

Abstract

The female vaginal microbiome is a delicate ecosystem susceptible to disruption by foreign elements like menstrual products, potentially leading to conditions such as bacterial vaginosis and urinary tract infections. This study investigated the microorganisms residing on four different menstrual pad brands, exploring their potential implications for women's vaginal health. We targeted bacteria like Staphylococcus Aureus, E. coli, and Candida, as well as assessed the pads' pH levels, moisture content, and antimicrobial properties. While challenges arose in assessing bacterial growth and antimicrobial effectiveness, the findings highlight the need for further research to fully comprehend the microbial ecosystem on menstrual pads and its impact on women's well-being.

Methods and Materials

For this research project, the same methods were used for all menstrual pads. All pads were tested on MacConkey Agar, Bovine Blood Agar, Sabouraud Agar, and Luria-Bertani Agar.

- Bacterial Growth:** Phosphate buffer solution was used in three different methods to identify bacterial growth.
- **Vacuum Filtration:** The PBS solution was filtered through a vacuum filtration system to quantify the amount of possible bacterial sample being collected.
 - **Media Plate Application:** 50-100 microliters of the PBS solution were dispensed onto all media plates and spread evenly.
 - **Quadrant Plates:** 15 microliters of each PBS solution were dispensed onto quadrant plates.

Zone of Inhibition: Pads were tested for potential antimicrobial properties on selective media coated with E. coli, Staphylococcus aureus, and Candida using the following methods:

- **Method 1:** A piece (0.1g) of each pad was introduced onto media plates separated into quadrants.
- **Method 2:** Filter paper soaked with the PBS solution was introduced onto media plates separated into quadrants.

This research project also tested the percentage of moisture for each pad and the pH reading of each pad after being suspended in DI water.

Conclusions

In conclusion, this research attempted to explore the microbial communities on various menstrual pad brands and their potential implications for women's vaginal health. Challenges were encountered in assessing bacterial growth, with concerns about contamination and slow-growing microorganisms. While the zone of inhibition tests showed some promise, filter paper-based methods were less effective. The pH and moisture content assessments underscored the importance of comfort and pH balance. Further, more comprehensive research is needed to fully understand the microbial ecosystem on menstrual pads and its impact on women's well-being.

Introduction

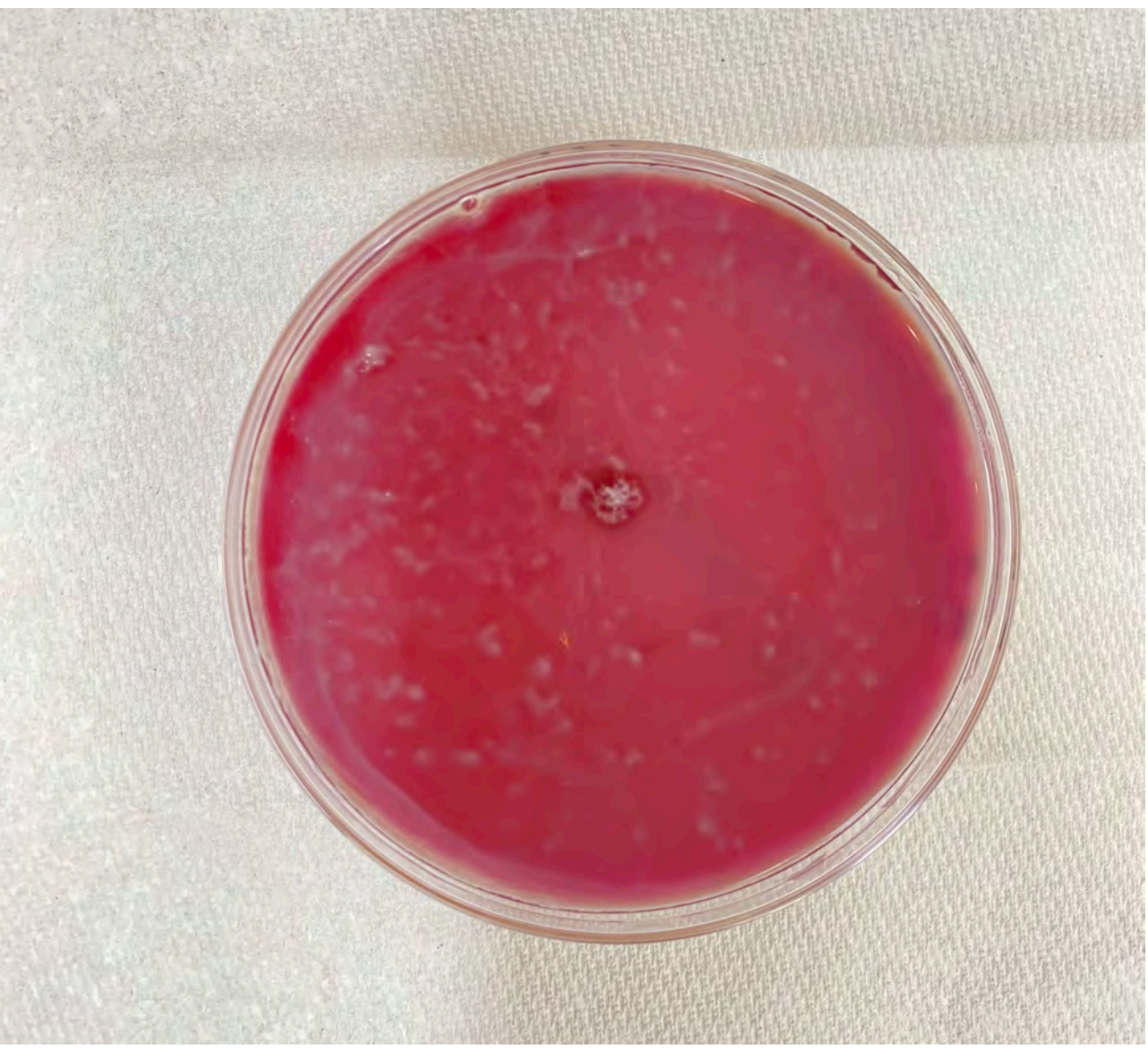
The female vaginal microbiome is a fragile ecosystem that varies among individuals. The introduction of foreign elements like menstrual products can disrupt that fragile ecosystem and leave women vulnerable to conditions such as bacterial vaginosis and urinary tract infections. Countless women use menstrual pads every month, but there is little understanding of what lives on those products. This research project attempts to explore the microscopic world that lives on menstrual pads. The study was conducted on four different menstrual pad brands—two of those being cotton-based, one hemp based, and one being a synthetic-based pad. This was an experimental procedure that tried to identify E. coli, Candida, and Staphylococcus Aureus and a zone of inhibition test to see if the pads were antimicrobial. Staphylococcus Aureus was selected as a test subject because its overgrowth is known to be a leading cause of toxic shock syndrome. E. coli was chosen due to its association with urinary tract infections, often stemming from inadequate menstrual and vaginal hygiene practices. Candida was also included in the study, as yeast overgrowth is a common trigger for yeast infections. The purpose of testing the pH of each pad was to identify any risk that could alter the natural pH of the vagina, which typically resides within the range of 3.8 to 4.2. An imbalance in this pH level could also potentially lead to infections such as bacterial vaginosis, yeast infections, and urinary tract infections. Similarly, if a menstrual product is antimicrobial, as claimed by hemp pads, it could potentially disrupt the balance of the healthy bacteria needed to maintain a healthy vaginal microbiome.

Results

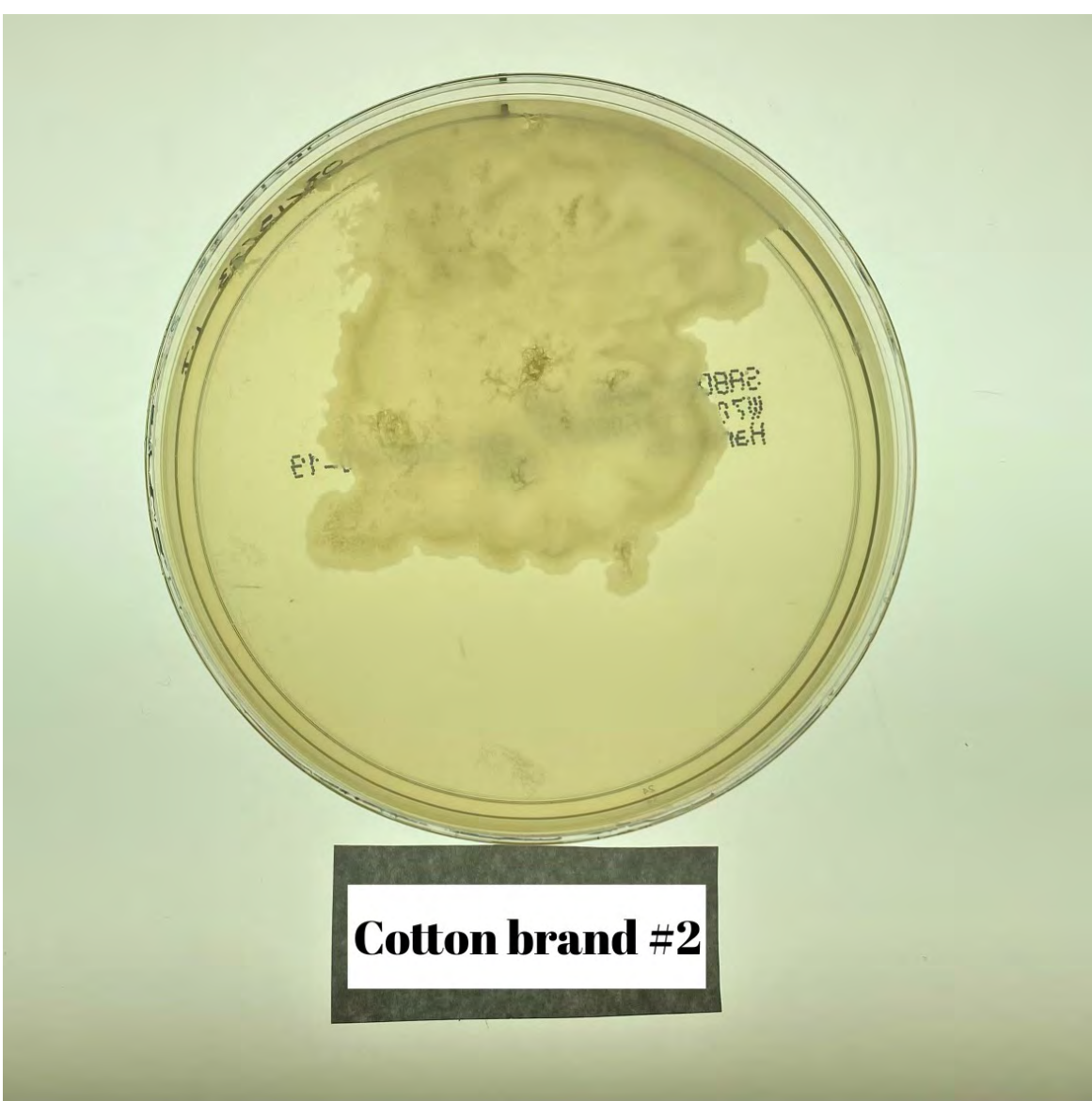
Bacterial growth was initially tested with the filtration system; however, further trials revealed possible contamination by filamentous bacteria. When using the media plate application method, minimal growth was observed on only a few of the agar plates. The quadrant plates with drop application displayed only one fully formed colony and potentially slow-growing bacteria.

The zone of inhibition for the 0.1-0.2g pads showed potential inhibition. However, the use of filter paper was unsuccessful this could be because the control bacteria was inadequately spread.

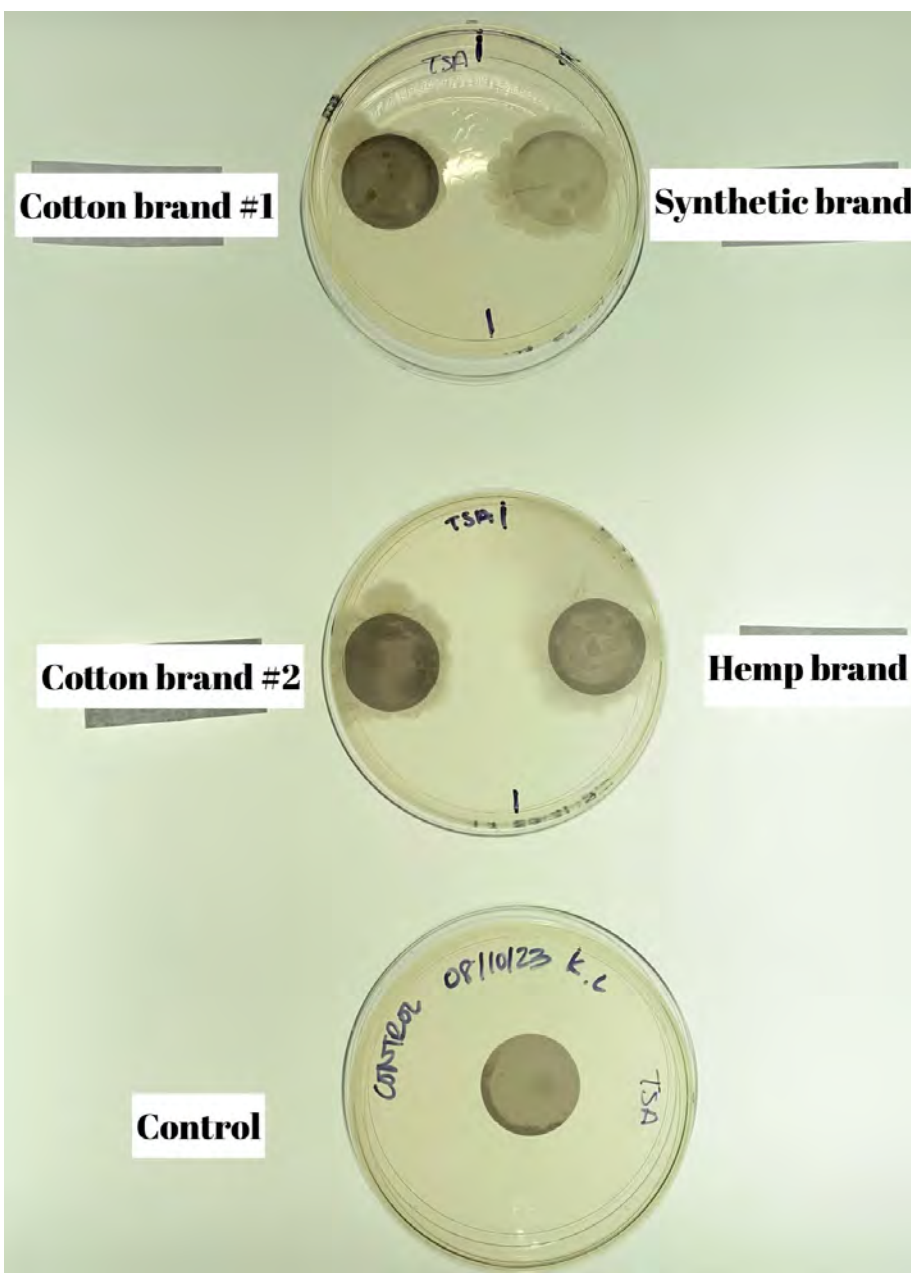
Pad	pH	Percent Moisture
Cotton 1	7.141	94.86%
Cotton 2	4.93	87.93%
Hemp	7.656	87.10%
Synthetic	6.935	90.34%



Bovine Blood Agar with potential yeast growth from hemp pads.



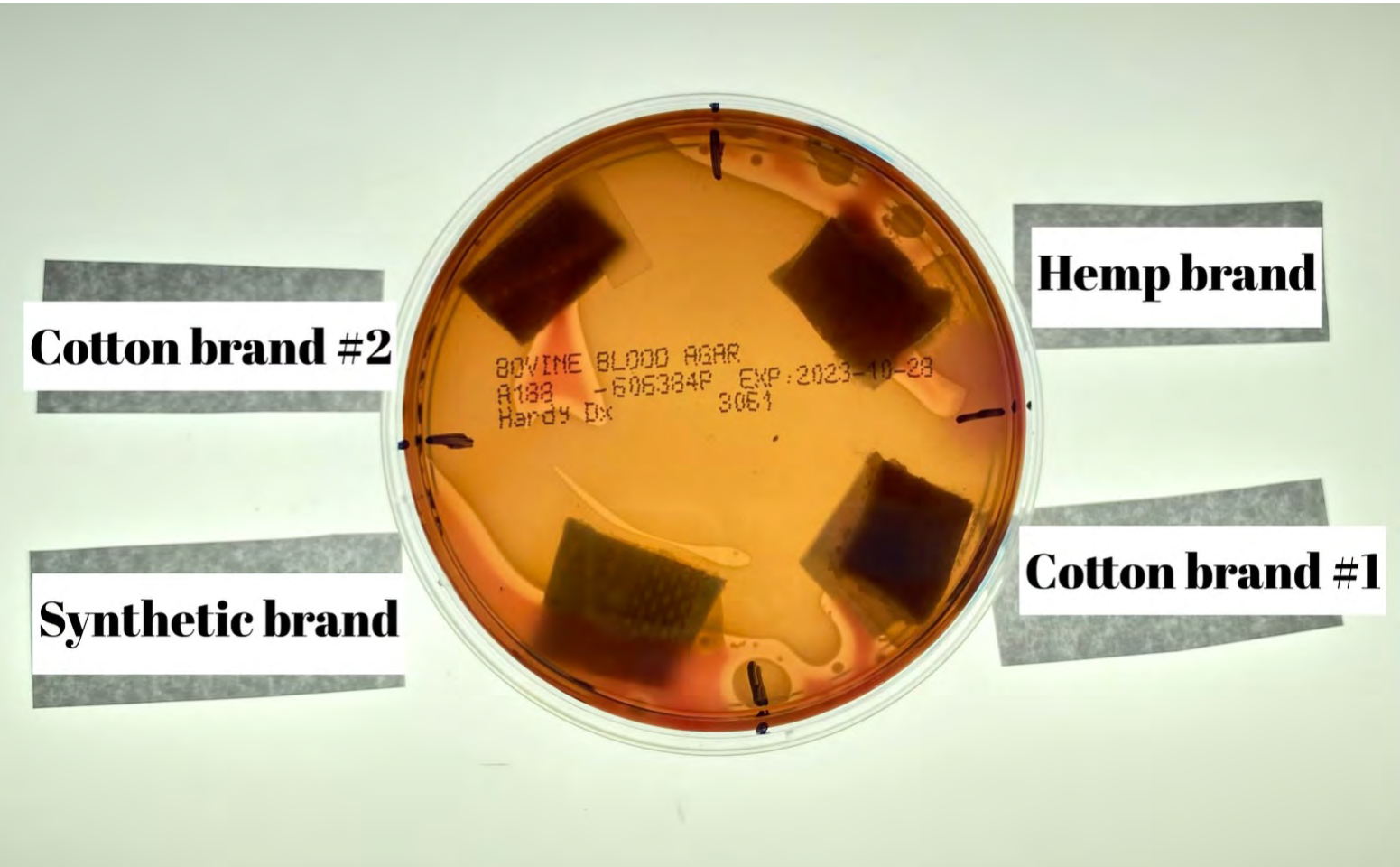
Potential yeast growth from cotton pad on Sabouraud Agar



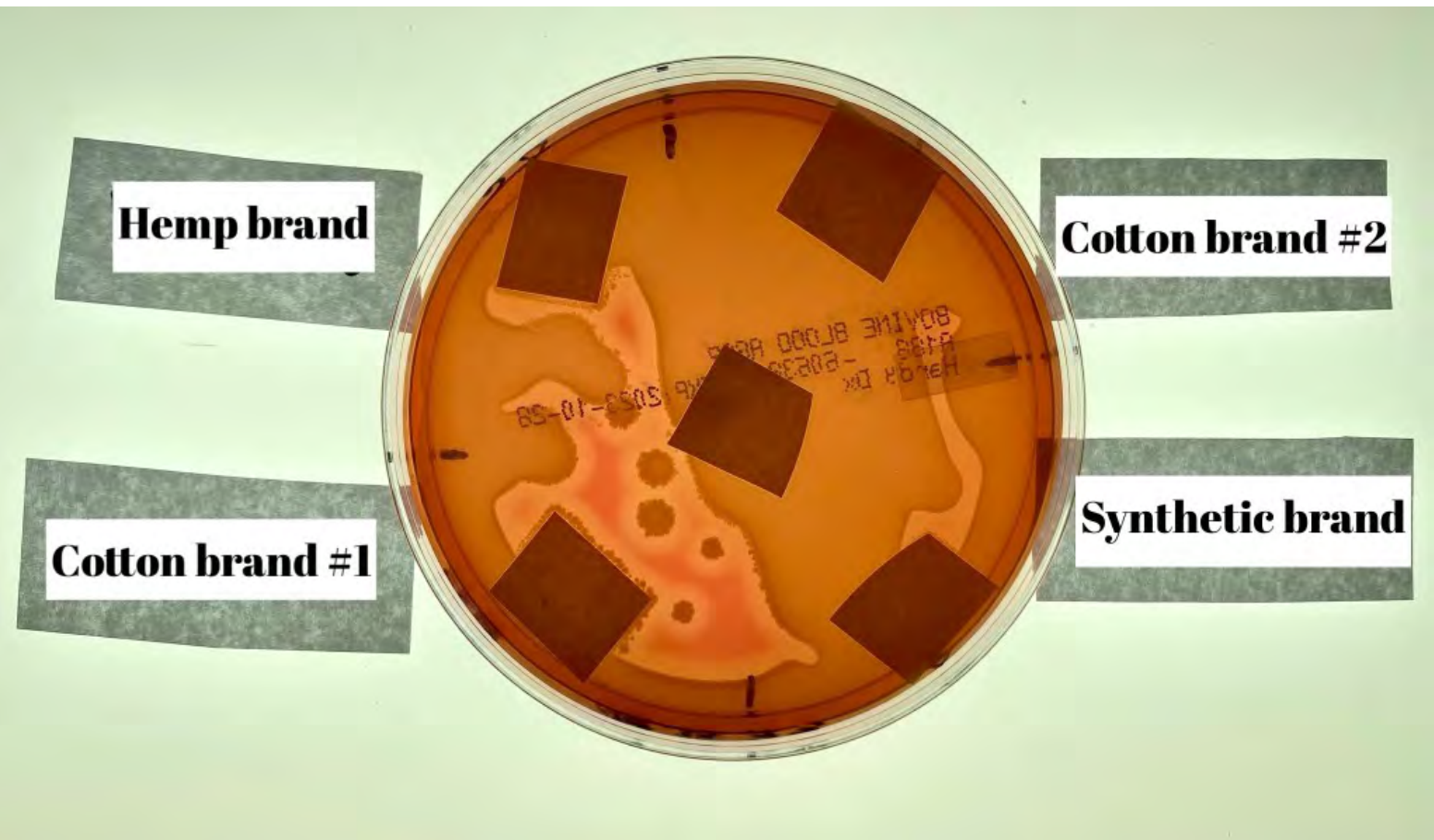
Possible contamination from filter unit plated on Luria-Bertani Agar

Future Directions

In the future, it would be valuable to further explore bacterial growth through experiments involving varying concentrations and media. Additionally, extending research to include tampons and other potentially more invasive menstrual products could provide valuable insights.



Zone of inhibition test using pad pieces on Bovine Blood Agar



Zone of inhibition using PBS Solution on Bovine Blood Agar

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References

Buchta V. Vaginal microbiome. Ceska Gynekol. 2018 Winter;83(5):371-379. English. PMID: 30848142., Yange Sun, Chao Li, Yan Yan, Ailuan Lai, Xiaoxiao Peng, Xiaohong Yue, Yanling Li, Junzhuo Liu, Yan Liu, "Effects of Hemp Sanitary Pads on the Vaginal Microecology", *Computational and Mathematical Methods in Medicine*, vol. 2022, Article ID 4435722, 6 pages, 2022. <https://doi.org/10.1155/2022/4435722>, Schlievert PM, Davis CC. Device-Associated Menstrual Toxic Shock Syndrome. Clin Microbiol Rev. 2020 May 27;33(3):e00032-19. doi: 10.1128/CMR.00032-19. PMID: 32461307; PMCID: PMC7254860.

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Would like to thank Dr. Paul Webster, Rebecca Caputo, Katie-lynn Howard, Maria Lizama, Jessica Uribe, Alexis Travanti, Lisa Wang, Dr. Marianne Smith, Oak Crest Institute of Science, and Citrus College for this opportunity.

Alternate Text

Karina Lizama, Bianca Cerda, Gabby Verde, & Daniela Gonzalez

Oak Crest

'Bacterial Presence on Menstrual Pads'

Abstract: The female vaginal microbiome is a delicate ecosystem susceptible to disruption by foreign elements like menstrual products, potentially leading to conditions such as bacterial vaginosis and urinary tract infections. This study investigated the microorganisms residing on four different menstrual pad brands, exploring their potential implications for women's vaginal health. We targeted bacteria like *Staphylococcus Aureus*, *E. coli*, and *Candida*, as well as assessed the pads' pH levels, moisture content, and antimicrobial properties. While challenges arose in assessing bacterial growth and antimicrobial effectiveness, the findings highlight the need for further research to fully comprehend the microbial ecosystem on menstrual pads and its impact on women's well-being

Introduction: The female vaginal microbiome is a fragile ecosystem that varies among individuals. The introduction of foreign elements like menstrual products can disrupt that fragile ecosystem and leave women vulnerable to conditions such as bacterial vaginosis and urinary tract infections. Countless women use menstrual pads every month, but there is little understanding of what lives on those products. This research project attempts to explore the microscopic world that lives on menstrual pads. The study was conducted on four different menstrual pad brands—two of those being cotton based, one hemp based, and one being a synthetic-based pad. This was an experimental procedure that tried to identify *E. coli*, *Candida*, and *Staphylococcus Aureus* and a zone of inhibition test to see if the pads were antimicrobial. *Staphylococcus Aureus* was selected as a test subject because its overgrowth is known to be a leading cause of toxic shock syndrome. *E. coli* was chosen due to its association with urinary tract infections, often stemming from inadequate menstrual and vaginal hygiene practices. *Candida* was also included in the study, as yeast overgrowth is a common trigger for yeast infections. The purpose of testing the pH of each pad was to identify any risk that could alter the natural pH of the vagina, which typically resides within the range of 3.8 to 4.2. An imbalance in this pH level could also potentially lead to infections such as bacterial vaginosis, yeast infections, and urinary tract infections. Similarly, if a menstrual product is antimicrobial, as claimed by hemp pads, it could potentially disrupt the balance of the healthy bacteria needed to maintain a healthy vaginal microbiome.

Methods and Materials:

For this research project, the same methods were used for all menstrual pads. All pads were tested on MacConkey Agar, Bovine Blood Agar, Sabouraud Agar, and Luria-Bertani Agar.

Bacterial Growth: Phosphate buffer solution was used in three different methods to identify bacterial growth.

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This research project also tested the percentage of moisture for each pad and the pH reading of each pad after being suspended in DI water.

Results: Bacterial growth was initially tested with the filtration system; however, further trials revealed possible contamination by filamentous bacteria. When using the media plate application method, minimal growth was observed on only a few of the agar plates. The quadrant plates with drop application displayed only one fully formed colony and potentially slow-growing bacteria. The zone of inhibition for the 0.1-0.2g pads showed potential inhibition. However, the use of filter paper was unsuccessful this could be because the control bacteria was inadequately spread.

Figure 1: Bovine Blood Agar with potential yeast growth from hemp pads

Figure 2: Potential yeast growth from cotton pad on Sabouraud Agar

Figure 3: Possible contamination from filter unit plated on Luria-Bertani Agar

Conclusions: In conclusion, this research attempted to explore the microbial communities on various menstrual pad brands and their potential implications for women's vaginal health. Challenges were encountered in assessing bacterial growth, with concerns about contamination and slow-growing microorganisms. While the zone of inhibition tests showed some promise, filter paper-based methods were less effective. The pH and moisture content assessments underscored the importance of comfort and pH balance. Further, more comprehensive research is needed to fully understand the microbial ecosystem on menstrual pads and its impact on women's well-being.

Future Directions: In the future, it would be valuable to further explore bacterial growth through experiments involving varying concentrations and media. Additionally, extending research to include tampons and other potentially more invasive menstrual products could provide valuable insights.

Figure 4: Zone of inhibition test using pad pieces on Bovine Blood Agar

Figure 5: Zone of inhibition using PBS Solution on Bovine Blood Agar

References: Buchta V. Vaginal microbiome. Ceska Gynekol. 2018 Winter;83(5):371-379. English. PMID: 30848142., Yange Sun, Chao Li, Yan Yan, Ailuan Lai, Xiaoxiao Peng, Xiaohong Yue, Yanling Li, Junzhuo Liu, Yan Liu, "Effects of Hemp Sanitary Pads on the Vaginal Microecology", *Computational and Mathematical Methods in Medicine*, vol. 2022, Article ID 4435722, 6 pages, 2022. <https://doi.org/10.1155/2022/4435722>, Schlievert PM, Davis CC. Device-Associated Menstrual Toxic Shock Syndrome. Clin Microbiol Rev. 2020 May 27;33(3): e00032-19. doi: 10.1128/CMR.00032-19. PMID: 32461307; PMCID: PMC7254860.

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