterium Activity of N-Alkyl-1,2-dihydro-2-thioxo-3

containing n-alkyl were selected. Two all purpose cleaners, Pine-Sol® (The Clorox Company, Oakland, CA, active ingredient: Pine Oil) and Lysol All Purpose Cleaner® (Reckitt and Colman Inc., Wayne, NJ, active ingredient: n-Alkyl (50% C_{14}, 40% C_{12}, 10% C_{16}) dimethyl benzyl ammonium chlorides) were selected.

Cleaners were prepared to .1X, 1X, and 10X recommended use dilution.

Table I. Recommended Use Dilution of Cleaning Products

Cleaning Product	Soap : Water Ratio
Liquid dish soap	0.125 ml : 50 ml
Hand soap	10 µl : 5 ml
Laundry detergent	30 ml : 250 ml
All purpose cleaners	6 ml : 400 ml

E. coli was subjected to all products at all dilutions. 0.1 ml of *E. coli* and 0.1 ml of each product was surface plated and incubated overnight at 37°C along with control plates inoculated with bacteria only. Colony forming units were then counted.

Results

The research was designed so that all data could be analyzed by ANOVA, completely randomized design, with the level of significance set at 5% before testing.

H_0: $T_i = 0$

H_1: $T_i \neq 0$

$\alpha = .05$

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pyridinecarbothioamides. Preliminary Toxicity and Pharmacokinetic Evaluation." Journal of Medicinal Chemistry. Volume 43, no. 2. January 2000. 199-204.

t = 7

b = 2

N = 14

Table II. Number of Bacteria Colonies Grown in Presence of Dish Soap

Product	# Colonies	# Colonies	Xi	Average Xi
Joy® .1X	77	49	126	63
Joy® 1X	37	34	71	35.5
Joy® 10X	29	3	32	16
Clout® .1X	249	63	312	156
Clout® 1X	9	6	15	7.5
Clout® 10X	0	2	2	1
No soap	191	59	250	125

Table III. ANOVA Results for Dish Soap

Source	Df	SS	MS	F	CR @ 1, 12
Total	13	71124.858			
Treatment	1	44373.858	44373.858	48.90	4.75
Error	12	26751.000	2229.250		

Decision: The calculated statistic lies in CR. Reject H_0 with 5% probability of Type I error.

Table IV. MRT Results for Dish Soap

Compare	Difference	SE	q_{calc}	Range of Means	q_{table}	Decision
7-1	155	33.386	4.643	7	4.950	DNR
7-2	148.5	33.386	4.448	6	4.751	DNR

7-3	140	33.386	4.193	5	4.508	DNR
7-4	120.5	33.386	3.609	4	4.199	DNR
7-5	93	33.386	2.786	3	3.773	DNR
7-6	31	33.386	0.929	2	3.082	DNR
6-1	124	33.386	3.714	6	4.751	DNR
6-2	117.5	33.386	3.519	5	4.508	DNR
6-3	109	33.386	3.265	4	4.199	DNR
6-4	89.5	33.386	2.681	3	3.773	DNR
6-5	62	33.386	1.857	2	3.082	DNR
5-1	62	33.386	1.857	5	4.508	DNR
5-2	55.5	33.386	1.662	4	4.199	DNR
5-3	47	33.386	1.408	3	3.773	DNR
5-4	27.5	33.386	0.824	2	3.082	DNR
4-1	34.5	33.386	1.033	4	4.199	DNR
4-2	28	33.386	0.839	3	3.773	DNR
4-3	19.5	33.386	0.584	2	3.082	DNR
3-1	15	33.386	0.449	3	3.773	DNR
3-2	8.5	33.386	0.255	2	3.082	DNR
2-1	6.5	33.386	0.195	2	3.082	DNR

Conclusion: There was no significant difference between the number of viable *E. coli* colonies in the presence of the dish soap Joy® at .1X, 1X, and 10X dilution and the dish soap Clout® at .1X, 1X, and 10X dilution.

Table V. Number of Bacteria Colonies Grown in Presence of Hand Soap

Product	# Colonies	# Colonies	Xi	Average Xi
Clean and Smooth® without	42	87	129	26

triclosan .1X				
Clean and Smooth® without triclosan 1X	33	34	67	4.5
Clean and Smooth® without triclosan 10X	53	34	87	0.5
Softsoap® with triclosan .1X	44	39	83	41.5
Softsoap® with triclosan 1X	36	31	67	33.5
Softsoap® with triclosan 10X	13	21	34	17
No soap	191	59	250	125

Table VI. ANOVA Results for Hand Soap

Source	df	SS	MS	F	CR @ 1, 12
Total	13	32047.214			
Treatment	1	23117.714	23117.714	3.769	4.75
Error	12	8929.5	744.125		

Decision: The calculated statistic does not lie in CR. Do not reject H_0 with β probability of Type II error.

Conclusion: There was no significant difference between the number of viable *E. coli* colonies in the presence of the hand soap Clean and Smooth® without triclosan at .1X, 1X, and 10X dilution and the hand soap Softsoap® with triclosan at .1X, 1X, and 10X dilution.

Table VII. Number of Bacteria Colonies Grown in Presence of Laundry Detergent

Product	# Colonies	# Colonies	Xi	Average Xi
Arm and Hammer® .1X	22	30	52	26
Arm and Hammer® 1X	0	9	9	4.5
Arm and Hammer® 10X	1	0	1	0.5
Tide® .1X	24	16	40	20
Tide® 1X	22	37	59	29.5
Tide® 10X	0	0	0	0
No soap	191	59	250	125

Table VIII. ANOVA Results for Laundry Detergent

Source	df	SS	MS	F	CR @ 1, 12
Total	13	32047.214			
Treatment	1	23117.714	23117.714	3.769	4.75
Error	12	8929.5	744.125		

Decision: The calculated statistic does not lie in CR. Do not reject H_0 with β probability of Type II error.

Conclusion: There was no significant difference between the number of viable *E. coli* colonies in the presence of the laundry detergent Arm and Hammer® at .1X, 1X, and 10X dilution and laundry detergent Tide® at .1X, 1X, and 10X dilution.

Table IX. Number of Bacteria Colonies Grown in Presence of All Purpose Cleaner

Product	# Colonies	# Colonies	Xi	Average Xi
Pine-Sol® .1X	3	2	5	2.5
Pine-Sol® 1X	1	2	3	1.5
Pine-Sol® 10X	1	0	1	0.5
Lysol® .1X	3	5	8	4
Lysol® 1X	2	4	6	3
Lysol® 10X	0	0	0	0
No soap	191	59	250	125

Table X. ANOVA Results for All Purpose Cleaner

Source	df	SS	MS	F	CR @ 1, 12
Total	13	34711.5			
Treatment	1	25994	25994	3.769	4.75
Error	12	8717.5	726.458		

Decision: The calculated statistic does not lie in CR. Do not reject H_0 with β probability of Type II error.

Conclusion: There was no significant difference between the number of viable *E. coli* colonies in the presence of the cleaner Pine-sol® at .1X, 1X, and 10X dilution and the cleaner Lysol® at .1X, 1X, and 10X dilution.

Discussion

This experiment reveals no difference between the ability of cleaning products containing known antibacterial agents and general cleaning products to kill the bacterium *E. coli*. All products at all dilutions demonstrated the ability to reduce numbers of viable bacteria forming colonies. There was no significant difference between the number of viable *E. coli* colonies in the presence of the dish soap Joy® at .1X, 1X, and 10X dilution and the dish soap Clout® at .1X, 1X, and 10X dilution. There was no significant difference between the number of viable *E. coli* colonies in the presence of the hand soap Clean and Smooth® without triclosan at .1X, 1X, and 10X dilution and the hand soap Softsoap® with triclosan at .1X, 1X, and 10X dilution. There was no significant difference between the number of viable *E. coli* colonies in the presence of the laundry detergent Arm and Hammer® at .1X, 1X, and 10X dilution and laundry detergent Tide® at .1X, 1X, and 10X dilution. There was no significant difference between the number of viable *E. coli* colonies in the presence of the cleaner Pine-sol® at .1X, 1X, and 10X dilution and the cleaner Lysol® at .1X, 1X, and 10X dilution.

With these experimental results and the known risk of the evolution of bacterial mutants as a result of antibiotic overuse, it can be concluded that the use of general cleaning products is a better choice than the use of products with the antibacterial agents triclosan and n-alkyl. The general cleaning product Pine-sol® reduced number of viable *E. coli* as effectively as did Lysol®, which contains n-alkyl. Pine-sol® contains pine oil, a natural disinfectant that destroys the membranes of bacterial cells. As it kills bacteria, pine oil targets multiple bacterial gene pathways, more than n-alkyl. It can be hypothesized that bacterial resistance to pine oil would be more difficult to achieve than resistance to n-alkyl.

The bacterium *E coli* is present in most homes. It is found in both dry (floors, clothing) and wet (sinks, baths, damp washcloths, dishcloths, sponges) areas.[4] The presence of *E. coli* can lead to disease. Consumers may be compelled to purchase cleaning products containing antibacterial agents because it makes them feel good and disease free. According to this experiment, consumers should feel good about using general cleaning products. As Bill Jordan, director of the EPA microbial division, has said, "Instead of surrounding themselves with an arsenal of antibacterials, people are better off washing their hands or using soap and water to clean."[5]

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[4] Parnes, Carol A. "Efficacy of sodium hypochlorite bleach and alternative products". Journal of Environmental Health. Jan/Feb 1997. Volume 59, Issue 6, 14-20.
[5] "Overuse of Triclosan may be creating resistant bacteria." Infectious Disease News. September 1998. Proquest. Online. 3 October 2000.

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