Growth of *Staphylococcus aureus* When Using Tampons of Different Compositions

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Introduction

Toxic shock syndrome (TSS) is a rare, but potentially deadly disease caused by the release of toxins produced by *Staphylococcus aureus* and *Streptococcus sordellii*. These bacteria are usually harmless and are typically found on the skin or on mucous membranes, but under certain conditions these strains can become harmful, or even deadly.[1] Anyone is capable of developing toxic shock syndrome, however, those who menstruate are more likely to develop TSS.

Menstrual toxic shock syndrome (mTSS) is the overgrowth of TSST-1-producing *Staphylococcus aureus.* The overproduction of this bacterium leads to the release of toxins, causing the body to drop in blood pressure and depriving organs of oxygen.[2] Those who die from mTSS are typically killed when certain organs stop working, such as their heart and lungs, as a result of the release of toxins. While toxic shock syndrome is rare, with only 1 out of every 100,000 people having it, the symptoms can occur quickly and suddenly. After only a couple days of the bacteria entering the bloodstream, the symptoms can come on, but some progress too quickly, leading to amputation of limbs or death. TSS also needs treatment to ensure that it goes away, otherwise it can remain in the body and continue to spread toxins.[2]

Staphylococcus aureus is typically found in the vaginia and for the majority of the time is harmless. However, if this bacterium is an environment that allows rapid growth, it can then release toxins into the bloodstream, becoming harmful to the human body.[3]

Due to the use of certain tampons acting as a catalyst to the growth of *Staphylococcus aureus*, tampons have been looked into on their effect in developing mTSS.[4] Tampons are commonly used during menstruation and are often used past their recommended time frame. An oversaturated tampon or a tampon past absorption recommendation can provide a better

environment for *Staphylococcus aureus* to grow, potentially leading to increased toxins being released into the bloodstream.[2] Along with absorbance, tampon use has also been found to cause microscopic tears along the vaginal wall if removed when blood flow is light. These tears offer the chance for toxins to enter the bloodstream more easily.[4] Tampon use has also been associated with creating an aerobic environment, rather than the typical anaerobic environment using whiting the vaginal ecosystem. The introduction of oxygen can promote the growth of *Staphylococcus aureus,* as the change in environment can reduce competing bacteria that cannot live in the presence of oxygen. Properties and use of tampons have been explored for any possible correlation between use and risk of developing mTSS. Studies have focused on tampon absorbency, recommended time use and tampon composition.

A tampon's absorbency was the initial characteristic looked into for any correlation with developing menstrual toxic shock syndrome. Super absorbent tampons were found to have the greatest association with mTSS, likely due to creating an environment that bacteria could grow easier. It was also found that using the wrong absorbency depending on one's flow could lead to scratches in the vaginal canal, such as using a tampon if the canal was too dry.[4] The time period of tampon use has also been explored, as companies have recommended time limits one should use, depending on the tampons absorbency. Most companies recommend a use time of six hours, with many encouraging the removal at eight hours as the maximum. However, if tampons are left in for a prolonged period of time, such as overnight,it can lead to promoted growth of bacteria.[4] Composition of tampons has also been explored, as previous ingredients had cancer-linked products in it, such as dioxin, which was used to bleach the tampons. Certain ingredients, such as cotton, viscose and rayon have long been used in tampons, with some research indicating that cotton and rayon affect the growth of *Staphylococcus aureus.*[5]

The purpose of this experiment is to determine the effect that tampon composition has on the growth of *Staphylococcus aureus,* and if certain ingredients lead to increased growth. Since there is both a lack in recent research on this concept, as well as differing results from similar studies, there are multiple gaps that need to be filled. This experiment is looking to identify which tampon ingredients may promote growth of bacteria, comparing different combinations of cotton, rayon and polyester. To carry this out, these tampons of varying composition will go through incubations of different time periods and then cultured to visualize growth of colonies. Tampons composed of just cotton are expected to have less growth of *Staphylococcus aureus* compared to combinations of other ingredients, indicating a lesser chance of developing menstrual toxic shock syndrome. While menstrual toxic shock syndrome is not common, it is still a preventable health risk to humans that can be reduced.

Methods

Composition of tampons and their effect on *Staphylococcus aureus* growth was tested using a modified sac method. The composition of tampons used included cotton only (CVS pure cotton), mixed rayon and cotton (Tampax) and mixed polyester and cotton (L.). *Staphylococcus aureus* was grown on a nutrient agar plate and used to create a nutrient solution of one colony of *S. aureus* per 10mL of nutrient broth. . Four tampons of each brand were used, with one being used as a control in a nutrient only solution. Tampons were put into individual sterile plastic bags, using sterile gloves, with 10 ml of nutrient solution and an *S. aureus* colony. To the control bags, 10 ml of nutrient only solution was added. Excess air was removed through manual deflation. Bags were incubated vertically for periods of 4hr, 6 hr and 8 hr at 37 degrees celsius. Control bags were incubated for a period of 8 hours. Following incubation, tampons were

kneaded for five seconds. For each sample, an inoculating loop was dipped in the expressed liquid and plated onto a nutrient agar plate using a four quadrant method. Colony growth was observed and counted 24 hours later. Data was compared using a t-test to determine which tampon had the highest growth rate.

Results and Discussion

All nine plates that were streaked with the nutrient broth and *Staphylococcus aureus* mixture resulted in some form of growth (Figure 1). The three control plates streaked with just nutrient broth had no growth. For the 4 hour incubation plates, colonies appeared to be noticeably smaller as compared to plates streaked with the 6 hour and 8 hour incubation period broth. The extent of growth for each incubation and tampon brand differed. This was recorded based on which quadrants showed growth and where growth stopped (Table 1). As seen in Table 1, there was growth in quadrant three for all 8 hour incubation plates for each tampon brand, as well for Tampax at 6 hours of incubation. The only plate that's growth was limited to quadrant one was Tampax at 4 hours of incubation. All other plates had growth that only reached quadrant two. The approximate number of colonies for each brand at the different incubation periods were estimated and recorded (Figure 2). There was no consistent trend of growth seen with any tampon brand, with colony numbers varying for each incubation period. Tampon brand, Tampax, saw an increase from 4 hour incubation (~150) to 6 hour incubation (~380). Tampon brands, L. and Pure Cotton, each saw an increase in growth from 6 hour incubation to 8 hour incubation. Tampon brand, L., saw the most significant increase, with ~150 colonies at 6 hour incubation ro ~1000 colonies at 8 hour incubation. Pure Cotton had the least amount of growth overall, with the highest number of estimated colonies occurring at 8 hours of incubation with ~250 colonies.

Despite growth of *Staphylococcus aureus* on all plates, aside from the controls, the data was unusable, making the results inconclusive. Therefore, the hypothesis that cotton only tampons would have the least amount of growth was neither supported or unsupported. However, the preliminary data from this study does suggest that cotton only tampons could have reduced colony growth in comparison to the other tampon brands and their composition. This was seen with the estimated colony counts in Figure 2, where the CVS Pure Cotton had the least number of colony growth overall compared to the other two brands. Despite the data being unusable, it was still surprising to see so much growth with the brand L., especially with the incubation period of 8 hours sample. Limitations such as time, lack of knowledge and human error led to the failed experiment.

If this study was recreated, changes would need to be made to ensure better results. This would include using an agar that was selective for *Staphylococcus aureus,* such as a MSA agar plate or PEA agar plate. This could help ensure that only *Staphylococcus aureus* grows, especially for the initial control plate. To promote better growth of *Staphylococcus aureus* in the broth solution, a broth better suited for the bacterium's growth should be used. Missiakas *et al.* (2018) suggests that Tryptic Soy Broth (TSB) and BHI are the preferred media to grow cultures of *Staphylococcus aureus.*[6] Another change would be to use a known amount of bacteria cells mixed into the 10 ml of nutrient broth. This would allow for more accurate results and data collection.

Citations

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Figures and Tables

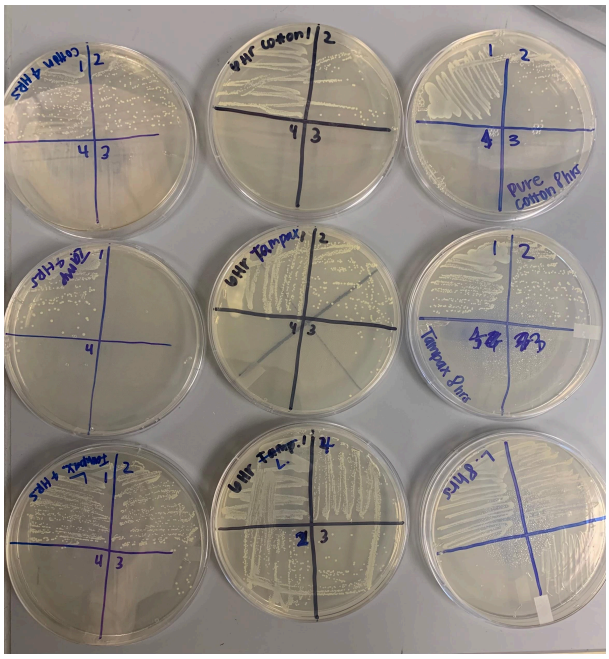

Figure 1. Nutrient Agar Plates 24hr After Streaking. Row one indicates CVS pure cotton (cotton only tampons), row two indicates Tampax (rayon and cotton) and row three indicates L. (polyester and cotton). Columns from top to bottom go from 4 hours, 6 hours and 8 hours of incubation. Controls had no growth and are not pictured above.

Table 1. Extent of Growth for each Tampon Brand. A four-quadrant method was used for streaking, the final quadrant where growth was seen was recorded. Controls had no growth and are not pictured below.

	Tampon Brand		
Incubation Period	Tampax	L.	Pure cotton
4hr	1st quadrant	2nd quadrant	2nd quadrant
6hr	3rd quadrant	2nd quadrant	2nd quadrant
8hr	3rd quadrant	3rd quadrant	3rd quadrant

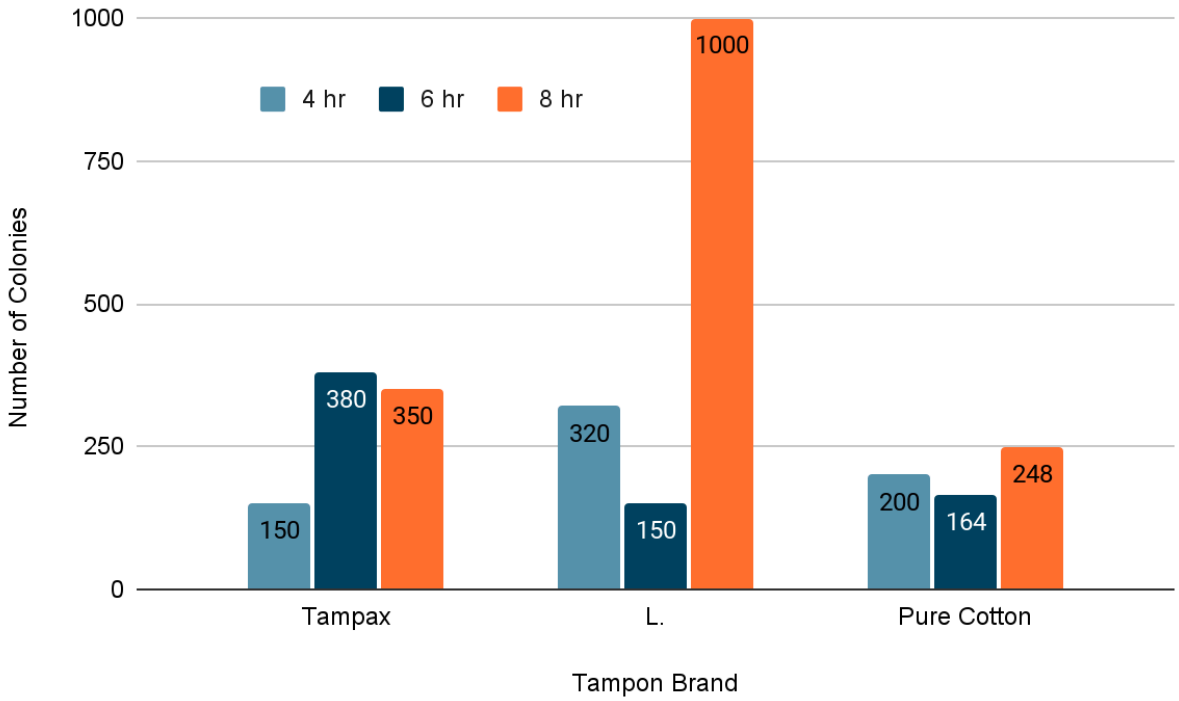

Figure 2. Approximate Number of Colonies for Each Brand at Different Incubation Periods. Colony numbers are estimated and not exact. The key represents the different incubation periods that each tampon went through. Controls had no growth and are not pictured above.