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A Natural Preventative to Menstrual-Related *Staphylococcus aureus* Toxic Shock Syndrome

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Abstract: Menstrual-related Toxic Shock Syndrome (TSS), a bacterial disease contracted by the growth of *Staphylococcus aureus* (*S. aureus*), affects over 10,000 women annually in the United States. *S. aureus* enters and infects the bloodstream via small lacerations made in the vaginal walls while inserting menstrual products, such as tampons and feminine sponges. TSS is often misdiagnosed, however if detected early and treated with antibiotics harbors the risk of bacterial mutation and resistance in the body. The purpose of this study was to investigate whether a natural preventative terminates the risk of contracting TSS. This experiment tested two natural substances: (1) green tea, a known antimicrobial, and (2) reduced glutathione (GSH), a byproduct of *Lactobacillus fermentum*, an essential bacterium needed for vaginal health. To model vaginal conditions in experimentation, all cultures of S. aureus were tested at the average vaginal pH 4 and measured in a spectrophotometer (OD 600). One-way analysis of variance technique indicated that green tea, GSH, and the combination of the two were inhibitors of the bacterium (P-value <0.01); moreover, the combination of both elements not only inhibited but also killed S. aureus. Furthermore, Tukey's multiple comparison method found that the inhibitory growths of bacteria were also statistically significant (P-value <0.01). In summary, the absence of the growth of *S. aureus* due to the combination of green tea and reduced glutathione support their potential use as a natural topical preventative to menstrual-related Toxic Shock Syndrome.

Introduction

In the United States, Toxic Shock Syndrome (TSS) occurs in approximately every 1 out of 100,000 menstruating women. Menstrual-related TSS is caused by a localized staph infection beginning in the vaginal area. The initial infection develops as a result of using insertion-based menstrual products (e.g. super absorbent tampons, feminine cups, and sponges) that cause microabrasions within the vaginal interior, allowing Staphylococcus aureus (S. aureus) to enter the bloodstream. Due to its rarity, TSS is often misdiagnosed which leads to further complications like bacterial resistance which may result in life threatening conditions or death. If TSS infections are caught in early stages, they can be treated with beta-lactam antibiotics like nafcillin or oxacillin through intravenous fluids. TSS staph infections present the chance of becoming antibiotic resistant if the proper course of treatment is not followed.

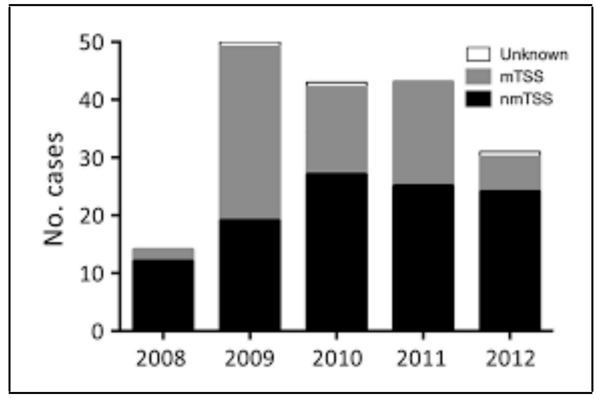


Figure 1: The following graphic from the Center for Disease Control (CDC) represents the different types of TSS recorded in the United States. mTSS (menstrual-related cases) shows a steady increase within the past decade, whereas nmTSS (non-menstrual cases) have a drastic decline.[1]

In the United States, legal measures—like banning highly absorbent tampons and abrasive materials in menstrual products—have been taken to minimize infections, but the threat of menstrual-related TSS is still a threat to women across the globe. This paper aims to investigate the properties of two low-cost, natural potential preventative solutions for TSS infections: (1) reduced reduced glutathione supplements and (2) green tea. For the purposes of experimentation, generic store-brand green tea ($1.99) was used and reduced glutathione supplement powder was purchased online ($22.99).

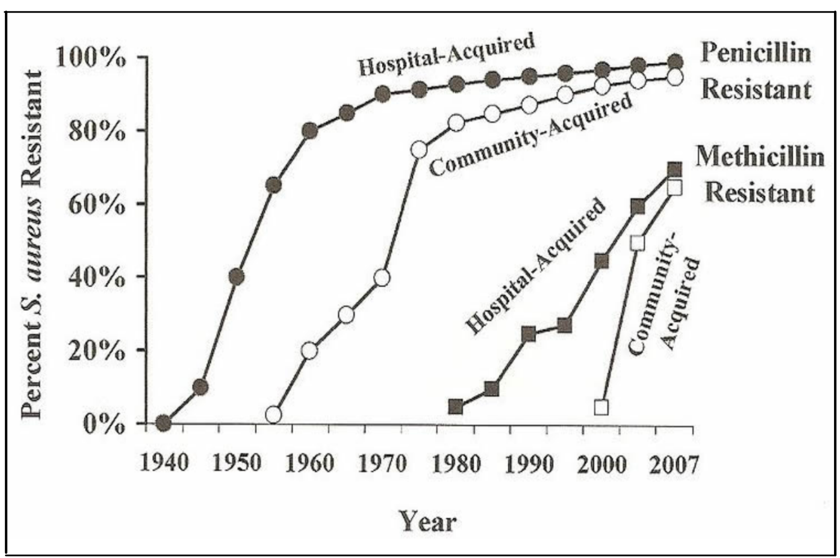
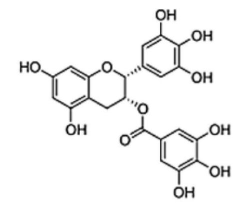


Figure 2: Sourced from the CDC depicts the increasing risk of antibiotic resistant bacteria in various settings. This causes for an emergent response since S. aureus is becoming more resistant under hospital settings.[1]

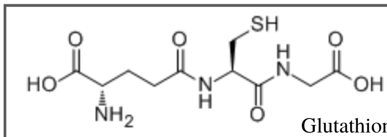
[1]Retrieved from https://wwwn.cdc.gov/nndss/conditions/toxic-shock-syndrome-other-than-streptococcal/case-definition/2011/

Significant Attributions of Natural Elements



Epigallocatechin-3-gallate (EGCg)

Green tea is known to have an antimicrobial effect against various organisms. This effect is the result of the four main catechins in green tea: epicatechin (EC), epicatechin-3-gallate (ECG), epigallocatechin (EGC), and epigallocatechin-3-gallate (EGCG). Within the components of the tea, the EGCG and ECG have shown to have a moderate potency against both Gram-negative and Gram-positive bacteria, like *S. aureus.* EGCG has shown the most potency against S. aureus, according to the WHO. However, for the purposes of developing a low-cost topical preventive for TSS generic, store-brand green tea was used. Green tea, in addition to containing many antioxidants when consumed, also has antibiotic properties when applied topically.



Glutathione

Lactobacillus fermentum is a probiotic essential for vaginal health and creates glutathione (GSH) as a byproduct. *S. aureus* relies on GSH from its host (vaginal area) as its sole sulfur source because it cannot synthesize the chemical itself. *S. aureus* is able to absorb a certain threshold of GSH in order to thrive. However, it is hypothesized that if the bacterium is placed in high concentrations of GSH then the *S. aureus* biofilms will be damaged and decrease the likelihood of occurrence and spreading. Prior to initial experimentation, different concentrations of GSH (in mg) were tested to determine the threshold for *S. aureus*. It was found that 50 mg of GSH supplement powder in distilled water had the least amount of bacterial growth when added to cultures. So, 50 mg of reduced GSH supplement was used in all other experimentations.

Objectives

This study analyzes the antimicrobial properties of green tea and high concentrations of reduced glutathione and its inhibitory effects on S. aureus at average vaginal pH levels. To further advance this field, this paper aims to:

1. Determine if green tea is an inhibitor of *S. aureus*
2. Asses and evaluate the threshold for GSH to inhibit the growth of *S. aureus*
3. Evaluate the inhibition rates of *S. aureus* and GSH with green tea at average vaginal pH
4. Identify whether the combination of green tea and GSH could be used as a natural preventative to TSS.

Materials and Methods

Materials: Prepared LB media, S. aureus cultures, generic store brand green tea, reduced glutathione supplement, Petri dishes, spectrophotometer, hydrochloric acid, sodium hydroxide, Pyrex glass cuvettes, pipettes, burner (for sterilization), distilled water.

To prepare the experiment, several liters of LB media were prepared and used as needed throughout experimentation. Cultures of S. aureus were grown in 100mL of LB media and the pH for all trials were adjusted to 4 using sodium hydroxide or hydrochloric acid. GSH powder was purchased online. Green tea solutions were created by steeping five tea bags in 500mL of water.

The following four trials were used in analysis:

1. Only *S. aureus* cultures
2. Only 5.0 g GSH in *S. aureu*s cultures
3. Only 20mL green tea in *S. aureus* cultures
4. 20mL Green tea and 50 mg GSH in *S. aureus* cultures

An initial reading was taken prior to placing cultures in the incubated shaker to track bacterial growth. The incubated shaker was set to 37°C at 180 rmp and hourly readings were taken with a spectrophotometer at OD 600.

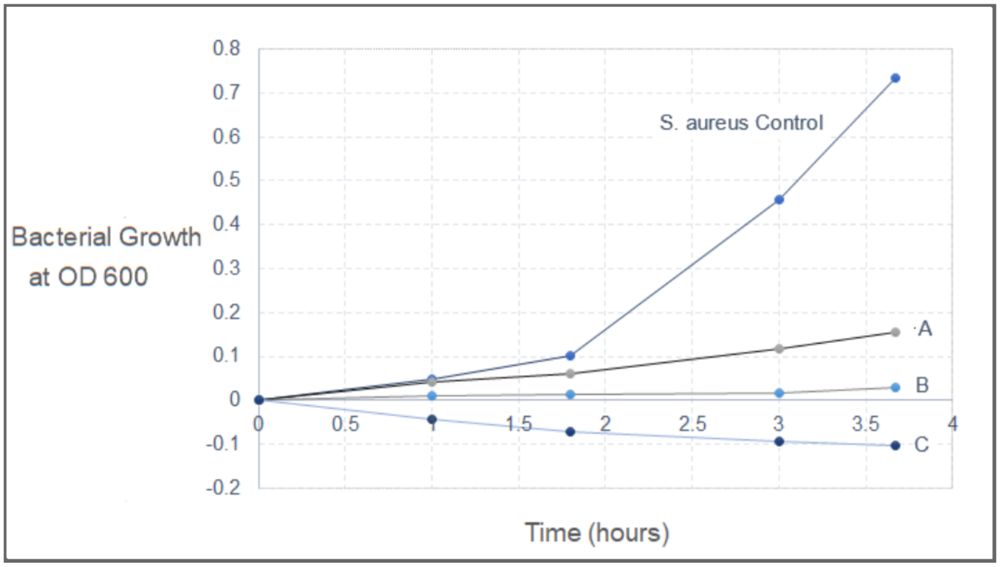
Results

To calculate the growth of *S. aureus,* the initial spectrophotometer reading (to account for background) was subtracted from the hourly culture readings. *Table 1* represents the growth of bacteria without the background.

Table 1: ***Average Growth of S. aureus in GSH and Green Tea***

Time (hours)	Positive Control: *S. aureus*	*S. aureus* and green tea (A)	*S. aureus* and 100mM GSH (at pH 4) (B)	*S. aureus* and 100mM GSH and green tea (at pH 4) (C)
0	0.0207	0.3548, 0.3766	0.0196, 0.0212	0.4340, 0.4405
1	0.0687	0.3964, 0.4218	0.0279, 0.0307	0.3863, 0.3998
1.8	0.1214	0.4124, 0.4413	0.0298, 0.0397	0.3687, 0.3612
3	0.4792	0.4675, 0.4976	0.0359, 0.0373	0.3444, 0.3409
3.67	0.7541	0.5134, 0.5277	0.0433, 0.0542	0.3379, 0.3313

Figure 3: ***Representative Data of Average Growth of S. aureus in GSH and Green Tea***



Additionally, *Figure 3* shows representative data of the average growth of *S. aureus* in GSH and green tea. The average growth times establish a visual of the bacterial growth curves. The positive control shows a significant increase. Green tea (A), GSH (B), and green tea and GSH (C) show comparatively slower growth curves than the positive control.

Analysis

One-Way Analysis of Variance (ANOVA)

One-way ANOVA is used here to test equality of three population means. The hypotheses are as follows:

- Null Hypothesis: $\mu1 = \mu2 = \mu3$, that is, no variation in means among three groups (A, B, and C).
- Alternative Hypothesis: At least one population mean is different.

Since P-value <0.01, there is statistically significant difference in the mean readings of A, B, and C. See the description of A, B, and C in Table 2.

Table 2: ***One-Way ANOVA Results***

Sources of Variation	Degrees of Freedom (df)	Sum of squares (SS)	Mean square (MS)	F	P-value
Groups	$k - 1 = 3 - 1 = 2$	SSG = 0.22592	MSG = SSG / df = 0.11296	MSG / MSE = 1847	< 0.01
Error	$n - k = 6 - 3 = 3$	SSE = 0.00018	MSE = SSE / df = 0.0006		

Tukey's Multiple Comparison Test

Tukey's multiple comparison test is used to compare the difference between all possible pairs of means with appropriate adjustments for multiple testing. The hypotheses are:

- Null Hypothesis: $\mu i = \mu j$
- Alternative Hypothesis: μi μj for all i j =1,2,3

Since P-value <0.01, the results revealed that all pairwise differences are statistically significant.

Table 3: ***Tukey's Multiple Comparison of Means Results***

Groups	Differences	95% Confidence Interval	P-value
B-A	-0.4718	(-0.5045, -0.4391)	0.0000*
C-A	-0.1859	(-0.21863, -0.1533)	0.0003*
C-B	0.2858	(0.2532, 0.3185)	0.0000*

Conclusion

Given the statistical significance, the results suggest that the growth of *S. aureus* is inhibited by the presence of GSH and green tea at the pH 4. The natural elements used, reduced GSH supplement and green tea, were relatively inexpensive at $22.00 and $1.99 respectively. Green tea, a known antimicrobial agent, prevented the growth of *S. aureus* in the LB media. But, in combination with reduced GSH supplements, there was the least amount of bacterial growth.

Line C in Figure 3 shows the representative growth of *S. aureus* in the presence of both natural elements. Rather the bacteria continuing to grow and have a positive trend, the line slopes downwards. This suggests in addition to reduced GSH and green tea inhibiting the growth of *S. aureus*, the combination of the natural elements killed the bacteria. Further testing is required to determine if the bacteria was actually decreasing in on the spectrophotometer or if the reading was attributed to artifact.

Discussion

This experiment aimed to find a combination of natural elements at a natural vaginal pH to be used as a potential topical preventative for TSS. While data from this experiment suggests that 50 mg of reduced GSH supplement and 20mL of five steeped green tea bags can inhibit the growth of S. aureus effectively at pH 4, it is necessary to test this combination on live tissue samples to determine the safety as a topical treatment. If found to be safe, this can become an ointment for any menstruating woman to use, especially those who are unable to change their insertion-based menstrual products frequently.

While menstrual-related TSS is rare in the United States, this infection remains a threat to women across the globe where menstrual products do not have to meet certain criteria (unlike strict regulations from the Food and Drug Administration). Additionally, this novel topical ointment has the potential to drastically decrease menstrual-related TSS cases internationally once further testing is undergone. Menstruation is a natural process that must be made safe for women across the globe and this novel topical preventative can do so.

Acknowledgments

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