

**Bacterial Vaginosis Biofilm Research and Disruption**

(Technical Paper)

**Women's Reproductive Health in the Military**

(STS Paper)

A Thesis Prospectus Submitted to the  
Faculty of the School of Engineering and Applied Science  
University of Virginia • Charlottesville, Virginia

In Partial Fulfillment of the Requirements of the Degree  
Bachelor of Science, School of Engineering

**Christina George**

Fall, 2022

Technical Project Team Members

Kaitlyn Gray

Peyton Johnston

On my honor as a University Student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments

Signature Christina George Date 11/30/2022

Author: Christina George

Approved Jason Papin Date 11/03/2022

Technical Advisor: Jason A. Papin, Ph.D., Department of Biomedical Engineering

Approved  Date 12/03/2022

STS Advisor: Richard D. Jacques, Ph.D., Department of Engineering & Society

## Introduction

Bacterial vaginosis (BV) is a polymicrobial infection caused by the overgrowth of multiple pathogenic anaerobes in the vagina. BV is the most common vaginal infection in reproductive-age women (Machado & Cerca, 2015). The global healthcare treatment costs for symptomatic BV are approximately \$4.8 billion annually (Peebles et al., 2019), likely due in part to the need for frequent recurrent treatment. Racial discrepancies exist regarding BV prevalence, with Hispanic and Black women having higher rates of occurrence compared to other racial groups within North America. Additionally, there are 20% higher rates of BV in women who have sex with women. Symptoms of BV include abnormal vaginal discharge, itching or burning, and odor. The cause of BV is not entirely known, although sexual activity and improper hygiene have been associated with the development of the condition. Untreated BV can lead to higher chances of contracting certain sexually transmitted infections (STIs) such as HIV, chlamydia, or gonorrhea. BV can also lead to Pelvic Inflammatory Disease (PID), which is an infection of a woman's reproductive organs that sometimes results in infertility and other reproductive complications (*STD Facts - Bacterial Vaginosis*, 2022).

*Gardnerella*, the dominant genus associated with BV, is capable of forming a biofilm by synthesizing extracellular polymeric substances (EPS) (Limoli et al., 2015). The cause of BV is still widely unknown as is the composition of this *Gardnerella* biofilm. Current BV treatment options include broad spectrum antibiotics, but research has shown that *Gardnerella* in the biofilm state is associated with decreased antibiotic susceptibility. The technical goals of this project are to 1) identify the primary polysaccharide components of the *Gardnerella* biofilm and 2) understand how carbon sources can modulate *Gardnerella* biofilm synthesis. Using these data, we will 3) predict and test *Gardnerella* biofilm disruptors.

Women's reproductive health is an area of study that has been readily overlooked and widely under-researched, especially when examining the lack of attention to reproductive health issues among women in the military. As minorities, women who serve in the military must deal with atypical hygienic and gynecological issues that are rarely considered by leaders and outsiders. This STS research will examine these research gaps. The technical aspect will help us understand more about a prevalent condition suffered by these women and hopefully prevent some of the recurring infections. Expanding the field of women's reproductive health research will improve the lives of these servicewomen who risk their lives for our country, as well as all women who suffer from related health issues.

### **Bacterial Vaginosis Biofilm Research and Disruption**

Despite its prevalence and lack of effective treatment, there continues to be insufficient information regarding the metabolic mechanisms of BV and the mechanisms underlying the high rate of recurrent infection. A healthy vagina is characterized by the dominance of the lactobacillus genus of bacteria. These bacteria produce lactic acid and drive the acidic environment that usually protects the vagina from the adhesion and growth of pathogenic organisms (Machado & Cerca, 2015). Studies have shown that BV is a result of the overgrowth of multiple harmful bacteria and their replacement of the beneficial bacteria that reside in the vaginal microflora. It was also determined that the *Gardnerella* species is the dominant microbe found in BV patient secretions (Bradshaw et al., 2006). In a disease state, *Gardnerella* adheres to the epithelial cells of the vagina and forms a biofilm that is associated with increased antibiotic resistance (Machado & Cerca, 2015). Bacterial biofilms are usually made of a mix of polysaccharides, proteins, and extracellular DNA. Biofilm formation usually occurs in multiple stages, beginning with initial adherence. The polysaccharide components of these biofilms provide structural support, protection, and help with adherence and continued growth. This

facilitates the creation of heterogeneous populations by recruiting other species into the biofilm (Limoli et al., 2015). These biofilms allow single-celled organisms to take advantage of some of the survival properties of multicellular organisms (Kostakioti et al., 2013). The specific polysaccharides that provide this support in *Gardnerella* biofilm are largely unknown and determining the components would give insight into the development of this infection as well as more effective treatment options.

The current standard treatments for BV are various antibiotics such as Metronidazole, Clindamycin, and Tinidazole, despite the decreased antibiotic susceptibility in this biofilm state. These treatments have an initial cure rate of around 80% to 90%. Although this seems promising, the main problem is seen when examining the high rate of recurrence of the infection. Depending on the type of antibiotic given and certain demographics, multiple studies have shown a recurrence rate ranging from 50% to 100% one year after initial treatment. The recurring *Gardnerella* and other pathogens may become even less susceptible to these antibiotics, further emphasizing the problems with antibiotic treatment (Wu et al., 2022). These issues highlight the need for a reevaluation of the way BV is treated with an emphasis on increased efficacy of antibiotic treatments.

A National Health and Nutrition Examination Survey (NHANES) (2001–2004) showed that 21.2 million women were affected by BV (*CDC - Bacterial Vaginosis Statistics*, 2021). This high prevalence and recurrence rate causes a high economic burden around the world. The medical cost per BV case is highest in North America at \$90.47. Total treatment cost in the United States accounts for \$1.3 billion of the \$4.8 billion global annual cost of BV. Within North America, black women have 33.2% higher rates of BV prevalence, and Hispanic women have 30.7% higher rates than other racial groups (Peebles et al., 2019). BV has also been identified as

a precursor for many chlamydia and gonorrhea cases among women in the military, who experience higher incidence of these STIs than civilian women (Bautista et al., 2018).

### **Women's Reproductive Health in the Military**

Women have been legally permitted to serve in the armed forces since the passage of the Women's Armed Services Integration Act in 1948 (*Women in the United States Army*, n.d.). The most recent Department of Defense (DoD) demographics report (2020) states that there are 229,933 female active-duty members of the United States military, making up 17.2% of total active-duty members. This number has increased by 14.7% from 200,440 in 2000 (*2020 Demographics Profile of the Military Community*, 2020). Although this is still a relatively small number compared to male membership, the increasing trend should predict a brighter future for servicewomen. However, various issues arise when examining the gender discrimination that still exists in today's military. A Government Accountability Office (GAO) report highlights the lower retention rates, lower promotion rates, and higher attrition rate among women in the United States military (U.S. Government Accountability Office, n.d.). Military personnel of all genders face a variety of challenges during their service, but women must deal with additional issues that make deployments and active-duty service difficult. Among these unique challenges faced by women are gynecological issues and pregnancy. Because these issues only affect women, they have historically been under-researched. A gap analysis of the research related to women's health in the United States military grouped their literature search results into eight different major topic areas, some of which were psychological health, injury, chronic illness, and readiness. This study found that of these eight topic areas, the obstetric-gynecologic health topic contained the most gaps and the highest percentage of low-quality articles (Englert & Yablonsky, 2019).

Servicewomen have many different challenges to consider when joining the military and living overseas, one of which is pregnancy. Most of the pregnancies of deployed women are unplanned and are the leading cause of servicewomen leaving combat zones. Research has shown that contraceptive failures are due to lack of knowledge and ineffective training. Additionally, social stigmas surrounding contraceptive use prevent military women from publicly purchasing them and from freely speaking to other members and even their doctors (Jacobson & Jensen, 2011). If pregnancy occurs, United States law prevents DoD medical facilities from performing abortions as well as use of DoD funds except where the mother's life is endangered or in cases of rape or incest (*10 USC 1093: Performance of Abortions: Restrictions*, n.d.). For deployed women living overseas, this means continuing your deployment would require seeking care outside United States military facilities in a foreign country, providing abortion is legal in that country. This also means that servicewomen seeking abortions must cover the associated costs themselves, while living on a military salary. These issues not only create problems for women, but the military as a whole when dealing with short notices and low retention rates.

Women in the military must also deal with personal hygiene problems and their influences on reproductive health. The most common health risks for deployed servicewomen are related to the reproductive system (Steele & Yoder, 2013). Deployment environments have been shown to drive unhygienic behavior that promote gynecological infections in women. Anatomical differences affect seemingly simple tasks such as urination in this heavily male dominated field. Limited numbers of bathrooms and showers, and lack of time and privacy drive unhealthy urination patterns among women in the military (Steele & Yoder, 2013). Menstruation and menstrual symptoms during deployment increase these difficulties, especially with

inadequate availability of tampons and sanitary napkins (Trego, 2007). These are just a few of the problems that contribute to the high urinary tract infection, bacterial vaginosis, and STI rates among women in the military (Bautista et al., 2018). More research is needed to improve the lives of these military women who suffer from these infections. One way we can do this is by understanding more about these infections, so that prevention methods can be optimized for women serving in the United States military.

### **Methods**

The first goal of this research is to identify the primary polysaccharide components of *Gardnerella* biofilm. This goal will be accomplished through lectin staining and accompanying literature reviews. Lectins are highly specific protein molecules that bind to certain carbohydrate groups (Vijayan & Chandra, 1999). Lectins will be chosen based on previous metabolomic data gathered using constraint-based reconstruction and analysis (COBRA) methods and computational predictions (Rawls et al., 2019). Using previously defined synthetic vaginal media (SVM), *G. vaginalis* type strain (ATCC 14018) and a more recently isolated *G. piotti* (JCP8151B) will be grown to a mature biofilm state under anaerobic conditions. The polysaccharide composition of the biofilm will be characterized utilizing *in vitro* immunofluorescent lectin staining. Fluorescence will be analyzed using a fluorescent plate reader and colocalization will be visualized using fluorescent microscopy.

The second goal of this research is to understand how different carbon sources can affect *Gardnerella* biofilm synthesis. The same *in vitro* staining techniques will be applied to *Gardnerella* grown in SVM supplemented with select carbon sources: arabinose, ribose, xylose, glucose, fructose, mannose, galactose, isomaltose, A-lactose, and maltose.

Finally, using all the data and information gathered from previous experiments in addition to independent literature reviews, we will design and test *Gardnerella* biofilm disruptors. SVM will be supplemented with identified compounds that we predict will disrupt biofilm, and co-incubated with mature *Gardnerella* biofilms. Biofilm alteration will be assessed using scanning electron microscopy. These methods will enable us to identify compounds capable of inducing *Gardnerella* biofilm disruption.

### **Conclusion**

The cause of BV is still not fully understood and the composition of the *Gardnerella* biofilm remains largely unknown. This research will increase our understanding of the structure of this biofilm and how certain carbon sources influence its growth. We will also determine compounds that disrupt *Gardnerella* biofilm. By disrupting the biofilm, we can increase the effectiveness of antibiotic treatments and hopefully decrease rates of recurring infections caused by residual biofilm after treatment. The disproportionate infection rate among minority groups as well as the staggering treatment costs of BV highlight the importance of this research in the realm of women's health. This field is readily overlooked and neglected; however, this type of research has the potential to promote equality in the healthcare industry. This research, coupled with research regarding improvements that can be introduced in military settings, will also improve gynecological health among servicewomen. Improving and expanding treatment possibilities for BV patients in and out of the military will help millions of women suffering from BV and reduce other serious complications that are associated with BV.



## References

- 10 USC 1093: Performance of abortions: Restrictions.* (n.d.). Retrieved October 31, 2022, from <https://uscode.house.gov/view.xhtml?req=granuleid:USC-prelim-title10-section1093&num=0&edition=prelim>
- 2020 Demographics Profile of the Military Community.* (2020). 222.
- Bautista, C. T., Wurapa, E. K., Sateren, W. B., Hollingsworth, B. P., & Sanchez, J. L. (2018). Longitudinal association of gonorrhea and bacterial vaginosis with repeat chlamydia diagnoses among U.S. Army women: A retrospective cohort analysis. *Military Medical Research, 5*(1), 37. <https://doi.org/10.1186/s40779-018-0184-3>
- Bradshaw, C. S., Tabrizi, S. N., Fairley, C. K., Morton, A. N., Rudland, E., & Garland, S. M. (2006). The association of *Atopobium vaginae* and *Gardnerella vaginalis* with bacterial vaginosis and recurrence after oral metronidazole therapy. *The Journal of Infectious Diseases, 194*(6), 828–836. <https://doi.org/10.1086/506621>
- CDC - Bacterial Vaginosis Statistics.* (2021, May 6). <https://www.cdc.gov/std/bv/stats.htm>
- Englert, R. M., & Yablonsky, A. M. (2019). Scoping Review and Gap Analysis of Research Related to the Health of Women in the U.S. Military, 2000 to 2015. *Journal of Obstetric, Gynecologic & Neonatal Nursing, 48*(1), 5–15. <https://doi.org/10.1016/j.jogn.2018.10.009>
- Jacobson, J. C., & Jensen, J. T. (2011). A Policy of Discrimination: Reproductive Health Care in the Military. *Women's Health Issues, 21*(4), 255–258. <https://doi.org/10.1016/j.whi.2011.03.008>
- Kostakioti, M., Hadjifrangiskou, M., & Hultgren, S. J. (2013). Bacterial Biofilms: Development, Dispersal, and Therapeutic Strategies in the Dawn of the Postantibiotic Era. *Cold Spring Harbor Perspectives in Medicine, 3*(4), a010306.

<https://doi.org/10.1101/cshperspect.a010306>

- Limoli, D. H., Jones, C. J., & Wozniak, D. J. (2015). Bacterial Extracellular Polysaccharides in Biofilm Formation and Function. *Microbiology Spectrum*, 3(3), 10.1128/microbiolspec.MB-0011–2014. <https://doi.org/10.1128/microbiolspec.MB-0011-2014>
- Machado, A., & Cerca, N. (2015). Influence of Biofilm Formation by *Gardnerella vaginalis* and Other Anaerobes on Bacterial Vaginosis. *The Journal of Infectious Diseases*, 212(12), 1856–1861. <https://doi.org/10.1093/infdis/jiv338>
- Peebles, K., Velloza, J., Balkus, J. E., McClelland, R. S., & Barnabas, R. V. (2019). High Global Burden and Costs of Bacterial Vaginosis: A Systematic Review and Meta-Analysis. *Sexually Transmitted Diseases*, 46(5), 304–311. <https://doi.org/10.1097/OLQ.0000000000000972>
- Rawls, K. D., Dougherty, B. V., Blais, E. M., Stancliffe, E., Kolling, G. L., Vinnakota, K., Pannala, V. R., Wallqvist, A., & Papin, J. A. (2019). A simplified metabolic network reconstruction to promote understanding and development of flux balance analysis tools. *Computers in Biology and Medicine*, 105, 64–71. <https://doi.org/10.1016/j.combiomed.2018.12.010>
- STD Facts—Bacterial Vaginosis*. (2022, June 2). <https://www.cdc.gov/std/bv/stdfact-bacterial-vaginosis.htm>
- Steele, N., & Yoder, L. H. (2013). Military Women’s Urinary Patterns, Practices, and Complications In Deployment Settings. *Urologic Nursing*, 33(2), 61–78. <https://doi.org/10.7257/1053-816X.2013.33.2.61>
- Trego, L. L. (2007). Military Women’s Menstrual Experiences and Interest in Menstrual

- Suppression During Deployment. *Journal of Obstetric, Gynecologic & Neonatal Nursing*, 36(4), 342–347. <https://doi.org/10.1111/j.1552-6909.2007.00166.x>
- U.S. Government Accountability Office. (n.d.). *Female Active-Duty Personnel: Guidance and Plans Needed for Recruitment and Retention Efforts*. Retrieved October 31, 2022, from <https://www.gao.gov/products/gao-20-61>
- Vijayan, M., & Chandra, N. (1999). Lectins. *Current Opinion in Structural Biology*, 9(6), 707–714. [https://doi.org/10.1016/S0959-440X\(99\)00034-2](https://doi.org/10.1016/S0959-440X(99)00034-2)
- Women in the United States Army*. (n.d.). Retrieved October 31, 2022, from <https://www.army.mil/women/history/>
- Wu, S., Hugerth, L. W., Schuppe-Koistinen, I., & Du, J. (2022). The right bug in the right place: Opportunities for bacterial vaginosis treatment. *Npj Biofilms and Microbiomes*, 8(1), Article 1. <https://doi.org/10.1038/s41522-022-00295-y>